Synthesis, Resolution, Absolute Stereochemistry, and Enantioselectivity of 3',4'-Dihydroxynomifensine

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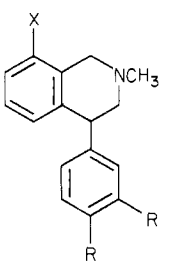
3',4'-Dihydroxynomifensine, 8-amino-1,2,3,4-tetrahydro-4-(3,4-dihydroxyphenyl)-2-methylisoquinoline (**1a**), is an agonist of dopamine receptors in central and peripheral systems. Since this dopamine receptor agonist bears an asymmetric center at position 4, its synthesis and resolution were undertaken as part of a study directed toward determining the mode of interaction of these agents with the receptor(s). The enantiomers of 3',4'-dihydroxynomifensine are of particular interest, as they provide additional probes of present conceptual models of the dopamine receptor(s). Initial attempts to prepare **1a** were inefficient or unsuccessful; instead, an isomeric compound, 1,2,4,5-tetrahydro-2-(3,4-dihydroxyphenyl)-4-methyl-3*H*-1,4-benzodiazepine (**9**), was obtained. For this reason, a new route to 3',4'-dihydroxynomifensine was employed. The racemic dimethoxy intermediate **1d**, thus obtained, was resolved. Methoxyl cleavage of the isomers of **1d** afforded the enantiomers of **1a**. Enantiomeric excess of these antipodes or appropriate derivatives was examined by NMR, CD, and HPLC methods. CD analysis suggests an enantiomeric excess greater than 99%. Determination of the absolute configuration of the enantiomers of **1a** was determined by single-crystal X-ray diffractometric analysis. Examination of the isomers in several pharmacological test systems revealed a high degree of enantioselectivity. D-1 dopaminergic activity resides almost exclusively in the *S* enantiomer. The findings of the study have been employed to suggest an accessory binding site on the dopamine receptor(s) that differs from that advanced earlier. This accessory binding site may be specific for the D-1 subpopulation of dopamine receptors.

Enantiomers of both dopamine (DA) receptor agonists[1-6] and antagonists[7-16] uniformly demonstrate pharmacological enantioselectivity of action. These observations have been coupled with structure–activity relationship (SAR) information to probe the nature of the dopaminergic pharmacophore and its mode of interaction with the DA receptor(s). Enantiomeric pairs are particularly useful for such probing because they have identical physical properties and, thus, unless they undergo differential metabolism, pharmacological differences[17-20] may reasonably be attributed to receptor-associated events.[21] Consideration of the general SAR requirements for DA receptor agonists,[22] as well as steric,[23] conformational,[24,25] and stereochemical[5,26,27] factors, has led to various conceptual models to rationalize the interaction of dopaminergic agents with the receptors(s). Many of these models[5,27-34] derived from DA receptor agonists suggest modes of binding and steric parameters. In order to further such enantiomeric probing of the DA receptor(s), the synthesis, resolution, determination of absolute configuration, and study of pharmacological enantioselectivity of DA-like action of the isomers of 3',4'-dihydroxynomifensine (**1a**) were performed.



1a, X = NH_2; R = OH
b, X = NH_2; R = H
c, X = R = H
d, X = NH_2; R = OCH_3

3',4'-Dihydroxynomifensine (**1a**) was originally considered as a potential metabolite[35] that might account for in vivo DA-like actions noted for the antidepressant drug nomifensine (**1b**).[36-43] Although detailed and careful studies have failed to detect **1a** as a metabolite of **1b**[35] but

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suggest instead that nomifensine itself inhibits presynaptic uptake of DA[44] and has weak direct DA receptor agonist actions,[45] the postulated metabolite **1a** has subsequently been shown to have selective D-1[46] or DA_1[47] receptor agonist actions. It stimulates DA-sensitive adenylate cyclase,[35] relaxes renal vasculature in phenoxybenzamine-treated dogs,[48] and has DA-like behavioral actions.[49] An interesting SAR observation is that the amino group in position 8 of **1a** is apparently not needed for DA-like activity; the deamino derivative **1c** is equipotent with **1a** as a stimulant of DA receptors.[50]

3',4'-Dihydroxynomifensine (**1a**) was considered a particularly informative probe of the DA receptor(s) because, in common with the tetrahydro-3-benzazepine class of DA receptor agonists which we have examined extensively,[51-57]

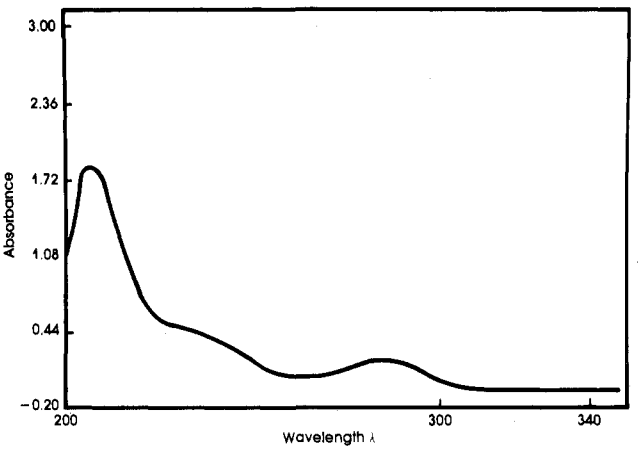

Figure 1. UV spectrum of **1a** in methanol.

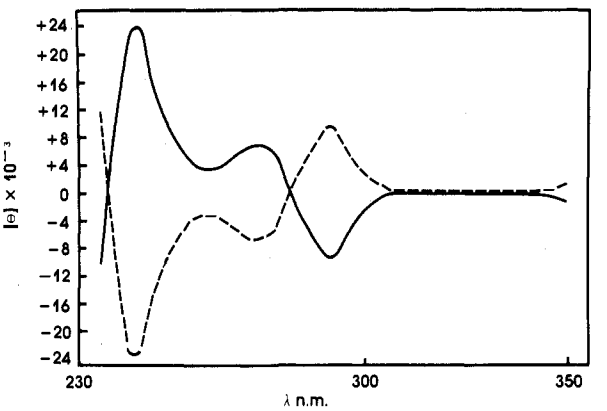

Figure 2. CD spectra of (*R*)- and (*S*)-**1a** in methanol (0.25 mg/mL). Each spectrum was the result of at least 10 computer-averaged scans: (- - -) *R*-**1a**; (—) (*S*)-**1a**.

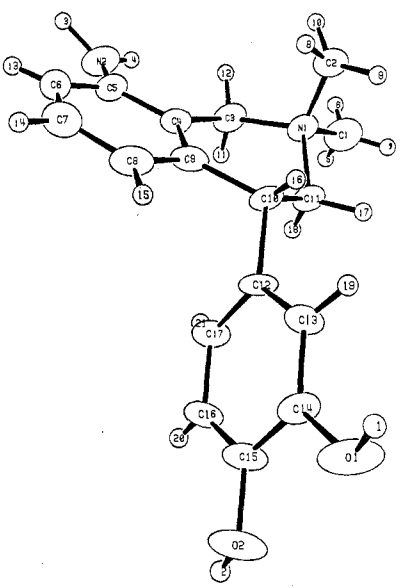

Figure 3. Single-crystal X-ray diffractometric determined structure of (-)-**1a** $[\alpha]^{25}_{D}$ -172° (*c* 1, DMF).

it seems to demonstrate selectivity for the D-1 or DA_1 subpopulation of DA receptors, and the asymmetric center

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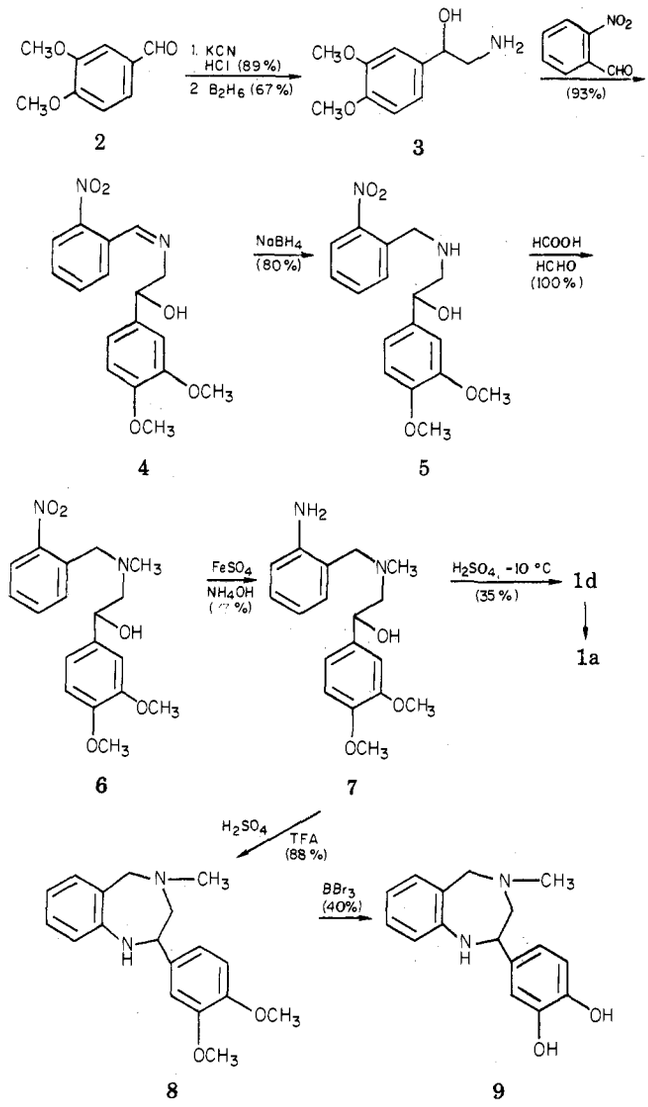
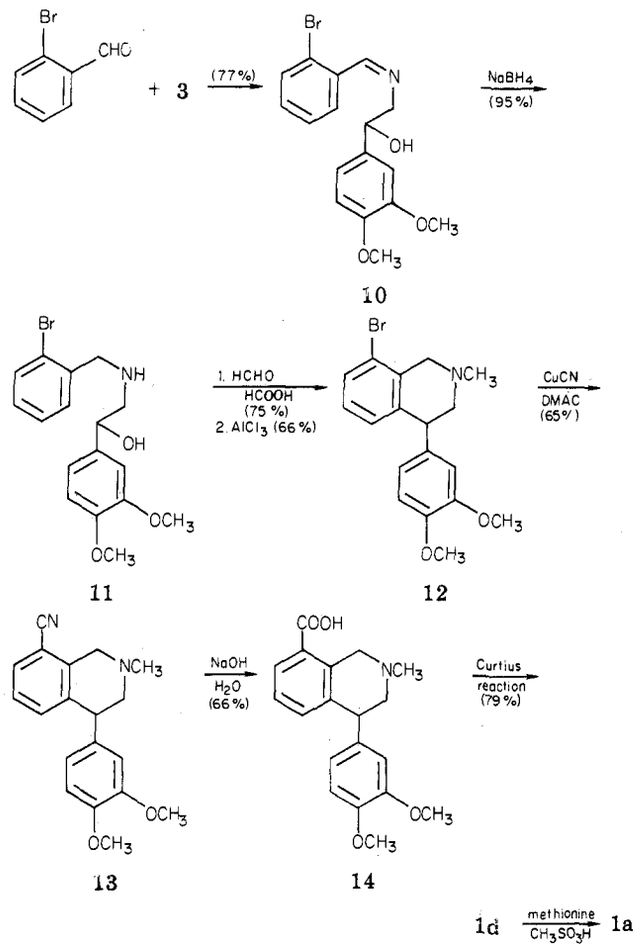
Scheme I



Scheme II



is located on the benzylic or β carbon of the embodied DA skeleton. This is distinct from most other enantiomeric probes, e.g., aminotetralins, aporphines,[2,34] ergolines,[60-62] and related compounds,[63] in which the center of asymmetry resides on the α carbon of the DA-embedded side chain.

In this article are described the synthesis, resolution, determination of absolute configuration, and the enantioselectivity of dopaminergic activity of the optical antipodes of 3′,4′-dihydroxynomifensine. The mode of interaction of these isomers with a conceptual model[5] of the DA receptor has been evaluated. On the basis of these results, coupled with earlier studies with enantiomers of dopaminergic 1-phenyltetrahydro-3-benzazepine derivatives,[53,54] a new location for an accessory binding site, perhaps selective for the D-1 subpopulation of DA receptors, is postulated.

Chemistry. Initially, the synthesis of 3′,4′-dihydroxynomifensine (**1a**) was carried out as described in the patent literature,[64] i.e., utilizing the general procedure reported for the preparation of nomifensine,[65] as outlined in Scheme I, employing **3** prepared from veratraldehyde (**2**) in a modification of the previously described method.[65] In this fashion, the dimethoxy precursor (**1d**) to **1a** was obtained in low and difficultly reproducible yields by cyclization of **7**. Modification of the cyclization conditions, i.e., utilizing sulfuric acid in trifluoroacetic acid, aluminum

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Table I. Biochemical Test Results for 3',4'-Dihydroxynomifensine (1a) and Its Enantiomers

test system	(*R*,*S*)-1a	(*R*)-1a	(*S*)-1a	DA
DA-sensitive adenylate cyclase stimulation: EC_{50},[a] μM	2.88 (1.85-4.31)	*b*	1.87 (0.95-3.05)	3.5
displacement of spiroperidol binding to rat caudate tissue: IC_{50},[a] μM	92 (71.2-119)	99.1 (73.9-133)	85.8 (65.0-113)	5.34 (4.18-6.82)
displacement of clonidine binding to rat cortical membranes: IC_{50},[a] μM	1.39 (1.17-1.66)	27.7 (22.9-33.7)	0.60 (0.52-0.69)	0.061 (0.052-0.071)

[a] Description of experimental methods and definition of EC and IC values are given in the Experimental Section. The values in parentheses are 95% confidence limits. For pharmacological evaluation, the bases were solubilized with an equivalent of aqueous HCl. [b] Inactive at a concentration of 10^{-4} M.

trichloride in methylene chloride,[50] or boron trifluoride etherate in chloroform, in each case gave 1,2,4,5-tetrahydro-2-(3,4-dimethoxyphenyl)-4-methyl-3*H*-1,4-benzodiazepine (8), which was cleaved to the dihydroxylated isomer of **1a**, i.e., **9**.

In order to afford a more reproducible source of **1a**, an alternative route, detailed in Scheme II, was developed.

The dimethoxy precursor (**1d**) to 3',4'-dihydroxynomifensine (**1a**) was consistently derived via the illustrated sequence (Scheme II). Resolution of **1d** was conveniently achieved by recrystallization of the diastereoisomeric di-*p*-toluoyl hydrogen tartrates from ethanol. Methionine in methanesulfonic acid[67] cleavage of the methoxyl groups of the enantiomers of **1d** afforded the enantiomers of 3',4'-dihydroxynomifensine (**1a**). Various methods, e.g., HPLC of a stereoisomeric derivative and NMR on precursor **1d** with chiral shift reagents, to establish the enantiomeric excess (ee) of the isomers of **1a** were not successful. However, the UV spectrum of **1a** clearly demonstrated absorption maxima at 245 and 292 nm (Figure 1). Comparison of the CD spectra of the enantiomers of **1a** (Figure 2) shows that the two are equally resolved ($[\theta]^{245}$ (−)/$[\theta]^{245}$ (+) = 1.004). This establishes that the two enantiomers are equally resolved and *suggests* that they are nearly optically pure. The magnitude of the molar ellipticities at the extrema are in good agreement with related compounds of known optical purity.[68] This observation, coupled with the pharmacological testing results, however, is the only evidence available for optical purity.

For determination of the absolute configuration of the enantiomers of **1a**, the isomer having $[\alpha]^{25}_{D}$ −172.1° (*c* 1, DMF) was converted to a methiodide. This quaternary salt was examined by single-crystal X-ray diffractometric analysis as described in the Experimental Section and the Supplementary Material to establish that its structure is that illustrated in Figure 3; i.e., this enantiomer has the *R* absolute configuration at position 4 of the tetrahydroisoquinoline.

The CD spectra for the enantiomers of **1a** are also in accord with this assignment of absolute configuration, as the CD extrema observed at 292 and 245 nm are very similar to those reported for a variety of rigid and conformationally labile benzylisoquinoline alkaloids.[68] If one assumes that the catechol ring of **1a** is pseudoequatorial and applies the quadrant rule,[68] then the sign of the A_{1g}–$B_{2\mu}$ 245 nm Cotton effect of the (−) enantiomer is positive and would be assigned the *R* configuration in agreement with the X-ray result. Conversely, relating the quadrant rule[68] with the known X-ray and CD data suggests that a considerable amount of the pseudoequatorial conformer of **1a** must be present in solution.

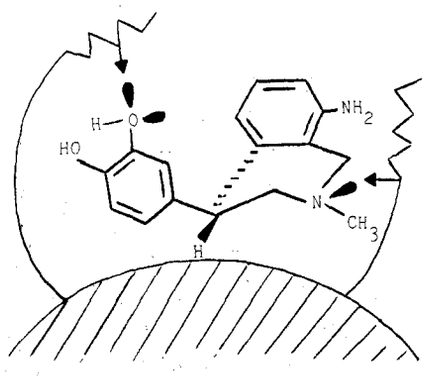

Figure 4. Binding mode of (*S*)-**1a** with the conceptual model of the dopamine receptor.

Results and Discussion

Several pharmacological test systems, namely, stimulation of rat striatal DA-sensitive adenylate cyclase (a D-1 DA receptor response[46]), inhibition of specific [^{3}H]-spiroperidol binding to rat striatal membrane preparations (probably involving D-2 DA receptors[69]), and inhibition of specific [^{3}H]clonidine binding to rat brain cortical membrane preparations (α-adrenoreceptor binding[70]), were employed to examine the biochemical actions of 3',4'-dihydroxynomifensine (**1a**) and its enantiomers, (*R*)- and (*S*)-**1a**. The results of these studies are summarized in Table I.

Clearly, enantioselectivity is observed with 3',4'-dihydroxynomifensine in its ability to stimulate striatal DA-sensitive adenylate cyclase. The *S* enantiomer is significantly more potent than the racemate, whereas (*R*)-**1a** is inactive. Enantioselectivity is also noted in the test for inhibition of specific [^{3}H]clonidine binding to rat brain cortical membranes. Here, (*S*)-**1a** is about twice as potent as the racemate, whereas the *R* enantiomer is nearly 20 times less effective. Perhaps significantly, in the inhibition of [^{3}H]spiroperidol binding test, **1a** is only weakly active, and no enantioselectivity is observed. These results suggest possible selectivity of (*S*)-**1a** for the D-1 subpopulation of DA receptors, combined with some affinity for α-adrenergic binding sites.

The enantioselectivity noted for 3',4'-dihydroxynomifensine is entirely consistent with the conceptual model of the DA receptor suggested by McDermed et al.[5-7] Thus, as illustrated in Figure 4, the *S* enantiomer of 3',4'-dihydroxynomifensine directs the bulk of the tetrahydroisoquinoline ring system away from the postulated site of steric occlusion, whereas in the *R* enantiomer, as shown in Figure 5, this bulk is directed precisely toward the site

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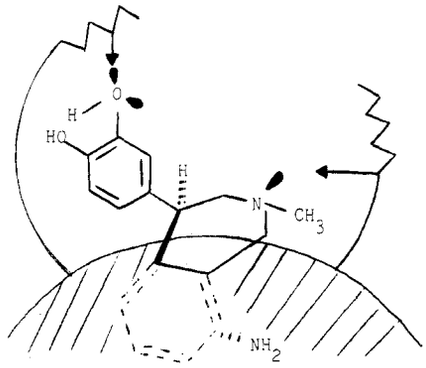

Figure 5. Exclusion of binding of (*R*)-**1a** with the conceptual model of the dopamine receptor.

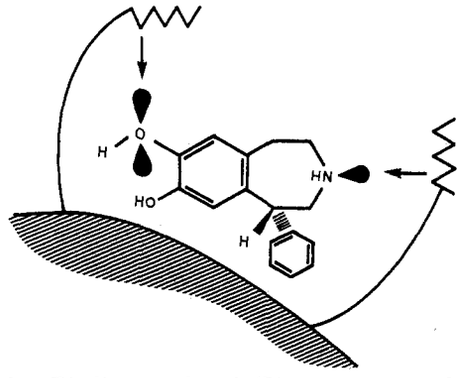

Figure 6. Binding mode of (*R*)-2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine with the conceptual model of the dopamine receptor.

of intolerance, indicated by the striped area. Interestingly, the benzo-fused ring of (*S*)-**1a** occupies a position above the plane of the postulated site of steric occlusion on the receptor that is virtually identical with the site occupied by the biologically significant 1-aryl substituent of the enantioselectively preferred (*R*)-2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepines, which also demonstrate D-1 DA receptor agonist activity,[53,54] as illustrated in Figure 6. This kind of an interaction requires that the 1-phenyl substituent on the tetrahydroazepine ring in a quasi chair conformation attain a somewhat unfavorable, albeit not prohibited, pseudoaxial orientation. Since the 1-phenyl group of the tetrahydro-3-benzazepine DA receptor agonists clearly enhances DA-like potency, it seems reasonable to suggest that, at least for the D-1 subpopulation of DA receptors, an accessory binding site may be located on the receptor in the vicinity of the presumably proteinaceous postulated site of steric hinderance, as illustrated in Figure 7. Such a postulated site might undergo interaction with the aromatic residues of Phe, Tyr, or Trp by $\pi-\pi$ stacking or by nonbonded interactions to enhance potency. For this reason, such a possibility is suggested. Unquestionably, location of such an accessory or secondary binding site is speculative; it is based on only two examples, i.e., the enantioselectivity of **1a** and the dopaminergic 1-aryltetrahydro-3-benzazepines. Further, it is in contradiction with previous suggestions that a secondary binding site might be located in an area above the 11-hydroxyl group of apomorphine and in the plane of the benzo-fused ring of this molecule, i.e., the dashed circle in Figure 7.[47,71] Instead, it suggests that, at least

(71) Nichols, D. E. "Dopamine Receptors" (*Am. Chem. Soc. Symp. Ser. no. 224*); Kaiser, C.; Kebabian, J. W., Eds.; American Chemical Society: Washington, DC, 1983; pp 201–218.

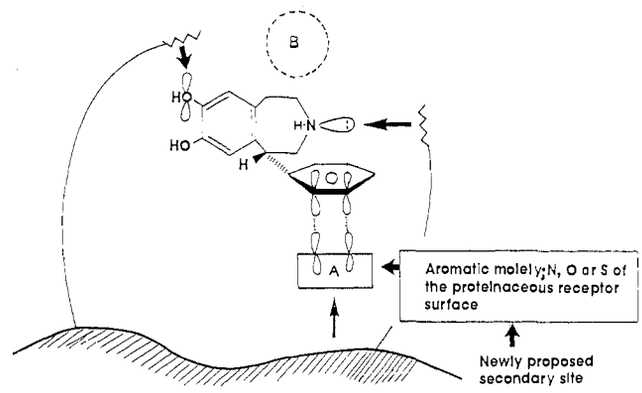

Figure 7. Proposed location of the accessory binding site (A) in the conceptual model of the dopamine (D-1?) receptor. Suggested location of the accessory binding site (B) in the conceptual model of the dopamine (D-2?) receptor (cf. ref 71).

in D-1 DA receptors, interaction is more favored by an orthogonal, even if somewhat disfavored, conformation of an aryl substituent. Additional experiments are clearly indicated to probe the validity of the interpretation that is being advanced.

Experimental Section

Melting points were determined in open capillary tubes on a Thomas–Hoover Uni-Melt apparatus and were not corrected. Elemental analyses were performed by the Analytical/Physical Chemistry Department of Smith Kline & French Laboratories. Where analyses are reported by symbols of the elements, results were within ±0.4% of the calculated value. IR spectra were obtained with a Perkin-Elmer 727 spectrophotometer. NMR spectra were derived with a Perkin-Elmer R-24 or a Bruker WM-360 spectrometer employing an internal standard of Me_4Si. Although IR and NMR spectral data are reported only where considered significant, these spectra were obtained for all compounds described in this section and were evaluated as consistent with the assigned structures. Mass spectra were also determined for all compounds on a Hitachi Perkin-Elmer RMV-6E spectrometer. Optical rotations were determined with a Perkin-Elmer 241 MC polarimeter. UV spectra were determined in methanol with a Perkin Elmer λ-5 spectrophotometer. CD spectra were obtained with a Jasco J-500C spectropolarimeter. Single-crystal X-ray crystallographic analyses were performed by the Molecular Structure Corp. staff.

Chemistry. α-(Aminomethyl)-3,4-dimethoxybenzyl Alcohol Hydrochloride (3).[66] Veratraldehyde was converted to α-hydroxy-3,4-dimethoxyphenylacetonitrile, mp 105–107 °C (89% yield), according to literature directions.[66] To this cyanohydrin (50.0 g, 0.26 mol) in 200 mL of THF was added *slowly* 550 mL of 1 M B_2H_6 in THF at 0 °C under argon. After the addition was completed, the solution was heated under reflux for 3 h. After the solution was cooled to 0 °C, methanol was added *dropwise* to the solution until bubbling ceased. The solution was concentrated, the residue was dissolved in 100 mL of methanol, gaseous HCl was added to pH 1, and then ether was added to the cloud point. When the solution cooled, 40.5 g (67%) of colorless crystals, mp 156–160 °C (lit.[66] mp 163–165 °C), was collected. Anal. ($C_{10}H_{16}ClNO_3$) C, H, N.

α-[[(2-Nitrobenzylidene)amino]methyl]-3,4-dimethoxybenzyl Alcohol (4). A solution of 71 g (0.3 mol) of α-(aminomethyl)-3,4-dimethoxybenzyl alcohol hydrochloride (**3**), 50.0 g (0.33 mol) of 2-nitrobenzaldehyde, and 45 mL of triethylamine in 200 mL of methanol was heated under reflux for 10 min. After the solution was cooled to 0 °C, yellow crystalline 4, 93 g (93%), mp 109–110 °C, after recrystallization from methanol, was collected. Anal. ($C_{17}H_{18}N_2O_5$) C, H, N.

α-[[(2-Nitrobenzyl)amino]methyl]-3,4-dimethoxybenzyl Alcohol (5). To a suspension of 46.5 g (0.14 mol) of α-[[(2-nitrobenzyliden)amino]methyl]-3,4-dimethoxybenzyl alcohol (**4**) in 250 mL of methanol was added *slowly*, in portions, 6.0 g (0.159

mol) of sodium borohydride. After the addition was completed, the mixture was stirred for 30 min at 0 °C. The precipitated colorless solid was collected on a filter and washed with methanol to give 37.0 g (80%) of **5**, mp 118–120 °C. Anal. ($C_{17}H_{20}N_2O_5$) C, H, N.

α-[[*N*-(2-Nitrobenzyl)-*N*-methylamino]methyl]-3,4-dimethoxybenzyl Alcohol (6). A mixture of 124.0 g (0.37 mol) of α-[[(2-nitrobenzyl)amino]methyl]-3,4-dimethoxybenzyl alcohol (**5**), 48 mL of 88% formic acid solution, and 77 mL of a 37% solution of formaldehyde in aqueous methanol (formalin, USP) was refluxed for 30 min. The resulting solution was added to about 500 mL of ice–water, and then aqueous ammonia was added to give a pH above 11. The mixture was extracted with ethyl acetate. After the extracts were washed with water and dried ($MgSO_4$), they were concentrated to give 130 g (100%) of crude **6**. A *hydrochloride* was prepared in acetone, and it was recrystallized from acetone–ether. Anal. ($C_{18}H_{23}ClN_2O_5 \cdot H_2O$) C, H, N.

α-[[*N*-(2-Aminobenzyl)-*N*-methylamino]methyl]-3,4-dimethoxybenzyl Alcohol (7). To a solution of 57.0 g (0.165 mol) of α-[[*N*-(2-nitrobenzyl)-*N*-methylamino]methyl]-3,4-dimethoxybenzyl alcohol (**6**) in 300 mL of methanol and 200 mL of 28% aqueous ammonia was added slowly 297.0 g (1.07 mol) of ferrous sulfate heptahydrate in 300 mL of boiling water. After the solution was refluxed for 30 min, the slurry was filtered. The filter cake was washed thoroughly with methanol. The combined filtrates were concentrated, and then 300 mL of water was added to the residue. After the mixture was extracted with ethyl acetate, the extracts were washed with water, dried ($MgSO_4$), and concentrated. Residual solid was recrystallized from ether–petroleum ether to give 40 g (77%) of crystals, mp 88–90 °C. Anal. ($C_{18}H_{24}N_2O_3 \cdot 0.125H_2O$) C, H, N.

1,2,4,5-Tetrahydro-2-(3,4-dimethoxyphenyl)-4-methyl-3*H*-1,4-benzodiazepine (8). A solution of 32.5 g (0.1 mol) of α-[[*N*-(2-aminobenzyl)-*N*-methylamino]methyl]-3,4-dimethoxybenzyl alcohol (**7**) in 325 mL of trifluoroacetic acid and 8.5 mL of concentrated sulfuric acid was refluxed for 20 min. After the solution was concentrated, the residue was diluted with ice–water, and the solution was made alkaline by the addition of concentrated aqueous ammonia. The mixture was extracted with methylene chloride. After being washed with water, the extracts were dried ($MgSO_4$) and concentrated to give 27.0 g (88%) of a solid, mp 142–144 °C, after recrystallization from 2-propanol: ^1H NMR ($CDCl_3$) δ 2.48 (s, 3 H, NCH_3), 2.90 (d, 2 H, NCH_2CH), 3.89 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.63–4.40 (m, 4 H, NH, benzylic CH, CH_2), 6.63–7.24 (m, 7 H, Ar H); TLC (silica G, 5:95 CH_3OH/CH_2Cl_2, I_2 vapor) R_f 0.53. Anal. ($C_{18}H_{22}N_2O_2$) C, H, N.

The same product was obtained (60% yield) upon treatment of **7** with excess boron trifluoride etherate in refluxing chloroform for 24 h and by $AlCl_3$ in methylene chloride ring closure of **7** (40% yield) according to the general procedure of Jacob et al.[50]

1,2,4,5-Tetrahydro-2-(3,4-dihydroxyphenyl)-4-methyl-3*H*-1,4-benzodiazepine (9) Hydrogen Fumarate. To a solution of 3.0 g (10.1 mmol) of 1,2,4,5-tetrahydro-2-(3,4-dimethoxyphenyl)-4-methyl-3*H*-1,4-benzodiazepine (**8**) in 100 mL of methylene chloride at 0 °C, under argon, was added dropwise 30 mL of a solution of 1 g of boron tribromide in sufficient methylene chloride to give a total volume of 2.5 mL [i.e., 12 g (47.9 mmol) of BBr_3]. The cooling bath was removed, and the solution was stirred at ambient temperature for 4 h; then 50 mL of methanol was added dropwise, and the solution was concentrated. The residue was dissolved in 100 mL of 50% aqueous methanol. This solution was added slowly to 200 mL of 5% aqueous sodium bicarbonate. The mixture was extracted repeatedly with ethyl acetate. The combined extracts were dried ($MgSO_4$) and concentrated. To a solution of the residual oil in 20 mL of methanol was added 1.2 g (10.3 mmol) of fumaric acid. A mixture of ethyl acetate–ether was added to bring the solution to the cloud point. While the solution cooled, 2.2 g (40%) of colorless crystals of the hydrogen fumarate, mp 213–214 °C dec, deposited. Anal. ($C_{16}H_{18}N_2O_2 \cdot C_4H_4O_4$) C, H, N.

α-[[(2-Bromobenzylidene)amino]methyl]-3,4-dimethoxybenzyl Alcohol (10). A solution of 20 g (85.8 mmol) of α-(aminomethyl)-3,4-dimethoxybenzyl alcohol hydrochloride (**3**), 10.9 mL (94 mmol) of 2-bromobenzaldehyde, and 12.0 mL (86.2 mmol) of triethylamine in 400 mL of methanol was refluxed for 10 min, and then water was added to the cloud point. After the solution was cooled at 0 °C for 12 h, 26.27 g (77%) of colorless crystals, mp 109–111 °C, was collected. Anal. ($C_{17}H_{18}BrNO_3$) C, H, N.

α-[[(2-Bromobenzyl)amino]methyl]-3,4-dimethoxybenzyl Alcohol (11). A solution of 60 g (0.165 mol) of α-[[(2-bromobenzyliden)amino]methyl]-3,4-dimethoxybenzyl alcohol (**10**) in 400 mL of methanol was cooled to about 20 °C, i.e., the point at which material began to fall from solution. At this point, sodium borohydride (9.3 g, 0.246 mol) was added in small portions. After all of the sodium borohydride had been added, the suspension was stirred at 0 °C for 30 min. Upon the addition of water, 56.9 g (95%) of crystals, mp 104–106 °C, was obtained. Anal. ($C_{17}H_{20}BrNO_3$) C, H, N.

α-[[*N*-(2-Bromobenzyl)-*N*-methylamino]methyl]-3,4-dimethoxybenzyl alcohol was prepared from **11** (75% yield) in the same manner described for the conversion of **5** to **6**. The crude crystalline material was employed for further reaction without purification. A sample was recrystallized from ether–hexane, mp 86–89 °C.

8-Bromo-1,2,3,4-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline (12) Hydrochloride. A solution of 21.0 g (55.1 mmol) of α-[[*N*-(2-bromobenzyl)-*N*-methylamino]-methyl]-3,4-dimethoxybenzyl alcohol in 200 mL of methylene chloride was added dropwise to a suspension of 36.5 g (0.274 mol) of $AlCl_3$ in 200 mL of methylene chloride at −50 to −60 °C. After the addition was completed, the mixture was stirred at 0 °C for 1 h and then poured into 1-*L* of ice–water. The stirred mixture was made alkaline with 2.5 N sodium hydroxide. After the methylene chloride layer was separated, the aqueous part was extracted several times with ethyl acetate. The organic extracts were combined, dried ($MgSO_4$), and concentrated. The residual oil was dissolved in 100 mL of methanol, and the solution was acidified with ethereal hydrogen chloride. Addition of ether gave 14.5 g (66%) of colorless crystals, mp 233–236 °C, after recrystallization from methanol–ethyl acetate–ether: TLC (alumina, 1:1 hexane/ethyl acetate) R_f 0.6. Anal. ($C_{18}H_{21}BrClNO_2$) C, H, N.

8-Cyano-1,2,3,4-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline (13). The 8-bromotetrahydroisoquinoline hydrochloride **12** (32.0 g, 80.3 mmol) was converted to an oily base by basification of an aqueous solution with ammonia, followed by extracting the mixture with ethyl acetate and drying ($MgSO_4$), and concentrating the organic extract. A mixture of the resulting base, 12.86 g (0.14 mol) of cuprous cyanide, and 150 mL of dimethylacetamide was stirred and refluxed for 4 h. The reaction mixture was diluted with 500 mL of water, 100 mL of ethylenediamine was added, and then the mixture was extracted with ethyl acetate. After the extracts were washed with water, dried ($MgSO_4$), and concentrated, the residual solid was recrystallized from ether to give 16.0 g (65%) of white crystals: mp 123–124 °C; TLC (silica G, 1:1 hexane/ethyl acetate) R_f 0.7. Anal. ($C_{19}H_{20}N_2O_2$) C, H, N.

1,2,3,4-Tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline-8-carboxylic Acid Hydrochloride (14). A mixture of 7.0 g (22.7 mmol) of 8-cyano-1,2,3,4-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline (**13**), 100 mL of methanol, and 150 mL of 8 N sodium hydroxide was stirred and refluxed for 24 h. The solution was adjusted to pH 4 by the addition of concentrated hydrochloric acid, and the resulting mixture was extracted continuously with chloroform. Concentration of the organic extracts afforded **14**·HCl: yield 5.5 g (67%); mp 254–256 °C. Anal. ($C_{19}H_{22}ClNO_4$) C, H, N.

8-Amino-1,2,3,4-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline (1d). Method A. To a stirred solution of 9.08 g (29.2 mmol) of 1,2,3,4-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline-8-carboxylic acid hydrochloride (**14**), 100 mL of acetone, and 100 mL of water at −5 °C was added dropwise 9.8 mL (70.4 mmol) of triethylamine, followed by 9.0 mL (73.1 mmol) of ethyl chloroformate. The mixture was stirred at −5 °C for 30 min, and then a solution of 9.0 g (0.14 mol) of sodium azide in 10 mL of water was added dropwise, and stirring was continued at −5 to 0 °C for 10 min. The reaction mixture was poured into 200 mL of ice–water, and the oily azide was extracted into 200 mL of toluene. The toluene solution was dried ($MgSO_4$) and then *gradually* brought to reflux. Refluxing was continued for 10 min after nitrogen evolution was completed, and

then the toluene solution was concentrated. Residual isocyanate was refluxed with 100 mL of 8 N hydrochloric acid for 30 min. The resulting solution was cooled to 0–10 °C and made alkaline with aqueous ammonia. After the mixture was extracted with ethyl acetate, the extracts were washed with water, dried ($MgSO_4$), and concentrated to give 6.9 g (79%) of an oil: TLC (silica G, 10:90 $CH_3OH/CHCl_3$) R_f 0.5; NMR ($CDCl_3$) δ 2.5 (s, 3 H, NCH_3), 2.5–3.1 (m, 2 H, NCH_2CH), 3.4 (d, 2 H, NCH_2Ar), 3.6 (br s, 2 H, NH_2), 3.75 (s, 3 H, CH_3O), 3.81 (s, 3 H, CH_3O), 4.20 (t, 1 H, Ar CH), 6.2–6.9 (m, 6 H, Ar H).

The base was dissolved in methanol and an equivalent of fumaric acid was added, followed by precipitation with ether. The resulting hydrogen fumarate melted at 184–188 °C after recrystallization from methanol–ether. Anal. ($C_{18}H_{22}N_2O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$) C, H, N.

Method B. α-[[*N*-(2-Aminobenzyl)-*N*-methylamino]-methyl]-3,4-dimethoxybenzyl alcohol (**7**; 3.0 g, 9.5 mmol) was added in small portions to 5 mL of stirred concentrated sulfuric acid at −10 °C. The resulting solution was immediately poured onto 300 mL of ice–water, the solution was made basic with aqueous ammonia, and the resulting mixture was extracted with ethyl acetate. The multicomponent mixture was chromatographed on a silica column, eluting with a 70:30 hexane/ethyl acetate mixture. Eluate was monitored by the TLC system described in method A. Concentration of fractions containing **1d** gave 1.0 g (35%) of an oily product, which was converted to a crystalline hydrogen fumarate, mp 184–188 °C, and having an IR spectrum identical with the product obtained by method A.

8-Amino-1,2,3,4-tetrahydro-4-(3,4-dihydroxyphenyl)-2-methylisoquinoline (3′,4′-dihydroxynomifensine, 1a) was prepared from **1d** (base) in 30% yield using boron tribromide under the same conditions described for methoxyl cleavage of **8** to **9**. The crystalline product (free base) melted at 206–209 °C after recrystallization from a small volume of cold methanol. Anal. ($C_{16}H_{18}N_2O_2 \cdot 0.125H_2O$) C, H, N.

Resolution of 8-Amino-1,2,3,4-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline [(+)- and (−)-1d]. To a solution of 6.9 g (23.1 mmol) of 8-amino-1,2,3,4-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline (**1d**) in 20 mL of ethanol was added 8.9 g (23.1 mmol) of di-*p*-toluoyl-*d*-tartaric acid ($[\alpha]^{20}_D$ −126° (*c* 1, EtOH), from natural tartaric acid) in 30 mL of ethanol. After the solution remained at 25 °C for 24 h, 6.0 g of a crystalline product, mp 180–184 °C, was collected on a filter. Recrystallization from 150 mL of ethanol gave 4.0 g of **1d** hydrogen di-*p*-toluoyl-*d*-tartrate: mp 186–188 °C; $[\alpha]^{25}_D$ −112.0° (c 1, CH_3OH). Anal. ($C_{18}H_{22}N_2O_2 \cdot C_{20}H_{18}O_8$) C, H, N. A suspension of these crystals in water was made alkaline with aqueous ammonia, and the resulting mixture was extracted with ethyl acetate. The extracts were washed with water, dried ($MgSO_4$), and concentrated to give 2.0 g (58% isomeric yield) of (−)-**1d** as a liquid $[\alpha]^{25}_D$ −83.6° (*c* 1, CH_3OH).

All mother liquors from the above isolation were combined and concentrated in vacuo. The residue was suspended in water. The suspension was made alkaline by the addition of aqueous ammonia to give a mixture, which was extracted with ethyl acetate. After the extracts were washed with water, they were dried ($MgSO_4$) and concentrated. The residual liquid was treated with an equivalent of di-*p*-toluoyl-*l*-tartaric acid monohydrate ($[\alpha]^{20}_D$ +132° (*c* 1, EtOH), from unnatural tartaric acid) as described for (−)-**1d**. After recrystallization from ethanol, (+)-**1d** hydrogen di-*p*-toluoyl-*l*-tartrate (4.0 g) was obtained as crystals, mp 186–188 °C. Anal. ($C_{18}H_{22}N_2O_2 \cdot C_{20}H_{18}O_8$) C, H, N. The base (+)-**1d** (2.0 g) was obtained as described for its enantiomer: $[\alpha]^{25}_D$ +82.1° (*c* 1, CH_3OH).

(*R*)-(−)-8-Amino-1,2,3,4-tetrahydro-4-(3,4-dihydroxyphenyl)-2-methylisoquinoline [(*R*)-(−)-3′,4′-Dihydroxynomifensine, (*R*)-(−)-1a]. A solution of 1.8 g (6.03 mmol) of (−)-8-amino-1,2,3,4-tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylisoquinoline [(−)-**1d**] and 9.0 g (60.4 mmol) of methionine in 100 mL of warm 98% methanesulfonic acid was stirred at 25 °C for 48 h; then it was poured into 700 mL of ice–water and adjusted to pH 8 with aqueous ammonia. The mixture was extracted with ethyl acetate. The extracts were washed with water, dried ($MgSO_4$), and concentrated. Trituration of the residue with 30 mL of methanol at 0 °C gave 0.8 g (49%) of colorless crystals: mp 220–222 °C dec; $[\alpha]^{25}_D$ −172.1° (*c* 1, DMF). Anal. ($C_{16}H_{18}N_2O_2 \cdot 0.125H_2O$) C, H, N.

A suspension of (−)-**1a** in ethanol was treated with methyl iodide to give colorless crystals of a (−)-**1a**·MeI, mp 249–254 °C, after recrystallization from methanol–ether. Anal. ($C_{17}H_{21}IN_2O_2$) C, H, N. These crystals were used for single-crystal X-ray diffractometric analysis, which established the absolute configuration as *R*.

(*S*)-(+)-8-Amino-1,2,3,4-tetrahydro-4-(3,4-dihydroxyphenyl)-2-methylisoquinoline [(*S*)-(+)-3′,4′-dihydroxynomifensine, (*S*)-(+)-1a] was prepared from (+)-**1d** in the same manner as described for the preparation of (*R*)-(−)-**1a**. The crystals melted at 220–222 °C dec: $[\alpha]^{25}_D$ +173.7° (*c* 1, DMF). Anal. ($C_{16}H_{18}N_2O_2 \cdot 0.125H_2O$) C, H, N.

Single-Crystal X-ray Analysis of (−)-8-Amino-1,2,3,4-tetrahydro-4-(3,4-dihydroxyphenyl)-2-methylisoquinoline Methiodide [(−)-1a·MeI].[72] (−)-**1a**·MeI occurred as needle-like crystals. Preliminary X-ray photographs showed orthorombic cell parameters of a = 9.301 (1), b = 10.834 (1), and c = 16.829 (2) Å. These were determined from a least-squares fit using the setting angles of 25 reflections in the range $5 < \theta < 19°$, measured by the computer-controlled diagonal slit method of centering. The space was determined to be *P*2-1 2-1 2-1 with an asymmetric unit of $C_{17}H_{21}IN_2O_2$; the calculated density was 1.61 g/cm^3. Preliminary examination and data collection were performed with Cu $K\alpha$ (1.54184 Å) radiation on a computer-controlled diffractometer equipped with a graphite crystal, incident beam monochromator. Of the 2095 reflections collected, 1998 were unique and not systematically absent.

The structure was solved by the Patterson heavy-atom method, which revealed the position of the iodine atom. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were located, and their positions were defined in least squares. The absolute configuration was checked by carrying out refinement with the larger data set containing Friedel pairs. The enantiomer shown (Figure 3, absolute stereochemistry at C-10 is *R*) was significantly lower; the *R* factor for the other enantiomer was significantly higher. Please consult supplementary material paragraph at the end of this article for additional details.

Pharmacology. Stimulation of dopamine-sensitive adenylate cyclase was measured by using rat caudate homogenates employing a modification of the procedures of Kebabian et al.[73] and Carenzi et al.,[74] as described previously.[52] The EC_{50} is defined as the micromolar concentration that gives half-maximal stimulation of cyclic AMP production over the concentration range tested.

Spiroperidol binding was determined by a modification of the method of Fujita and Saito,[75] as described previously.[53] The IC_{50} is the concentration of compound that produces 50% displacement of specific [^{3}H]spiroperidol binding to the rat caudate membrane preparations.

Clonidine binding was determined essentially as described by U'Prichard et al.[70] The IC_{50} is the concentration of compound that produces 50% displacement of specific [^{3}H]clonidine binding (the difference between total binding and binding observed in the presence of 10 μM norepinephrine, i.e., nonspecific binding) to rat brain cortical membranes.

Acknowledgment. We are grateful to Edith A. Reich of the Analytical/Physical Chemistry Department of Smith Kline & French Laboratories for determination of elemental analyses and specific rotation and to Dr. Tikam C. Jain for helpful discussions.

Registry No. (±)-**1a**, 87351-83-5; (*R*)-(−)-**1a**, 87419-65-6; (*R*)-(−)-**1a**·MeI, 87351-84-6; (*S*)-(+)-**1a**, 87419-66-7; (±)-**1d**, 87351-85-7; (±)-**1d** hydrogen fumarate, 87351-86-8; (*R*)-(−)-**1d**,

(72) These services were performed by the crystallographic staff of Molecular Structure Corp.: M. W. Extine, R. A. Meisner, J. M. Troup, and B. B. Warrington, MSC code MS2117.

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87419-67-8; (*R*)-(−)-**1d** hydrogen di-*p*-toluoyl-*d*-tartrate, 87419-68-9; (*S*)-(+)-**1d**, 87419-69-0; (*S*)-(+)-**1d** hydrogen di-*p*-toluoyl-*l*-tartrate, 87419-70-3; (±)-1 (X = N_3; R = OMe), 87351-87-9; (±)-**3**·HCl, 64124-23-8; (±)-**4**, 87351-88-0; (±)-**5**, 87351-89-1; (±)-**6**, 87351-90-4; (±)-**6**·HCl, 87351-91-5; (±)-**7**, 87351-92-6; (±)-**8**, 87371-37-7; (±)-**9**, 87351-93-7; (±)-**9** hydrogen fumarate, 87351-94-8; (±)-**10**, 87351-95-9; (±)-**11**, 87419-71-4; (±)-**11** (*N*-methyl derivative), 87419-72-5; (±)-**12**, 87351-96-0; (±)-**12**·HCl, 87351-97-1; (±)-**13**, 87351-98-2; (±)-**14**·HCl, 87351-99-3; 2-nitrobenzaldehyde, 552-89-6; 2-bromobenzaldehyde, 6630-33-7.

Supplementary Material Available: Description of experimental procedures (data collection, data reduction, and structure solution and refinement), tables of experimental details (crystal data, intensity measurements, and structure solution and refinement), positional and thermal parameters, general temperature factor expressions (*B*'s), bond distances, bond angles, torsional angles, intermolecular contacts up to 3.50 Å, least-square planes, intensity data, and a figure of a single molecule showing 40% probability ellipsoids are available (23 pages). Ordering information is given on any current masthead page.

Trypanocidal 1,3-Arylene Diketone Bis(guanylhydrazone)s. Structure–Activity Relationships among Substituted and Heterocyclic Analogues

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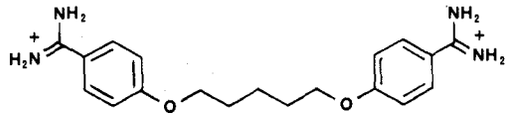
Based on the antitrypanosomal activity of 1,3-diacetylbenzene bis(guanylhydrazone) (**4**) and 2,6-diacetylpyridine bis(guanylhydrazone) (**17**), a number of substituted and heterocyclic 1,3-arylene diketone bis(guanylhydrazone)s were prepared and tested against *Trypanosoma brucei* infections in mice. A wide range of ED_{50} values was observed among 5-substituted derivatives of **4**. The 5-amino analogue **5** and 5-acetamido analogue **6** were about twice as active as **4**. 1,3,5-Triacetylbenzene tris(guanylhydrazone) (**12**) was about 9 times as active as **4** and was approximately one-half as active as the currently used trypanocide diminazene aceturate in this test system. Other 5-derivatives had activity equivalent to or less than that of the parent compound **4**. Three new heterocyclic analogues were all less active than 2,6-diacetylpyridine derivative **17** and benzene derivative **4**. Ring substitution ortho to the guanylhydrazone side chains was invariably detrimental to activity. Side-chain homologues 1,3-dipentanoylbenzene bis(guanylhydrazone) and 1,3-diacetylbenzene bis(2-imidazolin-2-ylhydrazone) were essentially inactive.

Trypanosomiasis kills over 3 000 000 head of cattle in Africa every year;[1] in addition, there are thought to be at least 10 000 new cases annually of human trypanosomiasis in Africa, although quantitation is difficult because new outbreaks tend to occur in areas of political and economic turmoil.[2] Over the last 25 years, research on the chemotherapy of African trypanosomiasis has been sufficiently quiescent that no new trypanocides have been brought into use.[3]

Bis(benzamidine) derivatives, such as pentamidine (**1**)



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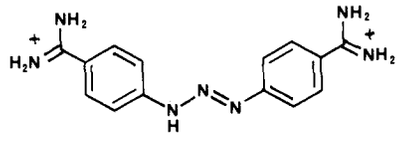

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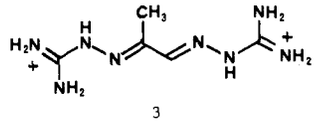

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isethionate and diminazene (**2**) aceturate, are an important class of currently employed antitrypanosomal agents.[4] Methylglyoxal bis(guanylhydrazone) (MGG, **3**) dihydrochloride, an antitumor agent that formally resembles **1** and **2** in having two terminal amidine moieties, has been shown to possess modest trypanosuppressive properties.[5] It occurred to us that simple aromatic analogues of **3** might be better trypanocides, based on the aromatic character of **1** and **2** and of other classes of cationic trypanocides, such as quinapyramine. This conjecture proved to be correct. In our preliminary study of aromatic bis(guanylhydrazone)s,[6] 1,3-diacetylbenzene bis(guanylhydrazone) (**4**) and 2,6-diacetylpyridine bis(guanylhydrazone) (**17**) showed substantial curative activity against *Trypanosoma brucei* infection in mice; **4** was also curative against *T. congolense*. Analogous dialdehyde derivatives and 1,4-isomers were less active.[6] The 1,3-arylene diketone bis(guanylhydrazone) series was therefore selected for further study. In an effort to identify structural modifications that would enhance antitrypanosomal activity in this series, we have synthesized and tested 12 ring-substituted analogues and two side-chain homologues of **4**, as well as three new heterocyclic derivatives.

Chemistry. Guanylhydrazones **4**–**22** (Table I) were routinely prepared from the corresponding diketone and a 10–20% excess of the appropriate aminoguanidine salt in aqueous ethanol containing a trace of excess acid.

A number of 1,3-phenylene diketones required for this study apparently had not been described previously (Table II). 5-Nitro-1,3-diacetylbenzene (**29**) was prepared from 5-nitroisophthaloyl dichloride by condensation with diethyl magnesiomalonate and subsequent hydrolytic decarboxylation, by analogy with a procedure for the synthesis of 2-nitro-1,3-diacetylbenzene.[7] 4-Nitro-1,3-diacetylbenzene (**30**) and 5-methyl-1,3-diacetylbenzene (**27**) were similarly prepared from their corresponding acid dichlorides.

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