5-HT_{1A} Receptor Binding Assay. 5-HT_{1A} receptor affinity was measured in hippocampal rat brain tissue by using a modification of the method of Hall et al.[27] Several rats were decapitated and the brains were rapidly removed. Hippocampal tissue was dissected and homogenized on ice in 40 volumes of buffer A (50 mM Tris·HCl, pH 7.7) with a Polytron homogenizer at setting 5 for three 15-s bursts. The homogenate was then centrifuged at 48000*g* for 10 min and the supernatant discarded. The pellet was resuspended in 40 volumes of the same buffer and incubated at 37 °C for 10 min to aid in the removal of endogenous serotonin. The homogenate was then centrifuged (as above) and the supernatant discarded. The pellet was then resuspended in 100 volumes of buffer B (50 mM Tris·HCl, pH 7.7 containing 0.1% ascorbate, 10 μM pargyline, and 4 mM $CaCl_2$) and sonicated. An aliquot was taken for protein determination by the Lowry method[25] and the remainder stored frozen at −70 °C until used.

The homogenate (500 μL; 0.4–0.6 mg of protein/sample) was incubated with 100 μL (1.5–1.8 nM) [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin and various concentrations of test drug in a final volume of 2 mL of buffer B for 10 min at 37 °C. At the end of the incubation, 3 mL of cold buffer A was added to each tube, and the contents were rapidly filtered through Whatman GF/B glass filters. The filters were then rapidly washed two times with 3 mL of the same buffer, placed in scintillation vials, and shaken for 15 min with 10 mL of Hydrofluor (National Diagnostics) scintillation cocktail. The vials were then counted in a Packard 460 CD scintillation counter.

Specific binding was defined at total binding less binding in the presence of 1 μM (+)-serotonin. Results of 5-HT_{1A} receptor binding studies were analyzed as previously stated for the D_2 receptor binding studies. The K_D value for [^{3}H]-8-OH-DPAT binding in hippocampus was 1.8 nM.

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Registry No. **4**, 25547-30-2; **5**, 114298-16-7; **6**, 6295-73-4; **7**, 6705-94-8; **8**, 114222-40-1; **9**, 23852-39-3; **10**, 17397-36-3; **11**, 33974-23-1; **12** (R^2 = 2-pyrimidinyl), 33386-20-8; **12** (R^2 = 2-pyrazinyl), 114222-98-9; **12** (R^2 = 6-chloro-2-pyrazinyl), 114222-99-0; **13**, 102995-93-7; **13**·$C_4H_4O_4$, 102995-94-8; **14**, 103066-37-1; **14**·$C_4H_4O_4$, 103066-38-2; **15**, 110944-39-3; **15**·HCl, 110944-62-2; **16**, 114222-41-2; **16**·HCl, 112050-66-5; **17**, 110944-42-8; **17**·HCl, 112025-11-3; **18**, 103066-39-3; **18**·$C_4H_4O_4$, 103066-40-6; **19**, 114222-42-3; **19**·2HCl, 114222-72-9; **20**, 114222-43-4; **20**·2HCl, 114222-73-0; **21**, 110944-33-7; **21**·2HCl, 110944-54-2; **22**, 110944-28-0; **22**·HCl, 110944-63-3; **23**, 110944-35-9; **23**·HCl, 114222-74-1; **24**, 110944-48-4; **24**·2HCl, 110944-68-8; **25**, 114222-44-5; **25**·2HCl, 114222-75-2; **26**, 114222-45-6; **26**·2HCl, 114222-76-3; **27**, 114222-46-7; **27**·2HCl, 114222-77-4; **28**, 114222-47-8; **28**·2HCl, 114222-78-5; **29**, 110944-29-1; **29**·2HCl, 114222-79-6; **30**, 110944-49-5; **30**·HCl, 110944-69-9; **31**, 114222-48-9; **31**·2HCl, 114222-80-9; **32**, 110944-50-8; **32**·HCl, 112025-10-2; **33**, 114298-17-8; **33**·2HCl, 114374-96-8; **34**, 114298-18-9; **34**·HCl, 114374-97-9; **35**, 114222-49-0; **35**·2HCl, 114298-21-4; **36**, 114222-50-3; **36**·2HCl, 114298-22-5; **37**, 114298-19-0; **37**·2HCl, 114374-98-0; **38**, 114222-51-4; **38**·2HCl, 114298-23-6; **39**, 114298-20-3; **39**·2HCl, 114374-99-1; **40**, 114222-52-5; **40**·2HCl, 114222-81-0; **41**, 114222-53-6; **41**·2HCl, 114222-82-1; **42**, 110944-41-7; **42**·2HCl, 110944-61-1; **43**, 110944-40-6; **43**·HCl, 114222-83-2; **44**, 114222-54-7; **44**·HCl, 114222-84-3; **45**, 110944-51-9; **45**·2HCl, 110944-70-2; **46**, 114222-55-8; **46**·2HCl, 114222-85-4; **47**, 110944-36-0; **47**·HCl, 114222-86-5; **48**, 114222-56-9; **48**·2HCl, 114222-87-6; **49**, 110944-31-5; **49**·HCl, 110944-57-5; **50**, 110944-32-6; **50**·2HCl, 110944-58-6; **51**, 110944-34-8; **51**·HCl, 114222-88-7; **52**, 110944-45-1; **52**·2HCl, 110944-65-5; **53**, 110944-46-2; **53**·HCl, 110944-66-6; **54**, 114222-57-0; **54**·2HCl, 110944-72-4; **55**, 114222-58-1; **55**·HCl, 114222-89-8; **56**, 114222-59-2; **56**·2HCl, 114222-90-1; **57**, 114222-60-5; **57**·2HCl, 114222-91-2; **58**, 114222-61-6; **58**·HCl, 114298-24-7; **59**, 114222-62-7; **59**·2HCl, 114298-25-8; **60**, 114222-63-8; **60**·2HCl, 114298-26-9; **61**, 114222-64-9; **61**·2HCl, 114298-27-0; **62**, 114222-65-0; **62**·2HCl, 114298-28-1; **63**, 114222-66-1; **63**·2HBr, 114222-92-3; **64**, 114222-67-2; **64**·HCl, 114222-93-4; **65**, 114222-68-3; **65**·HCl, 114222-94-5; **66**, 114222-69-4; **66**·HCl, 114222-95-6; **67**, 114222-70-7; **67**·HCl, 114222-96-7; **68**, 114222-71-8; **68**·HCl, 114222-97-8; maleimide, 541-59-3; maleic anhydride, 108-31-6; 1,3,5,7-cyclooctatetraene, 629-20-9; 1,3-cycloheptadiene, 4054-38-0; 1,3-cyclooctadiene, 1700-10-3; norbornadiene-2,3-dicarboxylic anhydride, 17397-31-8; cyclopentadiene, 542-92-7; sulfolene, 77-79-2; 2-(4-bromobutyl)hexahydro-4,7-etheno-1*H*-cyclobut[*f*]isoindole-1,3(2*H*)-dione, 114222-39-8; 1-(2-pyrimidinyl)piperazine, 20980-22-7; 4-pyridinylbutyl bromide, 109315-44-8; 2,6-dichloropyrazine, 4774-14-5; 1-(6-chloro-2-pyrazinyl)piperazine, 64022-27-1; 1-(3-chloro-2-pyrazinyl)piperazine, 85386-99-8.

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Synthesis and Dopamine Agonist and Antagonist Effects of (*R*)-(−)- and (*S*)-(+)-11-Hydroxy-*N*-*n*-propylnoraporphine

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The *R*-(−) and *S*-(+) enantiomers of 11-hydroxy-*N*-*n*-propylnoraporphine, (*R*)-**3** and (*S*)-**3**, were synthesized in six steps from 1-(3-methoxy-2-nitrobenzyl)isoquinoline. Neuropharmacological evaluation of the *R* and *S* isomers (by affinity to dopamine receptor sites in rat brain tissue, induction of stereotyped behavior, and interaction with motor arousal induced by (*R*)-apomorphine in the rat) indicated that, similar to the 10,11-dihydroxy congener **2**, both enantiomers can bind to dopamine receptors but that only (*R*)-**3** activates them, whereas (*S*)-**3** shows activity as a dopaminergic antagonist.

Absolute configuration is critically important for interactions at dopamine (DA) receptors.[1,2] Only the *R*-(−) enantiomer of apomorphine, obtained by the acid-catalyzed rearrangement of the natural product (−)-morphine, possesses DA agonist activity.[3] In contrast, (*S*)-(+)-bulbocapnine, a naturally occurring aporphine alkaloid, has DA receptor antagonist activity and its absolute configuration at carbon 6a is opposite to that of (*R*)-(−)-apomorphine.[4] It has been suggested that (*S*)-(+)-apo-

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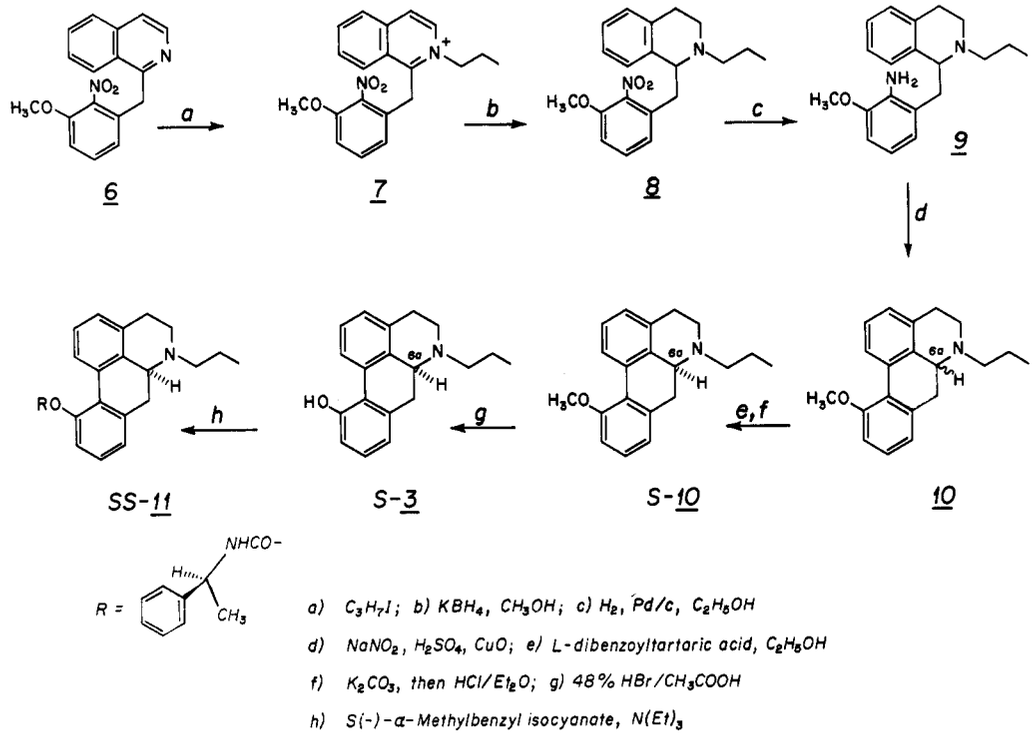
0022-2623/88/1831-1392$01.50/0

Scheme I



morphine, rather than being merely a weak DA agonist, may have activity as a DA receptor antagonist.[5,6]

The structural requirements for activity at DA receptors of a variety of aporphines and structurally related DA agonists prepared in our laboratories[4] led to an hypothesis relating to the mode of binding of such agonists to DA receptors.[7-10] For the hydroxylated aporphines, the configuration at the 6a chiral carbon atom, the location of the hydroxyl groups, and the presence of an *N*-alkyl substituent at the nitrogen atom all appear to contribute to optimizing the DA receptor (D-1, D-2, and DA agonist) interactions in radioreceptor assays and in functional or behavioral assays believed to represent DA-receptor-mediated effects.[4,8,9] Of particular interest to us were earlier observations that (*RS*)-(±)-11-hydroxyaporphine and (*RS*)-(±)-11-hydroxy-*N*-*n*-propylnoraporphine (11-OH-N-Pa) had DA agonist activity nearly as potent as the corresponding catechol aporphines (*R*)-(-)-apomorphine, **1**, and (*R*)-(-)-*N*-*n*-propylnorapomorphine, **2**.[11-13] This finding emphasized that a catechol moiety was not an absolute requirement for dopaminergic activity and presented an opportunity to test the prediction that only (*R*)-(-)-11-hydroxy-*N*-*n*-propylnoraporphine, **3**, would have agonist activity at brain DA receptors.

Other monohydroxyphenyl-containing compounds with DA receptor activity permit further analysis of structural and stereochemical relationships. These include 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP, **4**) and *cis*-1-methyl-5-hydroxy-2-(di-*n*-propylamino)tetralin (5-OH-MDAT, **5**). The observation that (3*R*)-**4** is a DA agonist whereas the antipode (3S)-**4** blocks postsynaptic DA receptors (but may be a presynaptic agonist at low doses[14]) challenged the widely held concept that only one enantiomer of a compound is active at DA receptors while the other is inactive. Furthermore, among the aminotetralin analogues of DA, the 1*S*,2*R* isomers of **5** has been found to be a presynaptic and postsynaptic DA antagonist while the 1*R*,2*S* antipode of **5** was a DA agonist.[15] Similarly, while (*R*)-(-)-apomorphine and its *N*-*n*-propyl analogue are DA agonists, the *S*-(+) antipodes of apomorphine[5] and *N*-*n*-propylnorapomorphine[6,16] recently have been found to be DA antagonists in behavioral indices of receptor activity. (*S*)-(+)-*N*-*n*-Propylnorapomorphine

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is a particularly potent antagonist and may be selective for limbic DA receptors.[6,16,17] Although (*R*)-(−)-11-hydroxy-*N*-*n*-propylnoraporphine, **3**, has been reported to have high affinity at DA agonist binding sites,[8,9] the *S*-(+) enantiomer has not until now been available for biological evaluation and neither isomer has been well characterized pharmacologically. This paper presents the synthesis and preliminary pharmacological characterization of (*R*)-(−)- and (*S*)-(+)-11-hydroxy-*N*-*n*-propylnoraporphine, **3**.

Chemistry

We reported previously[12] the synthesis of (*RS*)-**3**. In the present work, the synthetic procedure was modified to give better overall yields.[18] The precursor 1-(-methoxy-2-nitrobenzyl)isoquinoline, **6**, was synthesized from 2-nitro-3-methoxybenzoic acid and isoquinoline in a multistep sequence[19] and treated with propyl iodide to give the quaternary salt **7** (Scheme I). Reduction with potassium borohydride in absolute ethanol gave an 82% yield of the tetrahydroisoquinoline derivative **8**, which was easily reduced to corresponding amino compound **9** by catalytic hydrogenation. The Pschorr cyclization[12] of **9** afforded (*RS*)-11-methoxy-*N*-*n*-propylnoraporphine, **10**.

Resolution of (*RS*)-**10** was achieved by a modification of a procedure previously described for the resolution of (*R*)- and (*S*)-*N*-*n*-propylnorapomorphine, **2**.[10] The optical purity of the resolved compounds was determined by obtaining a constant specific rotation after three recrystallizations from a mixture of 2-propanol and absolute ethanol (1:1, v/v). The enantiomeric purity was established to be >99% by using the following procedure for the chiral derivatization and chromatographic resolution of the resulting diastereoisomers. Both (*R*)-**3** and (*S*)-**3** were treated with (*S*)-(−)-α-methylbenzyl isocyanate and converted to the diastereoisomeric carbamates (S,R)-**11** and (*S,S*)-**11**, which could be resolved chromatographically. The chromatograms of the carbamates derived from (*R*)-**3** and (*S*)-**3** had a similar pattern, with one major peak and only a trace of the antipode peak, whereas the mixture of two carbamates (*S,R*)-**11** and (*S,S*)-**11** derived from (*R*)-**3** and (*S*)-**3** gave two peaks with expected heights. Treatment of (*R*)-**10** with 48% aqueous HBr gave (*R*)-**3**; similarly, (*S*)-**10** was converted to (*S*)-**3**. The absolute configuration was established by comparison with authentic (*R*)-(−)-11-hydroxy-*N*-*n*-propylnoraporphine, designated as 6a*R*, since this compound was derived from (−)-morphine, by a procedure previously reported.[20]

Pharmacology

The binding of the isomers of compound **3** at dopamine receptor sites was evaluated with a membrane preparation of corpus striatum from rat brain and radiolabeled ligands selective for D-1, D-2, and dopamine agonist sites, by using methods reported in detail previously.[21-23] In competition with 1.5 nM [^{3}H]ADTN [(±)-6,7-dihydroxy-2-aminotetralin], a dopaminergic agonist ligand, compound **3** showed a strong (57-fold) preference and high affinity (K_i = 4 nM) in the *R* isomer form. There was similar affinity of (*R*)-**3** (K_i = 3 nM) and enantiomeric selectivity (39-fold) for sites labeled by the selective D-2 antagonist [^{3}H]spiperone (at 0.15 nM), but both lower affinity of (*R*)-**3** (K_i = 434 nM) and isomeric selectivity (3-fold) with the D-1 antagonist [^{3}H]-SCH-23390 (0.3 nM) (Table I). These observations indicate that the preferred isomer of **3** is *R*, but that (*S*)-**3** also has moderate affinity at D-2 sites (K_i = 105 nM) and to dopamine agonist sites (K_i = 229 nM). Receptor assay experiments also included isomers of compound **2**, the 10,11-dihydroxy analogue of compound **3**, for comparison (Table I). There were similar receptor affinity and isomeric preferences for **2** as found with **3**, in the following rank order: dopamine agonist ≥ D-2 > D-1.

Behavioral experiments in young adult, male Sprague–Dawley albino rats included assessment of the ability of the isomers of **3** to induce stereotyped behaviors (sniffing, licking, and gnawing) typical of dopaminergic agonists such as (*R*)-(−)-apomorphine, **1**, or (*R*)-(−)-*N*-*n*-propylnorapomorphine, **2**. In addition, we assessed the ability of the compounds to alter the behavioral arousal-inducing affect of a moderate standard dose of (*R*)-**1** by use of a micro-

Table I. Affinity of Enantiomers of Hydroxylated *N*-*n*-Propylnoraporphines to Dopamine Receptor Sites

compd	computed values of K_i,[a] nM: [^{3}H]-SCH-233390 (D-1)	[^{3}H]spiperone (D-2)	[^{3}H]ADTN (agonist)	D-1:D-2 potency ratio
(*R*)-**2**	340	2.4	1.1	142
(*S*)-**2**	1345	115	278	11.7
(*R*:*S* potency)	(4.0)	(48)	(253)	
(*R*)-**3**	434	2.7	4.0	161
(*S*)-**3**	1413	105	229	13.5
(*R*:*S* potency)	(3.3)	(39)	(57)	

[a] Binding competition experiments were carried out with membrane preparations from corpus striatum of rat brain; data are mean values for apparent K_i (nM), computed from K_d values and ligand concentrations, [L] of 0.34 nM for SCH-23390 (D-1 ligand), 0.30 nM for spiperone (D-2 ligand), and 1.5 nM for ADTN (DA agonist ligand), as well as from values for IC50 determined at six or more, concentrations of *N*-*n*-propylnorapomorphine, **2**, or 11-hydroxy-*N*-*n*-propylnoraporphine, **3** (above and below IC50), in triplicate, at the indicated [L], with data fit by a microcomputer program (ALLFIT) to obtain IC50 ± SEM (ref 16, 17). Variances for IC50 (as SEM) were consistently less than 10% of the mean. Values for K_i were computed from the relationship: $K_i = IC50/[1 + ([L]/K_d)]$.

Table II. Stereotyped Behavior in Rat Induced by Aporphines[a]

dose, mg/kg	(*R*)-**1**	(*R*)-**3**	(*S*)-**3**
0 (saline)	0.8 ± 0.4	0.8 ± 0.4	0.8 ± 0.4
0.3	9.7 ± 1.4*	5.7 ± 0.7*	0.6 ± 0.3
1.0	12.7 ± 2.3*	9.5 ± 0.5*	1.7 ± 0.3
3.0	17.5 ± 1.9*	13.8 ± 0.5*	1.3 ± 0.7

[a] Rats were given an aporphine (ip) and rated for stereotyped behavior every 10 min for 1 h; data are mean stereotypy scores (maximum possible score = 18.0). Compounds were (*R*)-**1** (apomorphine) and isomers of **3** (11-hydroxy-*N*-*n*-propylnoraporphine). Data are means ± SEM (N = 6); an asterisk indicates statistical significance at $p < 0.05$ or less by Student's t test.

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(19) The reduction of 2-nitro-3-methoxybenzoic acid (Aldrich) with diborane in THF did not go to completion under the reported conditions. After the reaction was continued over 3 days, no more starting material was left, which led to a significant increase in the formation of acid chloride (from 66% to 82%) in the following step. Another modification was involved the Reissert alkylation. Instead of using 55% of NaH, a alkylation of the Reissert compound with 65% NaH could afford a 95% yield of crude product (mp 168–171 °C) without trituration with cold 95% ethanol. Previously, after trituration, only a 50% yield of crude product (mp 170–173 °C) was obtained.
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Table III. Effects of Isomers of 11-Hydroxy-*N*-*n*-propylnoraporphine, **3**, on Motor Arousal Induced by (*R*)-(-)-Apomorphine, **1**

pretreatment (dose) + treatment (dose)[a]	counts/h[b]	% of control[c]
saline (0) + (*R*)-**1** (0.3)	505 ± 55	100
(*S*)-**3** (1) + (*R*)-**1** (0.3)	410 ± 62	81
(*S*)-**3** (3) + (*R*)-**1** (0.3)	218 ± 33*	43*
(*S*)-**3** (10) + (*R*)-**1** (0.3)	105 ± 09*	21*
(*R*)-**3** (3) + (*R*)-**1** (0.3)	764 ± 58*	151*

[a] Rats were given an enantiomer of **3** (11-hydroxy-*N*-*n*-propylnoraporphine) just before treatment with 1 [(*R*)-(-)-apomorphine, 0.3 mg/kg] and placed in a microcomputer-controlled electronic activity monitor for 1 h. [b] Data are mean activity counts per hour ± SEM (N = 6); doses are in mg/kg, ip. [c] An asterisk indicates different from saline + apomorphine control at $p < 0.05$ or less by Student's t test.

computer-controlled electronic activity monitor. The experimental methods involved have been discussed in detail previously.[6]

(*R*)-**3** induced strong stereotyped behavior in the rat, nearly as potently as (*R*)-**1** (apomorphine), whereas (*S*)-**3** had no ability to induce stereotypy, even at an acute dose as high as 3 mg/kg, intraperitoneally (ip) (Table II).

These behavioral observations (Table II) indicated that the *R* isomer of **3** has greater agonistic activity, as well as higher affinity (Table I), at dopaminergic receptor sites and accord with previous findings indicating that *S* isomers of 10,11-dihydroxyaporphines lack the intrinsic dopaminergic agonistic activity of their *R* enantiomers.[6,9,10,16]

Additional experiments indicated that (*S*)-**3** not only lacks activity as a dopaminergic agonist but may also have antagonistic activity. Thus, the *S* enantiomer of this 11-monohydroxyaporphine induced a dose-dependent inhibition of arousal induced by the classic dopaminergic agonist (*R*)-**1**, with an ED_{50} of approximately 5 mg/kg, ip (Table III). In contrast, (*R*)-**3** increased behavioral arousal induced by a moderate dose of (*R*)-**1** (0.3 mg/kg, ip) (ED_{50}, or dose yielding a 50% increase in activity counts per hour, of ca. 3 mg/kg; Table III). The antiapomorphine effect of (*S*)-**3** thus appears to extend the antiapomorphine, antiarousal, and antidopaminergic actions of the *S*-(+) enantiomers of apomorphine, (*S*)-**1**,[22] and of *N*-*n*-propylnorapomorphine, (*S*)-**2**, reported previously.[5,6,16]

Conclusions

The present findings support tentative conclusions: (1) the presence of only an 11-OH group (analogous to the *m*-OH of dopamine), but not a 10-OH,[9,13] in aporphines is sufficient to confer affinity and activity at postsynaptic dopaminergic receptors (effects of monohydroxylation on the activity of aporphines at putative presynaptic DA autoreceptors require further study); (2) as with 10,11-dihydroxy, or "catechol", aporphines, 11-monohydroxy congeners have higher affinity for dopaminergic receptors, as well as intrinsic agonistic activity, in the *R*-(-) configuration; (3) affinities for (*R*)-**3**, similar to dihydroxyaporphines, ranked as follows: DA agonist sites ≥ D-2 > D-1 receptors (possibly offering a clue to its pharmacologic activity); (4) as with *S*-(+) catechol aporphines, such as *N*-*n*-propylnorapomorphine, (*S*)-**3** was not only inactive as a dopaminergic agonist but also showed significant activity as a dopaminergic antagonist, possibly related to its moderate affinity and selectivity at D-2 sites.

Experimental Section

Melting points were determined in open capillary tubes in a Thomas-Hoover melting point apparatus and were not corrected. Elemental analyses were performed by Atlantic Microlab Inc. Atlanta, GA, and the analytical values obtained were within ±0.4% of calculated values. IR and NMR spectra data (not reported) were obtained for all compounds described in this section and found to be consistent with the indicated structures. ^{1}H NMR spectra were recorded on a Varian T-60 or XL-300 spectrometer (Me_4Si). Mass spectra were recorded on a Finnigan 4021 mass spectrometer. IR spectra were obtained with a Perkin-Elmer Model 700 spectrophotometer. TLC was done on E. Merck F-254 plastic-backed silica thin-layer gel plates. Optical rotation was determined with a Perkin-Elmer Model 241 polarimeter. HPLC was performed on a Supelcosil LC-8-DB column with 0.05 M KH_2PO_4 (pH 3.0) containing 35% (w/v) CH_3CN as the mobile phase, at a pressure range of 1000–3000 psi and flow rate of 1 mL/min; detection was with a UV III Monitor (Milton Roy, Riviera Beach, FL) by monitoring absorbance at 280 nm.

1-(3-Methoxy-2-nitrobenzyl)isoquinoline propiodide, 7, was prepared from **6** and 1-iodopropane as previously described[12]. Thus a solution of 14.5 g (0.049 mol) of **6** in 116 mL (66.6 g, 0.39 mol) of 1-iodopropane gave 15.4 g (yield, 79%) of **7**, mp 248-249 °C dec (lit.[12] mp 250 °C dec).

1-(3-Methoxy-2-nitrobenzyl)2-propyl-1,2,3,4-tetrahydroisoquinoline, 8. Reduction of 32 g of **7** with 4.8 g (0.089 mol) of potassium borohydride in absolute ethanol was carried out as described previously[12] to give 19.5 g (yield, 82%) of **8**, mp 95–97 °C (lit.[12] mp 94–95.5 °C).

1-(2-Amino-3-methoxybenzyl)-2-propyl-1,2,3,4-tetrahydroisoquinoline, 9. Hydrogenation of **8** was carried out in 1.9-g (5.6-mmol) batches. Thus to solution containing **8** dissolved in 125 mL of absolute ethanol was added 0.5 g of 10% Pd–C. The mixture was shaken at 50 psi of H_2 for 7 h. After removal of the catalyst by filtration, the ethanol was removed by rotary evaporation to yield 1.7 g (98%) of **9** as an oil, which showed only one spot on TLC. The oily product was used for further reaction without additional purification.

(*RS*)-11-Methoxy-*N*-*n*-propylnoraporphine, 10. Cyclization of 4 g of **9** was carried out as described previously[12] to give 2.7 g of **10** as an oil, which was purified by flash chromatography, eluting with a concentration gradient of 0.5–4.0% of methanol in methylene chloride. The desired compound **10** was converted to the hydriodide salt with 57% hydriodic acid in acetone to give 2.5 g (46.2%) of white crystals, mp 263–264 °C dec (lit.[12] mp 264 °C dec).

Resolution of (*RS*)-11-Methoxy-*N*-*n*-propylnoraporphine, 10. To a solution of 2 g (6.8 mmol) of (*RS*)-**10** as the free base, dissolved in 15 mL of ethyl acetate, was added a solution of 1.25 g (3.4 mmol) of (+)-dibenzoyl-D-tartaric acid [$[\alpha]^{20}_D$ +124° (*c* 1.2, EtOH)] dissolved in 15 mL of ethyl acetate. The mixture was heated to reflux for 1 h and then cooled, filtered, and washed with ethyl acetate to give 2.07 g of a white solid. This (+)-dibenzoyl-D-tartrate salt was recrystallized three times from a mixture of 2-propanol and absolute ethanol (1:1, v/v) to yield 600 mg of off-white crystals. Further recrystallization did not change the specific rotation of $[\alpha]^{25}_{578}$ +56.8 (*c* 0.08, *i*-PrOH). The crystalline salt was dissolved in a saturated aqueous solution of K_2CO_3, and the mixture was extracted with methylene chloride. After drying over $MgSO_4$, the extract was converted to the hydrochloride salt to give 320 mg (29%) of off-white crystals of (*R*)-(-)-**10**·HCl, mp 263–264 °C [$[\alpha]^{25}_{578}$ -79.3 (*c* 0.26, MeOH)].[20]

The combined mother liquors containing (*S*)-**10** were treated with saturated aqueous K_2CO_3 to liberate the free base, extracted with methylene chloride, and evaporated to dryness. The remaining 750 mg (2.5 mmol) of oil was dissolved in 10 mL of ethyl acetate and treated with 0.94 g (2.56 mmol) of (-)-dibenzoyl-L-tartaric acid [$[\alpha]^{20}_D$ -121.6 (*c* 1.5, EtOH)] to yield 1.65 g of a solid. Two recrystallizations gave 770 mg of (*S*)-**10** dibenzoyl-L-tartaric acid salt [$[\alpha]^{25}_{578}$ -56.6 (*c* 0.08, *i*-PrOH)], which was converted to the hydrochloride salt to give 450 mg (40%) of (*S*)-(+)-**10**·HCl, mp 263–264 °C [$[\alpha]^{25}_{578}$ +79.6 (*c* 0.2, MeOH)]; mass spectrum, *m/e* 293 (M^+). Anal. ($C_{20}H_{23}NO$·HCl·H_2O) C, H.

(*S*)-(+)-11-Hydroxy-*N*-*n*-propylnoraporphine Hydrochloride, (*S*)-3. To 200 mg (0.61 mol) of (*S*)-**10**·HCl dissolved in 5 mL of glacial acetic acid was added 5 mL of HBr (48% w/v). The reaction mixture was heated under an atmosphere of N_2 at 130 °C in an oil bath for 4 h and then allowed to cool under N_2. The solution was adjusted to basic pH condition with aqueous Na_2CO_3 solution, and the liberated free base was extracted from methylene chloride and converted to the hydrochloride salt to

yield 120 mg (yield 63%) of (S)-3·HCl: mp 257–258 °C $[[\alpha]^{25}_{578}$ +64.5 (c 0.11, MeOH)]; mass spectrum, m/e 279 (M^+). Anal. ($C_{19}H_{21}NO\cdot HCl\cdot H_2O$) C, H. (R)-3·HCl was obtained as described above from (R)-10·HCl, in a yield of 63%, mp 257–258 °C $[[\alpha]^{25}_{578}$ −64.0 (c 0.289, MeOH)].[20]

Determinations of Enantiomeric Purity. The enantiomeric excess of (R)-3 and (S)-3 was determined as follows: 10-mg samples of (R)-3 and (S)-3 were treated with 4 μL of triethylamine and extracted from ether. Each extract was evaporated to dryness with N_2 and allowed to react with triethylamine (1 μL) and (S)-(−)-α-methylbenzyl isocyanate (5 μL, Aldrich Chemical Co, Milwaukee, WI) for 5 h at room temperature. A sample of the reactive mixture was evaporated under a stream of N_2 and redissolved in 100 μL of mobile phase [0.05 M KH_2PO_4 containing 35% (w/v) CH_3CN, pH 3.0]. HPLC chromatographic separations were carried out with the mobile phase just described, and 0.2 μg of the derived carbamate was injected. The results of HPLC analyses showed that the diastereomeric excess of (S,R)-11 and (S,S)-11 were greater than 99%.

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Registry No. (S)-3, 114033-64-6; (S)-3·HCl, 113725-41-0; (R)-3, 88247-21-6; (R)-3·HCl, 114033-65-7; 6, 53055-08-6; 7, 53626-57-6; (±)-8, 114033-59-9; (±)-9, 114033-60-2; (±)-10, 113725-39-6; (±)-10·HI, 114033-61-3; (S)-10·HCl, 113678-77-6; (S)-10·dibenzoyl-L-tartrate, 114033-63-5; (R)-10·HCl, 83207-98-1; (SS)-11, 114033-67-9; (SR)-11, 114033-66-8; C_3H_7I, 107-08-4; (S)-$C_6H_5CH(CH_3)NCO$, 14649-03-7.

The Hypothetical Active Site Lattice. An Approach to Modelling Active Sites from Data on Inhibitor Molecules

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Microcomputer-assisted methods are described that allow the mathematical construction of a hypothetical active site lattice (HASL) which can model enzyme–inhibitor interactions and provides predictive structure–activity relationships for a set of competitive inhibitors. The inhibitor set can be structurally dissimilar, including acyclic and cyclic moieties normally refractory to classical parameter-based quantitative structure–activity relationship strategies. With use of three-dimensional Cartesian coordinates representing energy-minimized inhibitor conformations, a four-dimensional space-filling description is generated, wherein the fourth dimension can be a user-selected physiochemical property such as hydrophobicity or electron density. The multidimensional lattices thus generated are used to quantitatively compare molecules to one another. Composite lattices of more than one molecule are merged with binding data to form a HASL capable of predicting inhibitor binding and relative orientation. Details of the algorithms and assumptions utilized are illustrated for competitive inhibitors of yeast glyoxalase-I and *E. coli* dihydrofolate reductase.

Computer-assisted drug design is a constantly evolving area of inquiry that presently encompasses many distinctly unique approaches.[1-6] Some of these methods provide insight to active-site or receptor-binding requirements and include statistical techniques that yield quantitative relationships between structure and activity (QSAR) through the use of substituent-related physiochemical parameters,[7] pattern recognition techniques designed to classify bioactive compounds according to pharmacophores,[8-10] and discriminant analysis with fragment molecular connectivity to classify drugs.[11] In addition, considerable effort has been expended to relate bioactivity to molecular shape[12] or to occupied space by utilizing distance geometry methods that consider molecular conformational flexibility.[13] Although these methods represent powerful tools that provide insight to active site shape and binding requirements, they are necessarily biased since the results are based on arbitrarily chosen molecular overlays or binding-site points.

In the present investigation, a novel approach was formulated relying on a computer-assisted molecule to molecule match, which makes use of a multidimensional representation of inhibitor molecules. Furthermore, the results of such matching are used to construct a hypothetical active site by means of a lattice of points which is capable of modelling enzyme–inhibitor interactions. This technique is referred to herein as HASL.

Specifically, portions of the space occupied by an individual molecule are assigned parameter values corresponding to the physiochemical nature of the atom in that space. It is through such molecular four-dimensional (4D) representations that it becomes possible to quantify comparisons between different molecules. The information from selected structures is merged to yield a composite lattice of points (the HASL) which effectively captures the shape and binding properties of an active site. Quantitative predictions of inhibitor binding are obtained by means of computer-assisted fitting of molecules into the HASL. The power of the HASL approach is illustrated herein by the generation of enzyme/inhibitor models for yeast glyoxalase-I and *E. coli* dihydrofolate reductase.

Methods

Since the methods developed for the construction and use of a HASL are unique and somewhat complex, they will be presented in a stepwise format with appropriate

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