Structure-Activity Relationships in the *trans*-Hexahydroindolo[4,3-*ab*]phenanthridine ("Benzergoline") Series. 2. Resolution, Absolute Configuration, and Dopaminergic Activity of the Selective D_1 Agonist CY 208-243 and Its Implication for an "Extended Rotamer-Based Dopamine Receptor Model"

Max P. Seiler,* Philipp Floersheim, Rudolf Markstein, and Armin Widmer

Sandoz Pharma Ltd., Preclinical Research, CH-4002 Basel, Switzerland, and Sandoz Research Institute Berne Ltd., P.O. Box CH-3001 Berne, Switzerland

Received July 10, 1992

4,6,6a,7,8,12b-Hexahydroindolo[4,3-*ab*]phenanthridines ("benzergolines") was the first structural class of potent and selective dopamine D_1 agonists lacking a catechol group. In order to determine the enantioselectivity of the 7-methyl derivative in the adenylate cyclase assay, its 5,5a-dihydro precursor was resolved and both enantiomers oxidized to the final products. The biological activity was found to reside entirely in the (-)-enantiomer, (-)-1 (CY 208-243). An X-ray study of its (-)-mandelic acid salt revealed a 6a*R*,12b*R* absolute configuration, which, in confirmation of the structure hypothesis, corresponds to that of the ergolines. Unexpectedly, an axial conformation of the *N*-methyl group was observed in the crystal structure. In contrast, subsequently analyzed crystals of the free base of (-)-1 revealed an equatorial conformation of the *N*-methyl group, which, we assume, represents the bioactive conformation. Based on the determined absolute configuration, (-)-1 could be oriented in a previously described "rotamer-based dopamine receptor model", which allowed the localization of a "subtype selectivity-inducing site" (aryl binding site at the D_1 receptor, steric barrier at the D_2 receptor), marked by the conformationally fixed "additional" phenyl group of the benzergoline molecule.

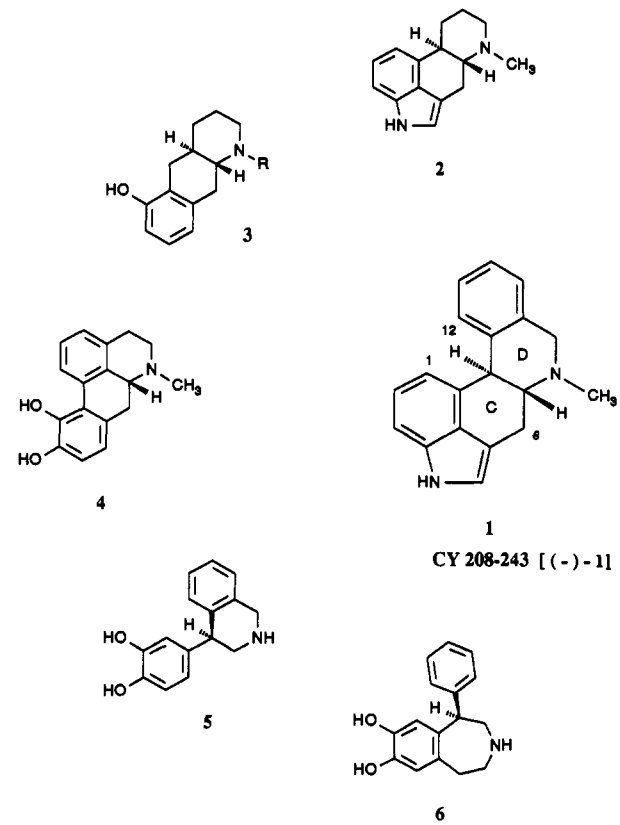
Introduction

We recently described *trans*-hexahydroindolo[4,3-*ab*]-phenanthridines ("benzergolines") as a new type of potent and selective dopamine (DA) D_1 receptor agonists with an unique structure in which the usual catechol group is lacking and in which a D_1 selectivity-inducing phenyl group is integrated into a fairly rigid pentacyclic skeleton.[1] The biologically active (-)-enantiomer of the *N*-methyl derivative 4,6,6a,7,8,12b-hexahydro-7-methylindolo[4,3-*ab*]-phenanthridine (CY 208-243, (-)-1) has been selected for development and its pharmacology in the CNS[2] and in the periphery[3] has already been described in some details. Here, we report resolution, absolute configuration, and enantioselectivity of the antipodes of 1.

The active enantiomers of several structural types of D_1 receptor activating drugs have been determined and their absolute stereochemistry has been elucidated. Among them are the unselective compounds *N*-methylergoline,[4] **2**, (4a*R*,10a*R*)-*N*-*n*-propyl-6-hydroxy-1,2,3,4,4a,5,10,10a-octahydrobenz[*g*]quinoline[5] ((4a*R*,10a*R*)-*N*-Pr-6-OH-OBGQ), (*R*,*R*)-**3** (R = *n*-propyl), and apomorphine,[6] **4**, and the selective D_1 agonists (*S*)-4-(3′,4′-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline[7] ((*S*)-DPTI), (*S*)-**5**, and (*R*)-SKF38393,[8] (*R*)-**6** (Chart I). The benzergolines combine the pharmacophore of the ergolines with that of DPTI in an orientation suggested by our previously described "rotamer-based DA receptor model",[5] which considers the absolute configuration at the asymmetric carbon adjacent to the basic nitrogen. According to this structure hypothesis, the absolute stereochemistry of the active enantiomer of 1 should correspond to that of the ergolines.

The D_1 selectivity and potency in 1, 5, and 6 is induced and enhanced, respectively, by a critically positioned "additional" phenyl group,[9] which appears to bind to a D_1 receptor-specific accessory aryl binding site. The counterpart to this site at the D_2 receptor presumably is a steric barrier, which prevents binding of DA agonists with such an additional aryl group. The localization of this "subtype

Chart I. Active Enantiomers of D_1 Receptor Activating Drugs (Absolute Configuration Shown)



0022-2623/93/1836-0977$04.00/0

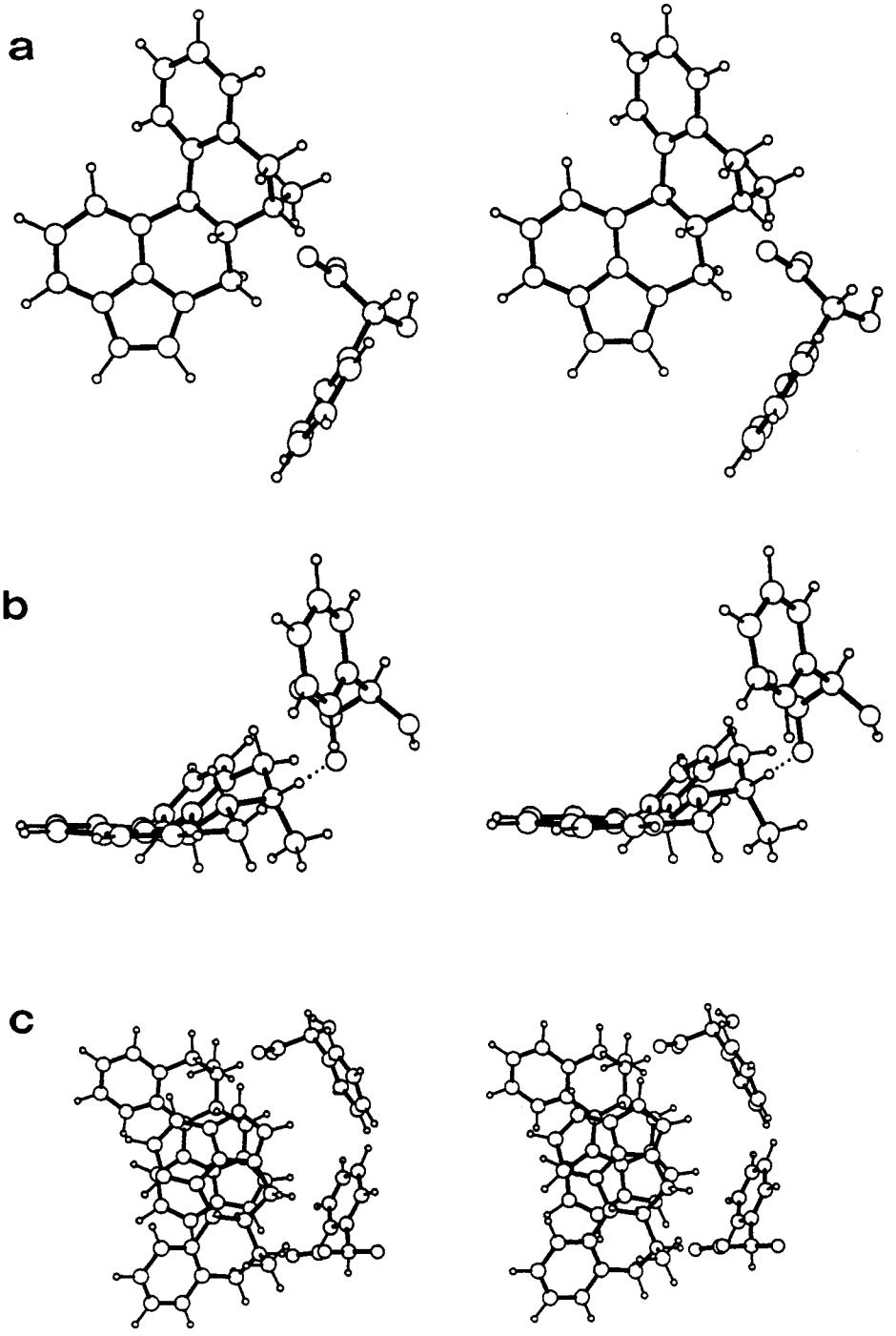


Figure 1. X-ray structure of (−)-*trans*-4,6,6a,7,8,12b-hexahydro-7-methylindolo[4,3-*ab*]phenanthridine (−)-mandelate. Stereoscopic representation of the molecule. (a) Top view. (b) Frontal view. (c) View along the crystallographic *a*-axis.

selectivity-inducing site" in the rotamer-based DA receptor model cannot be determined with the currently known enantioselective D_1 agonists, since the crucial phenyl group is freely rotating in DPTI, **5** (integrated in a tetrahydroisoquinoline), whereas in SKF38393, **6**, the dihydroxytetrahydrobenzazepine DA pharmacophore is symmetrical. The benzergolines, however, have an asymmetric DA pharmacophore, in which the additional phenyl group is conformationally fixed. The elucidated absolute configuration of the active enantiomer of **1** should thus allow unequivocal orientation of the molecule in the receptor model, thereby determining the position of this subtype selectivity-inducing site relative to the "major binding sites" for the amino and *m*-hydroxy group of DA.

Chemistry

Separation of the enantiomers of **1** was attempted using the conventional method of fractional crystallization of diastereomeric salts. Crystalline salts were readily obtained with several optically active acids, e.g. (−)-dibenzoyltartaric acid, (+)- and (−)-mandelic acid, and (−)-tartaric acid. However, no conditions could be found to induce significant resolution. Therefore, resolution of precursors of **1** was investigated. The 5,5a-dihydro derivative of **1**, in form of its mandelic acid salts, proved to be a suitable substrate for an efficient resolution, which required only a few recrystallizations. The resolved enantiomers were subsequently oxidized to the optically

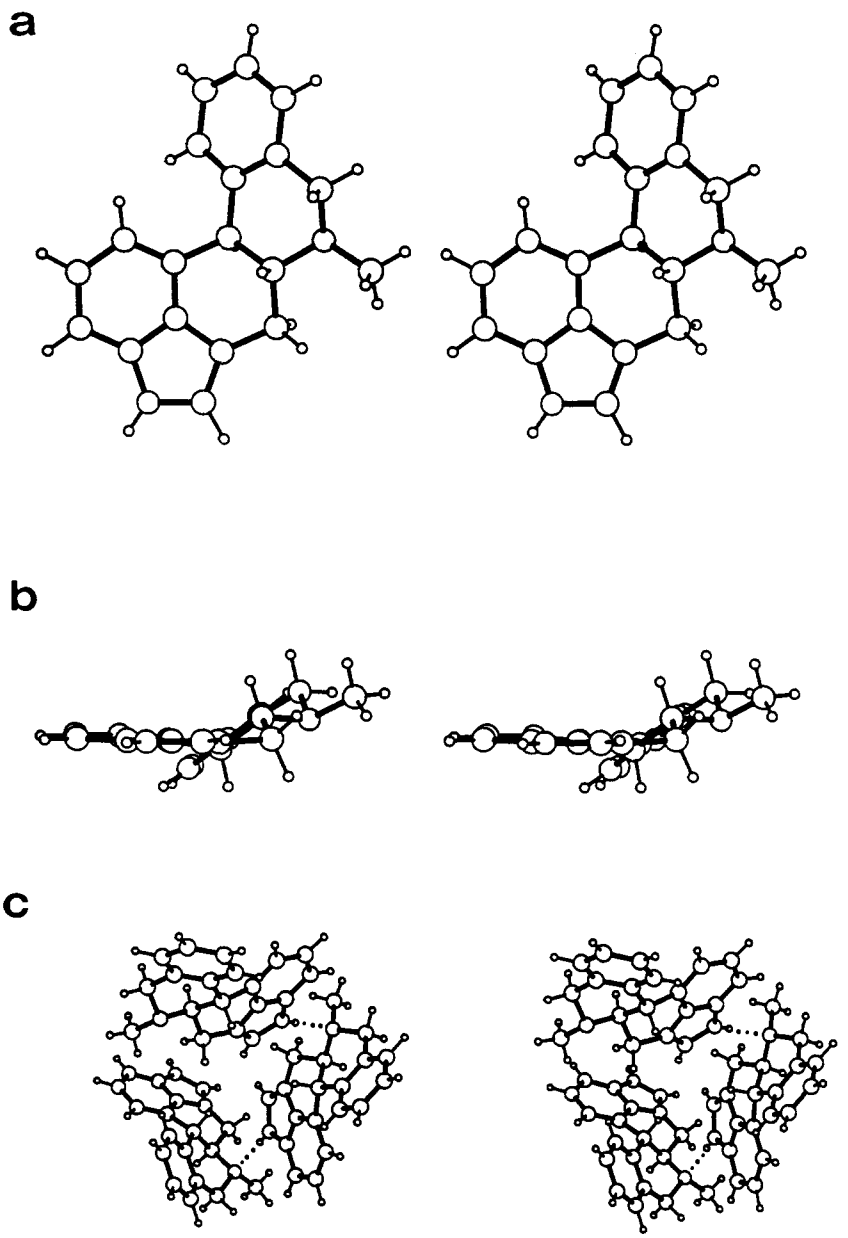


Figure 2. X-ray structure of (−)-*trans*-4,6,6a,7,8,12b-hexahydro-7-methylindolo[4,3-*ab*]phenanthridine. Stereoscopic representation of the molecule. (a) Top view. (b) Frontal view. (c) View along the crystallographic c-axis.

active benzergolines (+)- and (−)-1 as previously described for the racemate.[1]

The determination of the absolute configuration of the enantiomers of 1 was achieved by single-crystal X-ray analysis of its (−)-antipode in form of its (−)-mandelic acid salt. For conformational studies, an additional single-crystal X-ray analysis was performed with the free base of (−)-1.

Results and Discussion

Absolute Configuration, Solid-State Conformations, and Molecular Mechanics Calculation of (−)-1. The X-ray structure of the (−)-mandelic acid salt of (−)-1, is shown in Figure 1. The asymmetric unit contains two independent but conformationally similar benzergoline molecules. The protonated basic nitrogen participates in a hydrogen bond to a mandelate carboxyl oxygen (N···O = 2.604 Å). The second carboxyl oxygen is linked by another hydrogen bond to the indole nitrogen of a second benzergoline molecule (O···N = 2.765 Å). The absolute configuration of (−)-1 in the crystal could be determined as 6a*R*,12b*R*, based on the known *R* configuration of the (−)-mandelate anion.

The shape of the benzergoline molecules in the crystal merits some comments. Rings A, B, and C lay in a plane with the exception of C(6a), which forms an envelope.[10] A second plane is determined by rings D and E with the exception of N(7), which forms another envelope. The angle between these planes was measured as 44.46° and 44.94° for the two benzergoline molecules in the unit cell. The two hydrogens H(1) and H(12), facing each other on the indole and phenylen ring, are separated by 2.3 Å, indicating no steric interaction between these rings. Unexpectedly, the conformation of the *N*-methyl group of the piperidine ring was found to be axial. This could be due to the protonated nitrogen, or to the coexistence of both configurations at the asymmetric nitrogen, of which the form with the energetically favored crystal lattice would first crystallize. The special nature of this observation was confirmed by examination of the Cambridge X-ray data file,[11] which revealed no example of an axial *N*-methyl group in protonated α-unsubstituted *N*-methylpiperidine

Table I. Biological Activities

compd	adenylate cyclase[a] potency pD_2	adenylate cyclase[a] maximal stimulation,[b] % rel to DA	ACH release[a] potency pD_2	ACH release[a] maximal inhibition,[c] % of control
(±)-1	6.1	57		0
(+)-1		0		0
(−)-1 (CY 208-243)	6.9	68		0
reference compounds				
6-methylergoline (2)	5.2	24	7.5[d]	30
N-Me-6-OH-OBGQ[e,f] (3, R = Me)	6.15	14	7.3	62
apomorphine (4)	6.2	38	7.5	85
DPTI[e,g] (5)	6.0	86		0
SKF38393[e] (6)	6.6	58		0

[a] Mean values of two to six independent experiments performed in triplicate (SE < 10%). [b] Percent of maximal effect of 125 μM DA. [c] Change of S_2/S_1 versus control at 1 μM. [d] Partial agonist; inhibition of DA effect: $pK_i = 6.8$. [e] Racemic compounds. [f] N-Me-6-OH-OBGQ = N-methyl-6-hydroxy-1,2,3,4,4a,5,10,10a-octahydrobenzo[g]quinoline. [g] DPTI = 4-(3′,4′-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline.

partial structures and only two axial conformations within a group of 27 protonated α-monosubstituted N-methylpiperidines.[12,13] We thus decided to examine the X-ray structure of the free base of (−)-1 in addition to that of the mandelic acid salt.

In the crystal structure of the free base of (−)-1 (Figure 2), the indole NH forms a hydrogen bond to the basic nitrogen of a second benzergoline molecule (N(4)···N(7′) = 3.072 Å), which, according to the distances N(4)···H = 0.864 Å and H···N(7′) = 2.212 Å, can be considered as not protonated. In contrast to the crystal structure of the (−)-mandelic acid salt, this crystal form shows the expected equatorial conformation of the N-methyl group in accordance with all known X-ray structures of ergot derivatives, with the angle between the planes of the indole and phenyl rings being 39.01°. Apart from the inverted configuration at the asymmetric nitrogen, the overall shape of the benzergoline molecule is thus similar to the conformation found in the crystal of its (−)-mandelic acid salt.

In order to get additional information about the relevant conformation of the protonated N-methylbenzergoline molecule, the heat of formation was calculated using MOPAC optimization (AM1-Hamiltonian).[14] The axial conformation gave a value of 239.75 kcal/mol, whereas for the equatorial conformation a slightly lower value of 238.56 kcal/mol was determined. We might thus assume that the axial conformation found in the salt is indeed a crystallizing effect and that the relevant bioactive conformation of (−)-1, in accordance with the equatorial conformation found in all N-alkylpiperidine-containing DA agonists, e.g. octahydrobenzo[g]quinolines, ergolines, and apomorphine, corresponds to the equatorial form.

Structure–Activity Relationships (SAR). For a potent, large molecule having an asymmetric center adjacent to a crucial amino group, one would expect distinct enantioselectivity. This is indeed the case, since the (+)-enantiomer of 1 was found to be totally inactive, whereas the (−)-enantiomer showed a potency superior to the known unselective DA agonists apomorphine, 6-methylergoline, and N-Me-6-OH-OBGQ (racemic) and the racemic forms of the most potent selective D_1 agonists SKF-38393 and DPTI (Table I). With an efficacy of 68% relative to DA, (−)-1 should, however, be considered as a partial agonist in the adenylate cyclase paradigm.

The benzergoline molecule contains two asymmetric centers, both of which are adjacent to crucial functionalities, namely C(6a), which is α to the essential amino group, and C(12b), which is α to the D_1 selectivity-inducing phenyl group. In (−)-1, the determined R configuration of C(6a) corresponds to the absolute configuration of the respective asymmetric center in N-methylergoline, 2, apomorphine, 4, and in the active enantiomer of N-Pr-6-OH-OBGQ, 3 (R = n-propyl). The determined R configuration of C(12b), on the other hand, corresponds to the absolute configuration of the respective asymmetric center in the biologically active enantiomer of DPTI, 5, and SKF38393, 6 (Chart I). Thus, (−)-1 nicely links the two series of D_1 agonists with different types of asymmetric center. The results confirm our original structure hypothesis for the design of the benzergolines,[1] which relied on a combination of the ergoline and the DPTI molecule with the tetrahydroisoquinoline part of DPTI forming rings D and E of the benzergolines.

Extended Rotamer-Based Dopamine Receptor Model. Based on earlier ideas about DA receptor interactions of aminotetralins[15–17] (AT) and octahydrobenzo[f]quinolines,[18] in combination with new SAR data on octahydrobenzo[g]quinolines,[5] we recently suggested a rotamer-based DA receptor model,[5] which is valid for D_1 as well as for D_2 agonists. This model relies upon the two rotamer conformations of DA,[19] represented by α-rotameric (S)-5-hydroxy-2-aminotetralin and β-rotameric (R)-7-hydroxy-2-aminotetralin, which describe the bioactive conformations and minimal pharmacophores of DA in its N-unsubstituted ((R)-7-OH-AT) and di-N-n-propylated ((S)-5-OH-AT) form, respectively.[17] The increase in affinity/activity of 5-OH-AT upon di-N-n-propyl substitution has been explained by an interaction with "N-alkyl binding sites", which are accessible for α-rotameric compounds at both receptor subtypes, for β-rotameric compounds, however, only at the D_2 receptor.[16,17] New potential ligands are oriented in the model, guided by a set of criteria, along these two AT rotamers toward the two major binding sites (for amino and m-hydroxy group of DA). The elucidation of the absolute configuration of (−)-1 now allows an extension of the model, namely the localization of a subtype selectivity-inducing element, which consists of an aryl binding site at the D_1 receptor and its counterpart, a steric barrier at the D_2 receptor.

The construction of the "extended model" is shown in Figure 3. The two major binding sites for m-hydroxy and amino groups of DA are defined by β-rotameric (R)-7-OH-AT representing the bioactive conformation of DA. Its superimposition with α-rotameric (S)-5-OH-AT serves as a basic framework for the orientation of new structures (Figure 3a). The existence of a "general steric barrier" (part of which surrounds the "small N-alkyl binding site") is suggested by the inactivity of 8-OH-OBGQ and the observation that replacement of the n-propyl substituent in N-Pr-6-OH-OBGQ by n-butyl results in a loss in affinity/activity (Figure 3b).[5] Ergolines are oriented in the model in an α-rotamer type fashion along (S)-5-OH-AT (Figure 3c),[5] based on the corresponding absolute configuration of the asymmetric carbon adjacent to the basic nitrogen, the observed increase in potency upon N-n-propyl substitution (which, at the D_1 site, is found with α-rotameric but not with β-rotameric DA pharmacophores[16]) and the observed inactivity of the N-n-butyl derivative indicating that the N-alkyl substituent points toward the small N-alkyl binding site.[20] Since in the benzergoline series,

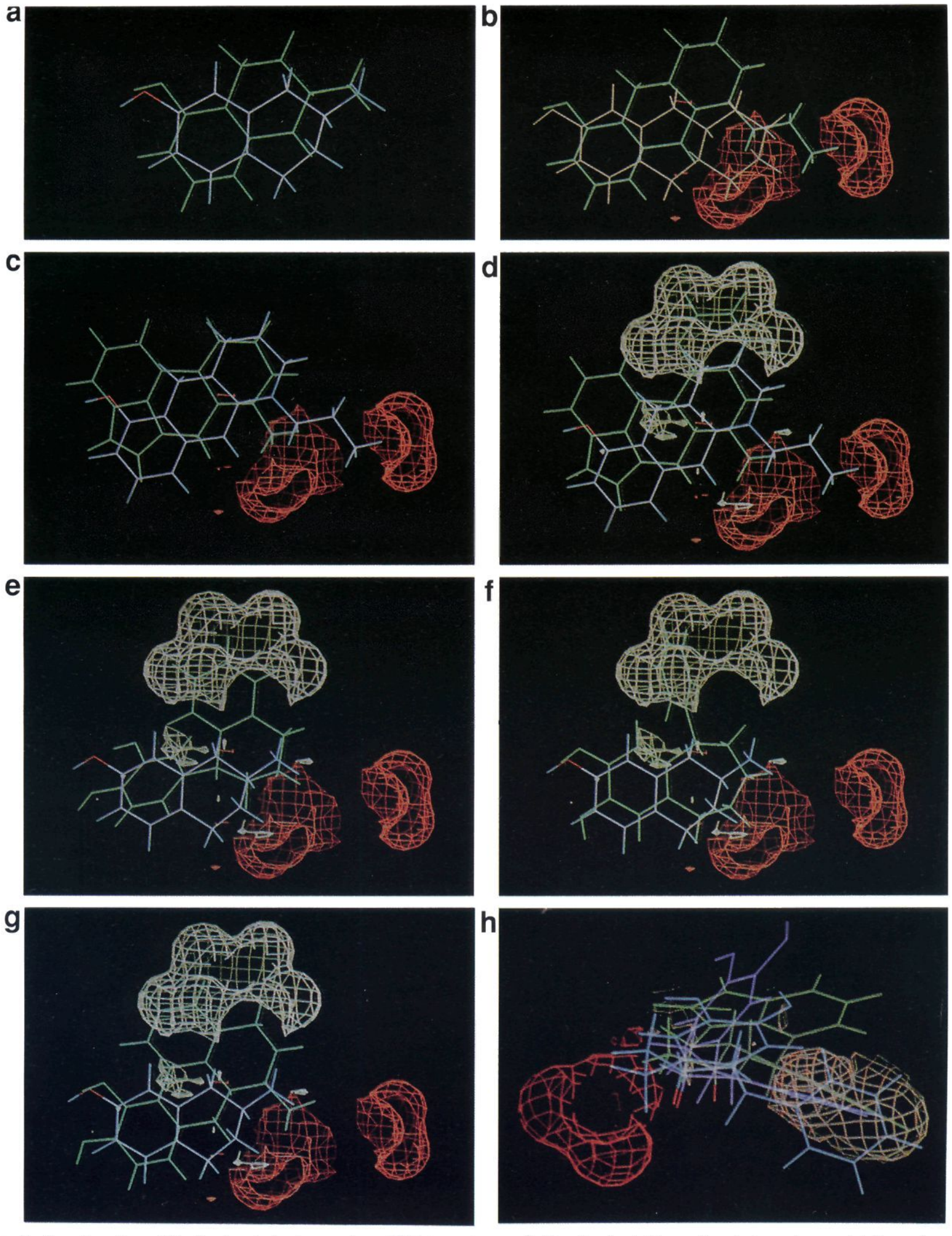


Figure 3. Construction of the "extended rotamer-based DA receptor model" and orientations of prototype drugs. (a) Superimposition of α- and β-rotameric aminotetralins: (*S*)-5-OH-AT (green), (*R*)-7-OH-AT (type-colored). (b) "General steric barrier" (red): volume of active compounds including (4a*R*,10a*R*)-*N*-Pr-6-OH-OBGQ (green) and (*R*)-7-OH-AT (not shown); volume of inactive compounds including (4a*S*,10a*S*)-8-OH-OBGQ (orange) and (4a*R*,10a*R*)-*N*-*n*-Bu-6-OH-OBGQ (not shown). (c) Receptor orientation of ergolines: *N*-methylergoline (green), (4a*R*,10a*R*)-*N*-Pr-6-OH-OBGQ (reference compound, type-colored). (d) "D_1 aryl binding site" coinciding with "D_2 steric barrier" (yellow): volume of active compounds = *N*-methylergoline (not shown); volume of inactive compounds = CY 208-243 (green); (4a*R*,10a*R*)-*N*-Pr-6-OH-OBGQ (reference compound, type-colored). (e) Orientation of (*S*)-DPTI with respect to the D_1 aryl binding site: (*S*)-DPTI (green), (*R*)-7-OH-AT (reference compound, type-colored). (f) Orientation of (*R*)-SKF38393 with respect to the D_1 aryl binding site: (*R*)-SKF38393 (green), (*R*)-7-OH-AT (reference compound, type-colored). (g) Orientation of apomorphine with respect to the D_1 aryl binding site: (*R*)-apomorphine (green), (*R*)-7-OH-AT (reference compound, type-colored). (h) Orthogonal view of the orientation of CY 208-243 (green), (*S*)-DPTI (violet) and (*R*)-SKF38393 (blue) with respect to the D_1 aryl binding site. Nitrogen lone pairs are marked red.

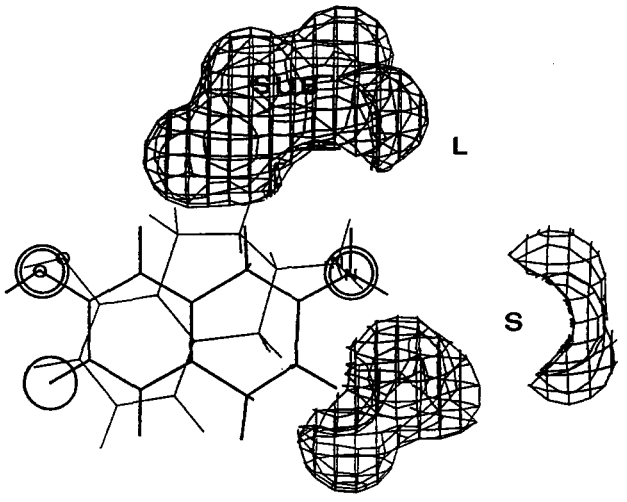


Figure 4. "Extended rotamer-based DA receptor model". β-Rotameric (R)-7-OH-AT (heavy) and α-rotameric (S)-5-OH-AT (light) superimposed, are oriented toward the two "major binding sites" for m-OH and amino group of DA (double circles), defined by (R)-7-OH-AT, and serve as basic framework for the orientation of new structures. The nitrogen lone pairs (N^+-H protons) are pointing down toward the amino binding site below the focal plane; its direction may be determined by using (4aR,-10aR)-N-Pr-6-OH-OBGQ in place of (S)-5-OH-AT. Accessory binding sites exist for the p-OH group of DA (circle) and for N-substituents, namely a "large N-alkyl binding site" (L) and a "small N-alkyl binding site" (S) with limited space due to a "general steric barrier", which partially surrounds it. The "subtype selectivity-inducing site" (SUB) consists of an aryl binding site at the D_1 receptor and a steric barrier at the D_2 receptor, respectively.

the SAR of N-alkyl substitution is similar to that found with the ergolines (the activity/efficacy is maintained upon N-ethyl/n-propyl substitution, but lost upon N-n-butyl substitution) and since the active benzergoline enantiomer (−)-1 has an absolute configuration identical with that of the ergolines, a similar receptor orientation is anticipated. The outlines of the subtype specificity-inducing site in the receptor model, marked by ring D of the benzergoline, can thus be defined (Figure 3d). The additional aryl groups of the selective D_1 agonists (S)-DPTI and (R)-SKF38393, which show β-rotamer type SAR of N-alkyl substitution,[7,21] are able to interact with this site at the D_1 receptor (Figure 3e,f,h), whereas the second aromatic ring of α-rotameric apomorphine only touches its borders (Figure 3g), thus explaining its lack of selectivity. The fit of SKF38393 is optimal in a chair conformation with equatorial phenyl group (shown in Figure 3f), the proposed bioactive conformation.[22,23] It should be noted that the D_1 aryl binding site tolerates quite a wide range of orientations of the aromatic ring as is demonstrated with the extremes: (−)-1 with an angle[24] between indole and phenyl ring of 48°, and SKF38393 with a corresponding plane angle of 90° (DPTI: 68°). This could indicate that π-stacking as well as orthogonal interactions between the additional aromatic ring of D_1 receptor ligands and the D_1 aryl binding site are feasible.

In summary, interpretation of SAR data of several classes of DA agonists, based on functional in vitro models representing D_1-like and D_2-like receptors,[25] led to the construction of the rotamer-based DA receptor model depicted in Figure 4, which is valid for both major families of dopamine receptor subtypes. Potential DA agonists are oriented in the model according to the following revised and extended criteria:

(I) Rotamer form: molecules containing a 5-OH-AT or a 7-OH-AT partial structure are fitted onto the corresponding AT, i.e., onto α-rotameric (S)-5-OH-AT and β-rotameric (R)-7-OH-AT, respectively.

(II) Absolute configuration of asymmetric center α to the basic nitrogen (β to phenol or catechol ring): it should correspond to the absolute configuration of the relevant aminotetralin of the basic framework (trans-fused ring systems).

(III) Influence of N-ethyl/N-n-propyl substitution: no change or increase in activity at the D_1 receptor (as compared to the corresponding NH/N-Me derivative) is suggesting an α-rotamer type orientation, a decrease in activity a β-rotamer type orientation.[26]

(IV) Influence of N-n-butyl substitution: preservation of, or increase in, activity indicates an orientation of the N-alkyl substituent toward the "large N-alkyl binding site", loss of activity indicates an orientation toward the small N-alkyl binding site.

(V) General steric barrier: it should neither be touched nor penetrated.

(VI) Subtype selectivity-inducing site: an increase in D_1 activity and selectivity upon introduction of an additional aryl group in the molecule may indicate an interaction with this site. The absolute configuration of a (3′,4′-di-OH) benzylic asymmetric center bearing this aryl group should correspond to that found in (−)-1, (S)-DPTI, and (R)-SKF38393.

The model is in agreement with the SAR's of all currently known DA agonists.[27] It should be of use, in a qualitative sense, for the design of new subtype-selective ligands and for the prediction of the absolute configuration of its biologically active enantiomers.

The concept of the postulated two major binding sites has recently been supported by site-directed mutagenesis studies in the case of the human D_2 receptor[28] which, as all currently known DA receptor subtypes, belongs to the superfamily of G protein-coupled receptors characterized by seven membrane-spanning domains. These investigations revealed a conserved aspartic acid residue in transmembrane domain three as binding partner of the amino group of DA, and two conserved serine residues in transmembrane domain five as binding partners of the catechol group. One of these serine residues, which is believed to form a hydrogen bond to the m-hydroxy group of DA, has a much greater impact on affinity than the other, believed to form a hydrogen bond to the p-hydroxy group of DA. In all cloned catecholaminergic G protein-coupled receptors (including the D_1 receptor[25]), two serines have been found conserved in transmembrane domain five, apart from the aspartic acid in transmembrane domain three. In all cases examined by site-directed mutagenesis the results implicated that these serine residues represent the binding partners of the catechol group.[28-30] A similar situation can also be expected for the D_1 receptor. The recent suggestion[31] of a charged residue in the D_1 receptor as a joint binding partner of the 8-OH group (corresponding to the m-OH group of DA) and the additional phenyl group of benzazepine D_1 agonists seems thus highly unlikely.

Experimental Section

Chemistry. Melting points were determined on a Büchi SMP-20 instrument and are not corrected. ^{1}H NMR spectra were measured on a Bruker Spectrospin 360 MHz (WH-360) instrument or 90 MHz (HX-90) spectrometer using Me_4Si as an internal standard. IR and mass spectra of the new compounds were

consistent with the proposed structures, and elemental analyses were within 0.4% of theoretical values. All reactions were followed by TLC carried out on Merck F254 silica plates. Solutions were dried over Na_2SO_4 and concentrated with a Büchi rotary evaporator at low pressure (water aspirator).

Resolution of (5a*S*,6a*R***,12b*R***)-4,5,5a,6,6a,7,8,12b-Octahydro-7-methylindolo[4,3-*ab*]phenanthridine.** A mixture of the racemic compound[1] (50 g, 0.18 mol) and (+)-mandelic acid (27.5 g, 0.18 mol) was dissolved at 70 °C in EtOH (500 mL) and the solution allowed to stand overnight at room temperature. The resulting crystals were collected by filtration, washed (50% Et_2O/EtOH, followed by Et_2O), and dried. The crystallisate thus obtained was dissolved in EtOH (400 mL) and left to stand overnight. A second crop of crystalline material was obtained, which was filtered, washed as described above, and dried: 29.1 g; mp 183–185 °C. Anal. ($C_{19}H_{20}N_2 \cdot C_8H_8O_3$) C, H, N. The colorless diastereomeric salt was converted by extraction with 1 N aqueous K_2CO_3/CH_2Cl_2 into the free base, which was recrystallized from 250 mL of hot EtOH to give 12.5 g of the (+)-enantiomer of the title compound: mp 184–186 °C; $[\alpha]^{25}_D$ = +378.4° (c = 1, DMF).

The filtrate from the original crystallization containing the (−)-enantiomer of the title compound was treated with 1 N aqueous K_2CO_3 to liberate the free base, extracted with CH_2Cl_2, and evaporated. The residue, together with 19.8 g (0.13 mol) of (−)-mandelic acid, was dissolved at 60 °C in 500 mL of EtOH, and the solution was allowed to stand overnight at room temperature. The crystalline product thus obtained was filtered off, washed as described for the (+)-mandelate salt, and dried: mp 183–185 °C. Anal. ($C_{19}H_{20}N_2 \cdot C_8H_8O_3$) C, H N. After conventional conversion into the free base, the (−)-enantiomer was recrystallized from 250 mL of hot EtOH to yield 13.3 g of colorless crystals: mp 184–186 °C; $[\alpha]^{25}_D$ = −381.0° (c = 0.7, DMF).

The optical purity of the enantiomers was determined by NMR in $CDCl_3$ using tris[3-(2,2,2-trifluoro-1-hydroxyethylidene)-*d*-camphorato]europium as a shift reagent. Based on completely separated signals of the 8-CH_2 group of both enantiomers, the optical purity of the (−)- as well as of the (+)-antipode was found to be ≥99%.

(−)-*trans*-4,6,6a,7,8,12b-Hexahydro-7-methylindolo[4,3-*ab*]phenanthridine ((−)-1). A stirred solution of the (+)-enantiomer of the octahydro derivative described above (6 g, 21.7 mmol) in CH_2Cl_2 (500 mL) was treated portionwise with activated MnO_2 (60 g, 690 mmol). Stirring was continued (at room temperature) until no more starting material could be detected by TLC. The reaction mixture was filtered (Celite), the filtrate evaporated, and the residue crystallized from 50% CH_2Cl_2/MeOH to give 3.3 g (55%) of the title compound: mp 212–215 °C; $[\alpha]^{25}_D$ −610.7° (c = 0.8, DMF). Anal. ($C_{19}H_{18}N_2$) C, H, N.

(+)-*trans*-4,6,6a,7,8,12b-Hexahydro-7-methylindolo[4,3-*ab*]phenanthridine ((+)-1). The title compound was prepared from the (−)-enantiomer of the octahydro derivative described above following the same procedure as for (−)-1: mp 212–215 °C; $[\alpha]^{25}_D$ = +611.2° (c = 0.8, DMF). Anal. ($C_{19}H_{18}N_2$) C, H, N.

Crystallography. Single-Crystal X-ray Analysis of (−)-*trans*-4,6,6a,7,8,12b-Hexahydro-7-methylindolo[4,3-*ab*]phenanthridine (−)-Mandelate. The compound crystallized from EtOH as thin, colorless prisms. Crystal data: $C_{19}H_{18}N_2 \cdot C_8H_8O_3$; formula weight = 426.5; space group $P2_1$; unit cell parameters a = 8.040(5), b = 22.946(6), and c = 12.431(2) Å; β = 100.89(4)°; V = 2252 Å^3; d_{calc} = 1.258 g/cm^3; Z = 4. Intensities were measured on an Enraf-Nonius CAD-4 diffractometer using monochromated Cu Kα radiation to θ < 60°. There was no measurable crystal decay, and no absorption corrections were applied. Of the 3251 measured independent reflections, 2973 had $F > 4\sigma(F)$ and were considered significant.

The structure was solved by direct methods using SHELX-86[32] and refined by blocked full matrix least squares using SHELX-76.[33] All hydrogen atoms (except the hydroxyl hydrogen, H(29′), in the second mandelic acid molecule) were located in a difference Fourier map after anisotropic refinement of the non-hydrogen atoms. Hydrogens were included riding (except for H(29), whose positional parameters were refined) on the non-H atom, with an isotropic temperature factor refined. H(29′) was not included in the final refinement. The final R was 0.0407 (2973 significant reflexions, 633 parameters).

Single-Crystal X-ray Analysis of (−)-*trans*-4,6,6a,7,8,12b-Hexahydro-7-methylindolo[4,3-*ab*]phenanthridine ((−)-1). The compound crystallized from MeOH as colorless polyhedra. Crystal data: $C_{19}H_{18}N_2$; formula weight = 274.3; space group $P3_2$; unit cell parameters a = 12.287(3), b = 12.287(2), and c = 8.486(2) Å, V = 1109 Å^3; d_{calc} = 1.232 g/cm^3; Z = 3. Intensities were measured on an Enraf-Nonius CAD-4 diffractometer using monochromated Cu Kα radiation to θ < 70°. There was no measurable crystal decay, and no absorption corrections were applied. Of the 1378 measured independent reflections, 1343 had $F > 4\sigma(F)$ and were considered significant.

The structure was solved by direct methods using SHELX-86 and refined by full matrix least squares. All hydrogen atoms were located in a difference Fourier map after anisotropic refinement of the non-hydrogen atoms and included in the final refinement with 3 positional and 1 isotropic thermal parameter. The final R factor was 0.029 (1343 significant reflexions, 261 parameters).

Molecular Modeling. Modeling was performed using SYBYL software (Tripos, version 5.4) on an Evans and Sutherland PS 390. All compounds were modeled from presently described or published[5,7b,34–36] X-ray coordinates except SKF38393, which gave a better fit when modeled from a conformation of the benzazepine core with C_s symmetry (chair conformation with equatorial phenyl group, suggested as bioactive conformation;[22,23] strain energy 10.3 kcal/mol) as compared to the modeled conformation, starting from the X-ray structure with C_2 symmetry[8] (twist conformation with equatorial phenyl group; strain energy 9.7 kcal/mol). The target compound (green for active, orange for inactive compounds, type-colored for reference compounds) was fitted to the relevant aminotetralin rotamer according to the rules previously published[5] (in place of the α-rotameric AT, (4a*R*,10a*R*)-*N*-Pr-6-OH-OBGQ was used in a second phase to define the direction of the lone pair of the basic nitrogen). Least squares fits (compounds with 5-OH-AT or 7-OH-AT partial structure) involved the oxygen of the *m*-hydroxy group, the orthogonal axis through the center of the phenolic/catecholic ring, the basic nitrogen atom, and with the exception of the AT's, the lone pair of the basic nitrogen. Manual fits were performed between N-methylergoline, (*S*)-DPTI, and (*R*)-SKF38393 and the reference compound involving the oxygen of the *m*-hydroxy group (DPTI, 8-OH in SKF38393), the basic nitrogen with the corresponding lone pair and the coplanarity of phenol/catechol/indole rings. In the case of the ergoline molecule, the indole NH was positioned between *m*- and *p*-OH group of the corresponding aminotetraline. (6a*R*,12b*R*)-*N*-Methylbenzergoline was fitted to *N*-methylergoline using the least squares fit procedure (indole N instead of meta O). In the case of DPTI, deviation from coplanarity had to be accepted at the expense of the superposition of the lone pair of the basic nitrogen. Steric barriers (receptor essential volumes) were constructed as the difference of the "volume of inactive compounds" (van der Waals volume generated by superposition of inactive structures) and the "volume of active compounds" (receptor excluded van der Waals volume generated by superposition of the active structures) as described in Figure 3.[5,37]

Pharmacology. The in vitro assays DA sensitive adenylate cyclase (bovine retina) and electrically evoked ACh release ACH (rat striatal slices) were performed as previously described.[5]

Reference Compounds. Apomorphine (4) was obtained from Sandoz. (−)-(5*R*,10*R*)-6-Methylergoline (2) and *N*-methyl-1,2,3,4,-4a,5,10,10a-octahydro-6-hydroxybenzo[*g*]quinoline (3, R = Me) were prepared as previously described by us.[1,5] SKF38393 (6) was synthesized according to the literature.[38] 4-(3′,4′-Dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline = DPTI (5) was the generous gift of Dr. D. E. Nichols, Purdue University, West Lafayette, IN.

Acknowledgment. We wish to thank B. Hartmann for his skillful technical assistance and our colleagues and their collaborators in the Physical Chemistry Department and the Analytical Laboratory for their help in measuring and interpreting the spectra and performing the elemental analysis, respectively. We are further grateful to Dr. F.

Braunschweiger and his collaborators for the supply of ample quantities of intermediate.

Supplementary Material Available: Tables of fractional coordinates, anisotropic vibration parameters, bond lengths, bond angles, and torsion angles and SYBYL mol files (29 pages). Ordering information is given on any current masthead page.

References

(1) Seiler, M. P.; Hagenbach, A.; Wüthrich, H.-J.; Markstein, R. trans-Hexahydroindolo[4,3-ab]phenanthridines ("Benzergolines"), the First Structural Class of Potent and Selective Dopamine D_1 Receptor Agonists Lacking a Catechol Group. *J. Med. Chem.* **1991**, *34*, 303–307.

(2) Markstein, R.; Seiler, M. P.; Vigouret, J. M.; Urwyler, S.; Enz, A.; Dixon, K. Pharmacologic Properties of CY 208-243, a Novel D_1 Agonist. In *Progress in Catecholamine Research, Part B: Central Aspects*; Sandler, M., Dahlström, A., Bellmaker, R. H., Eds.; Allan R. Liss, Inc.: New York, 1988; pp 59–64.

(3) Kohli, J. D.; Goldberg, L. I.; Glock, D.; Clark, B. J.; Seiler, M. P. CY 208-243 ((−)-4,6,6a,7,8,12b-Hexahydro-7-methylindolo[4,3-ab]-phenanthridine), a Benzergoline with DA_1 Dopamine Receptor Agonist Activity. *FASEB J.* **1989**, *3*, A588 (abstract 2107).

(4) For references and preparation see ref 1.

(5) Seiler, M. P.; Markstein, R.; Walkinshaw, M. D.; Boelsterli, J. J. Characterization of Dopamine Receptor Subtypes by Comparative Structure–Activity Relationships: Dopaminomimetic Activities and Solid State Conformation of Monohydroxy-1,2,3,4,4a,5,10,10a-octahydrobenz[g]quinolines and its Implications for a Rotamer-Based Dopamine Receptor Model. *Mol. Pharmacol.* **1989**, *35*, 643–651.

(6) Saari, W. S.; King, S. W. Synthesis and Biological Activity of (6a*S*)-10,11-Dihydroxyaporphine, the Optical Antipode of Apomorphine. *J. Med. Chem.* **1973**, *16*, 171–172.

(7) (a) Riggs, R. M.; Nichols, D. E.; Foreman, M. M.; Truex, L. L.; Glock, D.; Kohli, J. D. Specific Dopamine D-1 and DA_1 Properties of 4-(Mono- and dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline and Its Tetrahydrothieno[2,3-c]pyridine Analogue. *J. Med. Chem.* **1987**, *30*, 1454–1458. The absolute stereochemistry was determined of the *N*-methyl-8-amino derivative: (b) Dandridge, A. P.; Kaiser, C.; Brenner, M.; Gaitanopoulos, D.; Davis, L. D.; Webb, R. L.; Foley, J. J.; Sarau, H. M. Synthesis, Resolution, Absolute Stereochemistry and Enantioselectivity of 3′,4′-Dihydroxynomifensine. *J. Med. Chem.* **1984**, *27*, 28–35.

(8) Kaiser, C.; Dandridge, P. A.; Garvey, E.; Hahn, R. A.; Sarau, H. M.; Setler, P. E.; Bass, L. S.; Clardy, J. Absolute Stereochemistry and Dopaminergic Activity of Enantiomers of 2,3,4,5-Tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine. *J. Med. Chem.* **1982**, *25*, 697–703.

(9) See ref 1 for discussion and additional references.

(10) For a concise discussion concerning 5- and 6-ring envelope conformations, see: Dunitz, J. D. *X-Ray Analysis and the Structure of Organic Molecules*; Cornell University Press: Ithaca, 1979; pp 418–446.

(11) Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; Motherwell, W. D. S.; Rodgers, J. R.; Watson, D. G. The Cambridge Crystallographic Data Center: Computer-Based Search, Retrieval, Analysis and Display of Information. *Acta Crystallogr.* **1979**, *B35*, 2331–2339. Version 3.10 (January 1992).

(12) Hargrave, K. D.; Eliel, E. L. Structures of 8α- and 8β-t-Butyl-trans-decahydroquinoline Picrates and 8α,Nβ-Dimethyl-trans-decahydroquinoline p-Toluenesulfonate. *Isr. J. Chem.* **1980**, *20*, 127–132.

(13) Chengfu, X.; Guanghong, B.; Cunheng, H.; Shantian, M. Determination of Structure and Absolute Configuration of Humantenine Hydrobromide. *Acta Acad. Med. Sin* **1987**, *9*, 95–98.

(14) Stewart, J. J. P. MOPAC. A semiempirical molecular orbital program, QCPE 455.

(15) McDermed, J. D.; Freeman, H. S.; Ferris, R. M. Enantioselectivity in the binding of (+)- and (−)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene and related agonists to dopamine receptors. In *Catecholamines: Basic and Clinical Frontiers*; Usdin, E., Ed.; Pergamon Press: New York, 1979; pp 568–570.

(16) Seiler, M. P.; Markstein, R. Further Characterization of Structural Requirements for Agonists at the Striatal Dopamine D_1 Receptor. Studies with a Series of Monohydroxyaminotetralins on Dopamine-sensitive Adenylate Cyclase and a Comparison with Dopamine Receptor Binding. Mol. *Pharmacol.* **1982**, *22*, 281–289.

(17) Seiler, M. P.; Markstein, R. Further Characterization of Structural Requirements for Agonists at the Striatal Dopamine D_2 Receptor and a Comparison with Those at the Striatal Dopamine D_1 Receptor. Studies with a Series of Monohydroxyaminotetralins on Acetylcholin Release from Rat Striatum. *Mol. Pharmacol.* **1984**, *26*, 452–457.

(18) Wikstroem, H. B.; Andersson, B.; Sanchez, D.; Lindberg, P.; Arvidsson, L.-E.; Johansson, A. M.; Nilsson, J. L. G.; Svensson, K.; Hjorth, S.; Carlsson, A. Resolved Monophenolic 2-Aminotetralins and 1,2,3,4,4a,5,6,10b-Octahydrobenz[f]quinolines: Structural and Stereochemical Considerations for Centrally Acting Pre- and Postsynaptic Dopamine Receptor Agonists. *J. Med. Chem.* **1985**, *28*, 215–225.

(19) Cannon, J. G. Chemistry of dopaminergic agonists. *Adv. Neurol.* **1975**, *9*, 177–183.

(20) For details concerning SAR of *N*-alkyl substitution in the ergoline series see refs 1 and 5.

(21) Weinstock, J.; Hieble, J. P.; Wilson, J. W. The chemistry and pharmacology of 3-benzazepine derivatives. *Drugs Future* **1985**, *10*, 645–697.

(22) Weinstock, J.; Oh, H.-J.; DeBrosse, C. W.; Egglestone, D. S.; Wise, M.; Flaim, K. E.; Gessner; G. W.; Sawyer, J. L.; Kaiser, K. Synthesis, Conformation, and Dopaminergic Activity of 5,6-Ethano-Bridged Derivatives of Selective Dopaminergic 3-Benzazepines. *J. Med. Chem.* **1987**, *30*, 1303–1308.

(23) Pettersson, I.; Liljefors, T.; Bogeso, K. Conformational Analysis and Structure–Activity Relationships of Selective Dopamine D-1 Receptor Agonists and Antagonists of the Benzazepine Series. *J. Med. Chem.* **1990**, *33*, 2197–2204.

(24) The values of the plane angles are based on the modeled structures shown in Figure 3.

(25) Sibley, D. R.; Monsma, F. J., Jr. Molecular biology of dopamine receptors. *TiPS* **1992**, *13*, 61–69.

(26) Note: at the D_2 site, both rotamer forms lead to an increase in activity upon *N*-ethyl/*N*-*n*-propyl substitution.

(27) For α- and β-rotameric catechol analogs of benzergolines, see: Seiler, M. P.; Bölsterli, J. J.; Floersheim, P.; Hagenbach, A.; Markstein, R.; Pfäffli, P.; Widmer, A.; Wüthrich, H. J. Recognition at Dopamine Receptor Subtypes. In *Perspectives in Medicinal Chemistry*; Testa, B., Kyburz, E., Furrer, W., Giger, R., Eds.; Helv. Chim. Acta Publisher: Basel, 1993; pp 221–237.

(28) Mansour, A.; Meng, F.; Meador-Woodruff, J. H.; Taylor, L. P.; Civelli, O.; Akil, H. Site-directed mutagenesis of the human dopamine D_2 receptor. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1992**, *227*, 205–214.

(29) Strader, C. D.; Candelore, M. R.; Hill, W. S.; Sigal, I. S.; Dixon, R. A. F. Identification of Two Serine Residues Involved in Agonist Activation of the β-Adrenergic Receptor. *J. Biol. Chem.* **1989**, *264*, 13572–13578.

(30) Wang, C. D.; Buck, M. A.; Fraser, C. M. Site-Directed Mutagenesis of α_{2A}-Adrenergic Receptors: Identification of Amino Acids Involved in Ligand Binding and Receptor Activation by Agonists. *Mol. Pharmacol.* **1991**, *40*, 168–179.

(31) Pettersson, I.; Gundertofte, K.; Palm, J.; Liljefors, T. A. Study on the Contribution of the 1-Phenyl Substituent to the Molecular Electrostatic Potentials of Some Benzazepines in Relation to Selective Dopamine D-1 Receptor Activity. *J. Med. Chem.* **1992**, *35*, 502–507.

(32) Sheldrick, G. M. SHELX-86. Program for crystal structure determination. University of Göttingen, Germany, 1986.

(33) Sheldrick, G. M. SHELX-76. Program for crystal structure determination. University of Cambridge, England, 1976.

(34) Gieseke, J. The Crystal Structure of (+)-2-Dipropylamino-5-hydroxytetralin Hydrochloride. *Acta Crystallogr.* **1980**, *B36*, 110–114.

(35) Gieseke, J. The Crystal and Molecular Structure of Apomorphine Hydrochloride Hydrate. *Acta Crystallogr.* **1973**, *B29*, 1785–1791.

(36) Stadler, P. A.; Stuermer, E.; Weber, H. P.; Loosli, H.-R. 2-Aza-dihydroergotamine. *Eur. J. Med. Chem.* **1981**, *27*, 1405–1408.

(37) Marshall, G. R.; Barry, C. D.; Bosshard, H. E.; Dammkoehler, R. A.; Dunn, D. A. The Conformational Parameters in Drug Design: The Active Analog Approach. In *Computer-Assisted Drug Design*; Olson, E. C., Christoffersen, R. E., Eds.; ACS Symposium 112; American Chemical Society: Washington, D.C., 1979; pp 205–226.

(38) Walter, A.; Chang, W. K. Benzazepines. U.S. Patent 3 393 192 (16 July 1968) to Schering Corp.