(*R*)-(-)-10-Methyl-11-hydroxyaporphine: A Highly Selective Serotonergic Agonist

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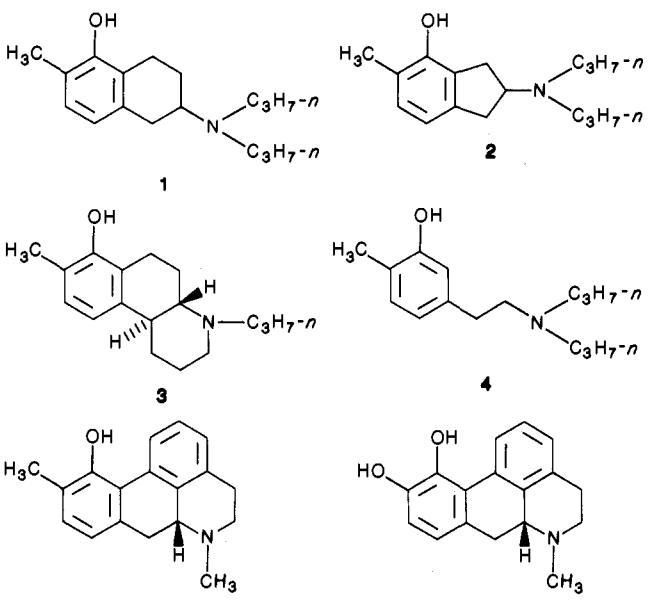
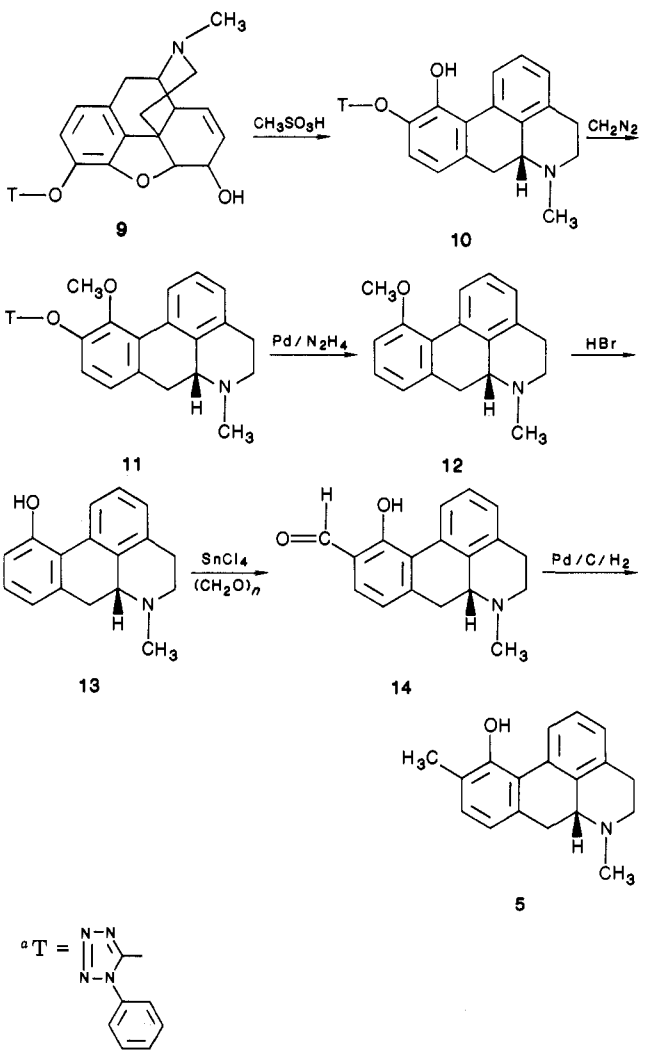
Prior work in these laboratories identified (±)-5-hydroxy-6-methyl-2-(di-*n*-propylamino)tetralin as a dopaminergic agonist prodrug. The ortho methyl hydroxy aromatic substitution pattern in this molecule has now been incorporated into the aporphine ring system to give a congener of the dopaminergic agonist apomorphine in which the position 10 OH group has been replaced by methyl. Preparation of the target compound involved acid-catalyzed rearrangement of the 3-(1-phenyltetrazolyl) ether of morphine and subsequent molecular modification of the product, the 10-(1-phenyltetrazolyl) ether of (*R*)-(-)-apomorphine. Surprisingly, the target compound elicited no responses in any assays for effects at dopamine receptors, but rather it displayed pharmacological properties consistent with its being a serotonergic agonist with a high degree of selectivity for 5-HT_{1A} receptors similar to the serotonergic agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin.

A series of previous papers[1a-d] described some pharmacological properties of congeners **1–4** of a number of dopaminergic agonists derived from several ring systems, in which the "para" hydroxy of a catechol moiety was replaced by a methyl group. The catechol congeners of all



of these derivatives are potent, highly active dopaminergic agonists.[2] Compounds **1–4** demonstrated few pharmacological properties in common. The tetralin derivative **1** is a relatively weak dopaminergic, but it is metabolically activated (by oxidation of the 6-methyl group) to a potent, active dopaminergic agonist;[1c] the indan system **2** is a weak dopaminergic agonist but apparently is not metabolically activated; the octahydrobenzo[*f*]quinoline derivative **3** is a moderately potent, short-acting DA_2 receptor *antagonist*; and the β-phenethylamine **4** is almost completely inactive as a dopaminergic agonist or an antagonist.[1d] In addition, compounds **2** and **3** produce behavioral responses in rats that were suggestive of involvement of serotonin receptors.[1d]

Scheme I. Preparation of (*R*)-(-)-10-Methyl-11-hydroxyaporphine[a]



The remarkable variation of pharmacologic actions in compounds **1–4** suggested possible interest in incorporating

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a 10-methyl, 11-hydroxy moiety into an aporphine ring system (structure **5**), to afford a congener of the potent, active, catechol-derived, dopaminergic agonist apomorphine.

Chemistry

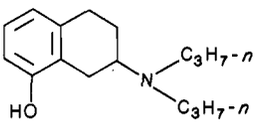
Preparation of the target compound **5** is illustrated in Scheme I. 11-Hydroxyaporphine (**13**) was prepared by a modification of a sequence of Ram and Neumeyer.[3] In the present work, acid-catalyzed rearrangement of the 3-(phenyltetrazolyl) ether of morphine **9** gave rise, in addition to the desired product **10**, to a second product, which was subsequently shown to be an aporphine derivative. This material could not be separated from the desired (and expected) phenolic product **10** by recrystallization or by chromatographic techniques. Treatment of the mixture with diazomethane permitted isolation of the methyl ether **11** of the desired aporphine product. The chemistry of the aporphine byproduct will be the subject of a future, separate paper. In the present study, the literature[3] method of reductive removal of the C-10 phenolic oxygen from the phenyltetrazolyl ether (**11** → **12**) using catalytic hydrogenolysis was undependable and frequently provided poor yields or failed completely. A preferrable procedure[4,5] utilized catalytic hydrogen transfer with hydrazine hydrate as the hydrogen donor. Attempted ortho-formylation of **13** by a method of Casiraghi et al.,[6] utilizing a Grignard reagent and paraformaldehyde, was unsuccessful. However, use of these authors' modification of their method utilizing stannic chloride and paraformaldehyde[7] led to the desired product, **14**. Spectral (IR, NMR, and MS) data for all intermediate products and for the target molecule were consistent with the proposed structures.

Results and Discussion

Electrical stimulation of the postganglionic fibers of the right cardioaccelerator nerve in the cat leads to a tachycardia, which is inhibited by dopaminergic DA_2 agonists[8,9] but not by the α_2 receptor agonist clonidine.[10] This inhibition is blocked by dopamine receptor agonists but not by α receptor antagonists,[8,9] and it does not involve antagonism of β receptors.[11] Thus, the cat cardioaccelerator nerve preparation was used to evaluate test compounds for dopamine receptor agonism.

Despite the close structural similarity between **5** and apomorphine, **5** did not show any apomorphine-like dopaminergic effects on the cat cardioaccelerator nerve at doses up to 10 times the dose of apomorphine required to produce a 50% inhibition of tachycardia. Electrical stimulation of the cardioaccelerator nerve led to an increase in heart rate of 17–40 beats/min above a base-line level of 110–150 beats/min, depending upon the particular animal. Apomorphine (10 μg/kg) decreased the response to electrical stimulation by approximately 50% ($p < 0.01$). Although multiple regression analysis suggested slight inhibition of the tachycardia by **5**, this effect was small (16%) and was neither significant ($0.1 > p > 0.05$) nor dose-dependent over the range of doses from 10 to 100 μg/kg ($p > 0.2$). Thus, it is unlikely that this slight trend reflects a real effect of **5** on dopamine receptors.

However, the pharmacological effects of **5** were remarkably similar to those of 8-hydroxy-2-(di-*n*-propylamino)tetralin (**6**), a serotonergic agonist.[12] In the present



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study, **6** was inactive in the cat cardioaccelerator nerve assay for dopaminergic effect.

Binding of [^{3}H]-**6** to rat cortical membranes was found to be saturable, to be linearly dependent upon protein concentration, and to have a dissociation constant (K_D) of approximately 1.9 nM, both in saturation experiments (1.9 nM, 95% CI = 1.5–2.4 nM) and in association kinetics experiments (1.8 nM, 95% CI = 0.9–7.0 nM). The aporphine-derived target compound **5** shifted the [^{3}H]-**6** saturation curve to the right in a parallel fashion, suggesting competition for the same binding site. Competition studies yielded a dissociation constant for **5** of 3.7 nM (95% CI = 2.1–6.5 nM).

The synthesis of a variety of neurotransmitters seems to be inhibited by feedback mediated by the receptors activated by those neurotransmitters. Compound **6** and LSD-*d* inhibit serotonin synthesis,[13] as does inhibition of serotonin uptake with chlorimipramine.[14] Reduction in the number of serotonin binding sites with kainic acid leads to an increase in serotonin turnover.[15] Thus, decreased serotonin turnover was used to indicate possible serotonin receptor agonism. A change in concentration of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin, reflects changes in serotonin turnover.[15] To account for the variation in 5-HIAA content due to variation in steady-state levels of serotonin, the 5-hydroxytryptamine (5-HT)/5-HIAA ratio better reflects the serotonin turnover.

The decrease in 5-hydroxytryptamine turnover when rats were pretreated with either **5** or **6** is shown in Table I. Two-way analysis of variance showed a significant dependence of 5-HT concentration on drug treatment ($p < 0.001$) and on brain region ($p < 0.001$) but no interaction between the two variables ($p > 0.2$). Multiple regression analysis showed a significant elevation in 5-HT concentration both when pretreatment with compound **5** was compared to control ($p < 0.001$) and when **6** was compared to control ($p < 0.05$). Similarly, the 5-HIAA concentration and the 5-HT/5-HIAA ratio each depended upon both

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Table I. Influence of **5** and **6** on 5-HT and 5-HIAA Concentrations (nmol/g) in Various Brain Regions

regions	control (*n*)	compound **5** (*n*)	compound **6** (*n*)
5-HT			
caudate (C)	3.3 ± 0.3 (8)	4.4 ± 0.3 (7)	3.6 ± 0.5 (5)
olfactory tubercle (OT)	8.7 ± 0.4 (8)	9.1 ± 0.3 (8)	8.3 ± 0.3 (5)
frontal cortex (FC)	3.7 ± 0.2 (8)	4.4 ± 0.1 (7)	4.2 ± 0.2 (5)
parietal cortex (FC)	2.8 ± 0.3 (8)	4.1 ± 0.2 (7)	3.1 ± 0.4 (5)
hippocampus (Hip)	3.1 ± 0.2 (8)	4.9 ± 0.6 (7)	4.2 ± 1.1 (5)
septum (Sep)	4.3 ± 0.6 (5)	4.5 ± 0.2 (7)	4.5 ± 0.7 (5)
amygdala (Am)	4.5 ± 0.5 (5)	5.1 ± 0.1 (7)	4.9 ± 0.4 (5)
hypothalamus (Hypo)	6.3 ± 0.5 (5)	6.7 ± 0.2 (7)	7.1 ± 0.4 (5)

analysis of variance results			individual treatment effects			
source	*F*	*p*	treatment	estimated effect[a]	*F*	*p*
treatment	10.32	<0.0001	**5**	+0.86	21.76	<0.0001
brain region	67.54	<0.0001	**6**	+0.47	5.46	<0.05
interaction	0.93	>0.2				

regions	control (*n*)	compound **5** (*n*)	compound **6** (*n*)
5-HIAA			
C	2.7 ± 0.2 (8)	2.9 ± 0.2 (7)	2.6 ± 0.5 (5)
OT	2.2 ± 0.2 (8)	1.5 ± 0.1 (7)	1.4 ± 0.6 (5)
FC	1.0 ± 0.1 (8)	0.9 ± 0.1 (7)	0.8 ± 0.2 (5)
PC	0.9 ± 0.1 (8)	1.0 ± 0.1 (7)	0.8 ± 0.1 (5)
Hip	1.6 ± 0.1 (7)	1.4 ± 0.2 (7)	1.7 ± 0.3 (5)
Sep	1.7 ± 0.1 (5)	1.3 ± 0.1 (7)	1.5 ± 0.2 (5)
Am	1.4 ± 0.1 (4)	1.0 ± 0.1 (7)	1.0 ± 0.2 (5)
Hypo	2.6 ± 0.5 (5)	1.6 ± 0.1 (7)	1.9 ± 0.2 (5)

analysis of variance results			individual treatment effects			
source	*F*	*p*	treatment	estimated effect[a]	*F*	*p*
treatment	7.37	<0.001	**5**	−0.29	10.96	<0.05
brain region	33.63	<0.0001	**6**	−0.28	8.82	<0.005
interaction	1.25	>0.2				

regions	control (*n*)	compound **5** (*n*)	compound **6** (*n*)
5-HT/5-HIAA ratio			
C	1.2 ± 0.1	1.5 ± 0.1	1.5 ± 0.5
OT	4.1 ± 0.3	6.1 ± 0.5	5.9 ± 0.4
FC	3.5 ± 0.3	5.2 ± 0.5	5.8 ± 1.1
PC	2.9 ± 0.2	4.1 ± 0.3	4.1 ± 0.6
Hip	1.9 ± 0.2	3.6 ± 0.7	2.5 ± 0.5
Sep	2.4 ± 0.2	3.6 ± 0.3	3.0 ± 0.4
Am	3.1 ± 0.2	5.5 ± 0.5	5.8 ± 1.3
Hypo	2.8 ± 0.3	4.0 ± 0.2	3.9 ± 0.4

analysis of variance results			individual treatment effects			
source	*F*	*p*	treatment	estimated effect[a]	*F*	*p*
treatment	27.40	<0.0001	**5**	+1.46	46.09	<0.0001
brain region	25.82	<0.0001	**6**	+1.30	30.57	<0.0001
interaction	1.16	>0.2				

[a] Regression coefficient for drug effect compared to control.

pretreatment and brain region with no interaction. The 5-HIAA concentration was significantly decreased from control with pretreatment with either **5** or **6**, and the 5-HT/5-HIAA ratio was significantly increased with either pretreatment. Thus, **5** and **6** decreased the turnover of 5-HT.

Compound **6** has been shown to be highly selective for 5-HT_{1A} binding sites.[16] The parallel shift of the [^{3}H]-**6** saturation curve by **5** suggests competition for the same binding site. The competition data suggest that **5** and **6** bind with comparable affinity to this site. Furthermore, these two compounds decreased 5-HT turnover in the brain, suggesting that both are agonists at the 5-HT_{1A} receptor. Thus, it appears that replacement of the hydroxyl group in the 10-position of apomorphine with a methyl group drastically reduces or abolishes DA_2 receptor agonism but introduces 5-HT_{1A} receptor agonism.

Analysis of molecular models does not reveal obvious structural similarities between the aporphine derivative **5** and 8-hydroxy-2-(di-*n*-propylamino)tetralin (**6**), which would account for their qualitative and quantitative pharmacological similarity at serotonin receptors. Distances between the basic nitrogen atom and the phenolic moiety (considered to be an important structural parameter in a variety of types of neurotransmitter molecules) are dissimilar in the two molecules. Attempts to superimpose the two molecules upon each other or upon the serotonin molecule do not reveal coincidence of aromatic ring(s), basic nitrogen, and phenolic OH groups, moieties which seem likely to be of prime importance in agonist–receptor interactions. Structure–activity correlations are further complicated by the finding[12] that both enantiomers of **6** have high serotonergic potency and activity. The aporphine derivative **5** is an optically pure enantiomer, having the same absolute configuration as apomorphine.

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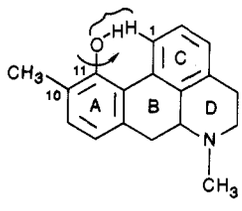
On the basis of present knowledge, it does not seem possible to present hypotheses to explain the similar serotonergic activities of **5** and **6**, nor is it possible to explain the weak serotonergic effects of the indan derivative **2** and of the octahydrobenzo[*f*]quinoline derivative **3**.

A related problem addresses a structural rationalization for the complete lack of dopaminergic effect of the aporphine derivative **5**. The "meta" hydroxyl group of catechol-derived dopaminergic agonists has been cited[2] as having an important role in agonist–receptor interactions, and van de Waterbeemd et al.[17] have postulated that a conformation in which the "meta" OH of dopamine is coplanar with the aromatic ring is an important factor in the recognition process between the neurotransmitter and its receptor.

Studies of Dreiding models as well as of space-filling models strongly suggest that the ring proton at position 1 of the aporphine ring system offers considerable steric opposition to the assumption by the C-11 OH of coplanarity with the A ring when the OH is in one rotameric disposition (structure **7**), and, similarly, the physical bulk of the C-10 methyl group presents steric hindrance to coplanarity of the OH group with the A ring when the OH group is in the alternate rotameric disposition (structure **8**). Inspection of molecular models suggests that the least



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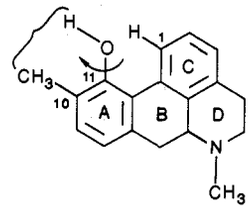

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sterically unfavored disposition of the OH group in 10-methyl-11-hydroxyaporphine (**5**) is one in which the plane of the OH is approximately perpendicular to the plane of the A ring. It may be speculated that such steric disposition of the "meta" OH group does not permit effectual interaction with the proper subsite(s) on the dopamine receptor(s), and hence this aporphine system is dopaminergically inert. Analysis of conformations of **5** using the CHEMGRAF (Chemical Design, Ltd.) molecular modeling–computer graphics program led to the conclusion that the phenolic OH of **5** exists in very high energy conformations when it is coplanar with the A ring. However, a low-energy conformation exists in which the OH group is out of the plane of the A ring. The program utilizing data based upon van der Waals radii of the atoms suggested that the plane of the OH group makes a torsion angle of approximately 120° with the plane of the A ring of the aporphine system.

NMR data support the conclusions derived from study of molecular models and computer-based molecular modeling. In the present study, the signal for the phenolic proton in 11-hydroxyaporphine (**13**) appeared at δ 10.03; the signal for the phenolic proton in 10-methyl-11-hydroxyaporphine appeared upfield at δ 8.76. This latter phenolic proton is considerably more shielded; the position of its signal is interpreted as indicating that the OH group is held above or below the plane of the aromatic A ring, not coplanar with it, as contrasted with the OH group in 11-hydroxyaporphine. Solvent and concentration effects on the position of the phenolic proton signal were minimized by using the same solvent for both compounds and the same concentrations of solutions.

It is proposed that the dopaminergic inactivity of 10-methyl-11-hydroxyaporphine is due to the inability of its "meta" OH to assume coplanarity with the aromatic ring to which it is attached; this proposal, if valid, lends powerful support to the concept[17] that an aromatic ring coplanar conformation of the "meta" OH of dopamine (and of other phenol-substituted dopaminergic agonists) is a critical factor in agonist–receptor recognition and/or interaction(s).

It should be noted that the o-methyl phenolic molecules cited previously (structures **1–4**) lack a bulky region equivalent to the C ring of the aporphine system. Thus, in these molecules there is no barrier to assumption by the "meta" OH of a conformation coplanar with the aromatic ring. Compounds **1–3** were cited as having some effect (agonist or antagonist) at dopamine receptors; the inactivity of the simple β-phenethylamine derivative **4** remains unexplained.

Experimental Section

Pharmacology. Methods. Competition for [^{3}H]-8-Hydroxy-2-(di-*n*-propylamino)tetralin (6) Binding Sites. The procedure was very similar to that of Peroutka and Snyder,[18] except that [^{3}H]-**6** was used as the radioligand. Whole cortex from adult rats was homogenized in sucrose solution (20:1 v:w) and was centrifuged for 10 min at 700*g*. The supernatant was then centrifuged at 20000*g* for 10 min, and the pellet from the second centrifugation was resuspended in 50 mM Tris buffer, pH 7.5, and was incubated for 10 min at 37 °C. After another centrifugation at 20000*g*, the pellet was resuspended in Tris buffer containing 10 μM pargyline, 0.1% ascorbic acid, and 4 mM $CaCl_2$. It was incubated with appropriate concentrations of [^{3}H]-**6** (Amersham, specific activity 185–223 Ci/mmol) and competitor for 15 min. The assay mixture was then poured over Whatman GF/B filters and washed three times with ice-cold buffer. Filters were placed in vials with Beckman Ready-Solv scintillation cocktail overnight and then were analyzed in a Beckman LS 4301 scintillation counter. Nonspecific binding was defined as binding remaining in the presence of 10 μM 5-HT. Data were analyzed by weighted nonlinear least-squares curve fitting with the LIGAND program.[19] Association kinetic experiments were done similarly except that aliquots were pipetted from a single assay mixture at measured time intervals and were filtered as described above. Data from these experiments were analyzed by a weighted nonlinear least-squares program written to allow for simultaneous analysis of all kinetic experiments.

Determination of 5-HT and 5-HIAA in Rat Brain. Male Sprague–Dawley rats (150–250 g) received 4 mg/kg of drug sc. This dose was selected for both compounds because either compound at this dose consistently produced behavioral effects such as reciprocal forepaw trading suggestive of serotonergic activation, and the binding studies suggested that the compounds were of comparable potency. The rats were sacrificed 30 min later. The brain was removed, and selected regions were dissected out: olfactory tubercle (OT), frontal (FC) and parietal (PC) cortex, caudate (C), septum (Sep), amygdala (Am), hippocampus (Hip), and hypothalamus (Hypo). Tissue samples were weighed and homogenized in 0.01 N $HClO_4$. Samples were allowed to stand on ice for 15 min and then were centrifuged at 13000*g* for 2 min. Supernatant (100 μL) was injected into the HPLC column for determination of 5-HT and 5-HIAA concentrations. The HPLC-EC determination of 5-HT and 5-HIAA was based upon the method of Nielson and Johnston[20] and Mefford et al.,[21] with modifications. The mobile phase buffer was 0.1 M sodium phosphate buffer with 0.312 g/L of EDTA, 0.150 g/L of 1-octanesulfonic acid, and 10% MeOH. The pH of the buffer was adjusted to 3.8 with phosphoric acid. The column used was a 25-cm C-18, BAS reverse-phase column. Oxidation was achieved by using a glassy carbon electrode with the potential set at +0.65

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V with respect to the Ag/AgCl reference electrode. Oxidation was monitored with a BAS LC-4B amperometric detector. Peak heights of known standards were used in the quantification of tissue levels. Data were analyzed initially by two way analysis of variance. In all cases, there were significant treatment effects and no significant interaction effects, so multiple regression analysis with treatment as one set of independent variables and brain region as another set were used to compare the individual effects of **5** and **6** with control.

Cat Cardioaccelerator Nerve Stimulation. Cats (2–3 kg) of either sex were anesthetized with pentobarbital sodium (30 mg/kg, ip). The right femoral artery was cannulated for arterial pressure and heart rate measurement with a Statham pressure transducer and a Beckman cardiotachometer. The left femoral vein was cannulated for drug administration. A bilateral vagotomy was performed, and the right postganglionic cardioaccelerator nerves were attached to bipolar electrodes. Electrical stimulation consisted of 5-ms pulses at 2 Hz and 25 V. Since the response to cardioaccelerator nerve stimulation clearly depended upon the prestimulation heart rate, data were analyzed by multiple linear regression analysis with prestimulation heart rate as one independent variable and log dose as another.

Chemistry. Melting points were determined in open glass capillaries with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are indicated by the symbols of the elements, analytical results were within ±0.4% of the theoretical values. NMR spectra were recorded on a Varian Associates EM 360A spectrometer and on a Brucker–IBM NR80 instrument. Mass spectra were recorded with a Ribermag R10-10C mass spectrometer. Qualitative HPLC analyses were performed with a Beckman 110A pump and Model 336 detector and an Altex 210 injector.

(*R*)-10-*O*-(1-Phenyltetrazol-5-yl)apomorphine (10). 3-*O*-(1-Phenyltetrazol-5-yl)morphine (**9**)[3] (1.29 g, 2.78 mmol) was heated with 4.2 mL of 98% methanesulfonic acid at 90–95 °C for 1 h. The cooled reaction mixture was neutralized with 10% $NaHCO_3$, and the aqueous solution was extracted with four 25-mL portions of $CHCl_3$. The pooled extracts were washed with two 25-mL portions of H_2O, and then they were dried (Na_2SO_4) and filtered, and the filtrate was taken to dryness under reduced pressure. The green solid residue (1.13 g, 99%) was chromatographed on a short silica gel column and was eluted with Me_2CO to afford, after removal of solvent and drying under reduced pressure, 0.974 g of green crystals. A 0.75-g portion of this material was chromatographed on silica gel and was eluted with hexane–EtOAc–Me_2CO (7:5:3) to yield a solid, which was recrystallized from 50% EtOH to afford 0.400 g of a green powder, mp 132–134 °C dec. HPLC analysis (0.2 mg of sample in 0.75 mL of $CHCl_3$, 10-μL injections, Alltech silica gel column, $CHCl_3$ solvent flow rate 2.0 mL/min) of this material indicated that it contained two major components, of which (as was subsequently shown) the desired product **10** was the major one. Efforts to attain efficient separation of this mixture were not successful, and the mixture was used in the next step without further treatment.

(*R*)-(-)-10-*O*-(1-Phenyltetrazol-5-yl)-11-methoxyaporphine (11). The mixture resulting from preparation of **10** (1.1 g, 2.68 mmol) was added in one portion to an ethereal solution containing ca. 3 g (71 mmol) of CH_2N_2, and the resulting mixture was stirred at room temperature for 24 h. Excess CH_2N_2 was destroyed with AcOH, the resulting solution was filtered, and the filtrate was evaporated under reduced pressure to yield a brown oil. This material was treated with excess 10% $NaHCO_3$ solution, and the resulting mixture was extracted with four 30-mL portions of $CHCl_3$. The pooled organic extracts were washed twice with 40-mL portions of H_2O, dried (Na_2SO_4), and filtered, and the filtrate was evaporated under reduced pressure. TLC analysis of the residue (silica gel; hexane–EtOAc–Me_2C, 7:5:3) revealed the presence of two components, one with R_f 0.31 (major component) and one with R_f 0.17 (minor component). The residue mixture was subjected to chromatographic separation by means of a Chromatotron apparatus (silica gel; hexane–EtOAc–Me_2CO, 7:5:3). One eluate fraction contained material corresponding to the component with TLC R_f 0.31. This eluate was taken to dryness under reduced pressure to provide a yellow crystalline residue, which was recrystallized from 50% EtOH to afford 0.87 g (65%) of **11** as fine white needles: mp 153–154 °C; MS, *m/e* 425 (M^+); $[\alpha]^{23.9}_{578}$ −115.4° (*c* 0.92, MeOH).[22] Anal. ($C_{25}H_{23}N_5O_2$) C, H, N.

A second eluate fraction from the Chromatotron treatment corresponded to the R_f 0.17 material observed in the TLC analysis (vide supra). This material was treated as described above for **11** and provided 0.140 g of an off-white powder: mp 148–150 °C; MS, *m/e* 425; $[\alpha]^{23.9}_{578}$ +47.0° (*c* 0.98, MeOH).

(*R*)-(-)-11-Methoxyaporphine Hydrochloride (12). Compound **11** (0.78 g, 1.8 mmol) and 0.5 g of 5% Pd/C were vigorously stirred in 62 mL of a mixture of benzene–EtOH–H_2O (7:3:2), and 8 mL of 64% (v/v) hydrazine hydrate (prepared by diluting 85% hydrazine hydrate with H_2O) was added dropwise. The reaction mixture was then heated under gentle reflux for 4 h. The cooled reaction mixture was filtered through Celite, and the filtrate was taken to near dryness under reduced pressure. The residue was diluted with 75 mL of H_2O, and this mixture was extracted with four 50-mL portions of $CHCl_3$. The pooled organic extracts were washed with two 60-mL portions of H_2O, dried (Na_2SO_4), and filtered, and the filtrate was taken to dryness under reduced pressure. The residue (0.6 g) was subjected to chromatographic separation on a Chromatotron apparatus (silica gel; hexane–EtOAc–Me_2CO, 7:5:3) to afford 0.27 g (56%) of **10**. This material was treated with ethereal HCl to provide a beige powder, which was washed with copious amounts of Et_2O and dried for several hours under reduced pressure: mp 246–248 °C dec (lit.[3] mp 235–242 °C dec); MS, *m/e* 265 (M^+ − HCl); $[\alpha]^{26.7}_{578}$ −85.2° (*c* 0.55, MeOH) [lit.[3] $[\alpha]^{22}_{578}$ −74.9° (*c* 0.0494, MeOH)]. Anal. ($C_{18}H_{20}ClNO$) C, H, N.

(*R*)-(-)-11-Hydroxyaporphine (13). Compound **12** (0.150 g, 0.50 mmol) was heated in 3 mL of 48% HBr at 120 °C for 4 h. The reaction mixture was then allowed to come to room temperature, and the solid material that separated was collected on a filter and was washed with Et_2O to give 0.142 g (86%) of a gray powder, mp 293–294 °C (lit.[3] mp 280–281 °C). This material was treated with excess 10% $NaHCO_3$, and the resulting aqueous mixture was extracted with three 15-mL portions of $CHCl_3$. The combined organic extracts were washed with two 15-mL portions of H_2O, dried (Na_2SO_4), and filtered, and the filtrate was evaporated under reduced pressure. The residue (0.117 g, 94%) was **13**: mp 232–234 °C; MS, *m/e* 251 (M^+); $[\alpha]^{25}_{578}$ −71.2° (*c* 0.52, MeOH); NMR (HCl salt, 0.005 g in 0.4 mL of Me_2SO-d_6) δ 10.03 (s, 1 H, O*H*, exchanged with D_2O). Anal. ($C_{17}H_{17}NO$) C, H, N.

(*R*)-(-)-10-Formyl-11-hydroxyaporphine Hydrochloride (14). A method of ortho-formylation of Casiraghi et al.[7] was employed. To an efficiently stirred suspension of 0.54 g (2.15 mmol) of **13** in 25 mL of freshly distilled toluene was added 178 μL (0.163 g, 1.34 mmol) of 2,4,6-collidine. After 30 min, 324 μL (0.72 g, 2.77 mmol) of anhydrous $SnCl_4$ was added, and the resulting mixture was stirred for 20 min. Paraformaldehyde (0.27 g, 9.0 mmol) and 23 mL of toluene were added, and the resulting mixture was heated at 100 °C for 8 h. The yellow-green reaction mixture was cooled to ambient temperature and acidified with 10% HCl, and the resulting mixture was stirred for 10 min. This mixture was then treated with excess 10% $NaHCO_3$ and was extracted with six 30-mL portions of Et_2O and then with three 30-mL portions of $CHCl_3$. The pooled extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The brown residue (0.67 g) was chromatographed on silica gel and eluted with EtOAc–95% EtOH (1:1) to afford (after removal of volatiles) 0.125 g of a white solid, which was taken up in MeOH and treated with ethereal HCl. The solid that separated was recrystallized from MeOH–Et_2O to provide 0.110 g (14%) of a finely divided powder: mp 260–262 °C dec; MS, *m/e* 279 (M^+ − HCl); $[\alpha]^{23.8}_{578}$ −103.2° (*c* 0.33, MeOH). Anal. ($C_{18}H_{18}ClNO_2$) C, H, N.

(*R*)-(-)-10-Methyl-11-hydroxyaporphine Hydrochloride (5). The free base of **14** (0.142 g, 0.51 mmol) was hydrogenated at 50 °C for 26 h in 45 mL of EtOH and 11 drops of concentrated HCl over 0.227 g of 5% Pd/C at an initial pressure of 45 psig. The reduction mixture was filtered through Celite, and the filtrate

(22) Ram and Neumeyer, ref 3, characterized this compound as the HCl salt and listed no data for the free base.

was evaporated to dryness under reduced pressure. The residue was taken up in anhydrous EtOH, and Et_2O was added to the cloud point. On prolonged cooling, the solution deposited crystals, which were collected on a filter, washed with anhydrous Et_2O, and dried under reduced pressure. Two additional recrystallizations provided 0.090 g (59%) of material: mp 270–272 °C dec; MS, m/e 265 (M^+ – HCl); $[\alpha]^{26.7}_{578}$ –85.2° (c 0.55, MeOH); NMR (0.005 g in 0.4 mL of Me_2SO-d_6) δ 8.76 (s, 1 H, O*H*, exchanged with D_2O). Anal. ($C_{18}H_{20}ClNO$) C, H, N.

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Registry No. **5**, 111635-19-9; **5**·HCl, 111635-21-3; **9**, 55592-66-0; **10**, 40169-97-9; **11**, 40169-98-0; **12**·HCl, 83207-97-0; **13**, 88247-20-5; **13**·HBr, 83247-89-6; **14**, 111635-20-2; **14**·HCl, 111635-22-4.

(Acyloxy)alkyl Carbamates as Novel Bioreversible Prodrugs for Amines: Increased Permeation through Biological Membranes

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(Acyloxy)alkyl carbamates of the type R_1R_2N–CO–O–CHR_3–OCO–R_4 are described as novel bioreversible prodrugs for primary and secondary amines. These were prepared either by a one-step reaction involving nucleophilic attack on *p*-nitrophenyl α-(acyloxy)alkyl carbonates with displacement of *p*-nitrophenol or by reaction of α-haloalkyl carbamates with silver or mercury salts of carboxylic acids. Enzymatic hydrolysis of the ester bond in these ester carbamates leads to a cascade reaction resulting in rapid regeneration of the parent amine. Permeability measurements of such nonionic derivatives of atenolol, betaxolol, pindolol, propranolol, and timolol through fuzzy rat skin and rabbit cornea mounted on diffusion cells show that derivatization of the hydrophilic β-blockers results in several-fold increase in permeation through these biological membranes. However, prodrug modification of the lipophilic β-blockers leads to little advantage in permeability characteristics.

Chemical modification of drugs into labile derivatives (prodrugs[1]) with improved physicochemical properties that enable better transport through biological barriers is a useful approach for improving drug delivery. Such transformation is practiced more often on ionizable molecules such as carboxylic acids[2] and amines in order to modify their ionization at physiological pH and to render desirable partition and solubility properties. Amino functional drugs have been converted[3] in the past to amides,[4] enamines,[5] Schiff bases,[6] oxazolidines,[7] Mannich bases,[8] hydroxymethyl derivatives,[9] *N*-(acyloxy)alkyl derivatives,[10] and carbamates[11] for these purposes. A necessary requirement of this approach is the capability of the prodrug to revert to the parent drug in the body by enzymatic or chemical action, quantitatively, and at a desirable rate. Many of the above amino derivatives are relatively stable to enzymatic hydrolysis and have to depend on acid or base catalysis for reconversion to the parent amines. In addition to this lack of adequate control in regeneration, some of the above methods are limited in their application only to amines with specific features such as low basicity, or with structural requirements such as mono- or trisubstitution on the nitrogen, or the presence of another neighboring functional group (such as hydroxyl).

We chose to study carbamylation as a means of prodrug modification since no bioreversible, chemically stable, and generally applicable prodrug strategy exists for primary and secondary amines. A priori carbamates have the following advantages: (a) carbamylated amines do not ionize and hence are more compatible with organic and lipoidal systems, (b) the modification is applicable to primary and secondary amines essentially irrespective of basicity, (c) there is potential for chemical selectivity in the presence of competing functionalities such as hydroxyl, and (d) they are chemically stable. However, success with a carbamate as a latentiating group requires that the prodrug by hydrolyzed in the body to an alcohol and a carbamic acid. The latter is unstable under physiologic pH and decomposes to the parent amine and carbon dioxide. Though several carbamate prodrugs with simple alcohol leaving groups in the carbamate moiety have been described in literature,[11] the approach has not often met

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