Preparation and Pharmacological Evaluation of Enantiomers of Certain Nonoxygenated Aporphines: (+)- and (-)-Aporphine and (+)- and (-)-10-Methylaporphine

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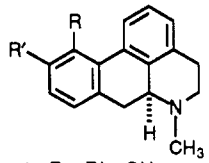
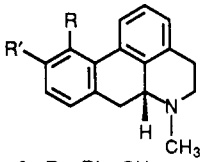
The subject compounds were prepared as a part of a continuing structure–activity study of the contrasting actions (agonism–antagonism) of (+)- and (-)-11-hydroxy-10-methylaporphine at serotonin (5-HT_{1A}) receptors. None of the targeted nonoxygenated aporphine derivatives demonstrated significant activity in assays for any effects at serotonin 5-HT_{1A} receptors. It is concluded that, in the aporphine series, serotonergic agonist or antagonism requires an alkyl group ortho to a phenolic OH group in the A ring.

The literature reveals examples of aporphine ring derivatives in which enantiomers display the relatively rare property of manifesting opposite pharmacological effects at the same receptor. Thus, (*S*)-(+)-apomorphine (**1**) is an antagonist at dopaminergic D-1 and D-2 receptors, where (*R*)-(-)-apomorphine (**2**) is an agonist.[1] (*R*)-11-Hydroxyaporphine (**3**) activates dopamine receptors, whereas the (*S*)-enantiomer **4** shows activity as a dopaminergic antagonist.[2] Neither enantiomer displayed actions consistent with effects at serotonin 5-HT_{1A} receptors in the isolated guinea pig ileum preparation.[3] Work from

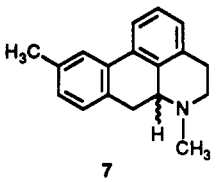


1: R = R′ = OH
4: R = OH; R′ = H
6: R = OH; R′ = CH_3



2: R = R′ = OH
3: R = OH; R′ = H
5: R = OH; R′ = CH_3

our laboratories[4,5] has demonstrated that (*R*)-(-)-11-hydroxy-10-methylaporphine (**5**) is a potent agonist at 5-HT_{1A} receptors, whereas the (*S*)-(+)-enantiomer **6** is a potent 5-HT_{1A} antagonist. Neither enantiomer demonstrated significant activity in assays for effects at dopamine receptors. (*RS*)-11-Hydroxy-10-methylaporphine demonstrated almost no pharmacological activity at 5-HT_{1A} receptors;[3] the two enantiomers nullify each other's effects. The remarkable difference in receptor specificity exhibited by the 11-hydroxyaporphines **3** and **4** (activity at dopamine receptors, but inactivity at serotonin 5-HT_{1A} receptors) and the 11-hydroxy-10-methylaporphines **5** and **6** (activity at serotonin 5-HT_{1A} receptors but inactivity at dopamine receptors) suggests an unusually significant role for the 10-methyl group in **5** and **6** in determining site of pharmacological effect. Weisbach et al.[6] reported routine screening data indicating that large doses of (*RS*)-10-methylaporphine (**7**) showed slight central nervous system (CNS) depressant effects in mice and some antiinflammatory activity in rats. The compound also showed some hypotensive effect in the cat. Possible dopaminergic and/or serotonergic effects were not addressed. To study further the pharmacological significance of the combination of C-10 methyl group and the C-11 hydroxyl group on the aporphine ring, we targeted for preparation the enantiomers of 10-methylaporphine (**7**).



7

8

The unsubstituted aporphine ring compound **8** was reported[7] to be inert in an assay for cyclic AMP production in rat striatal tissue, an assessment of dopaminergic effect. The stereochemistry of the compound used in this study was not specified, and the present work was based on the assumption that these pharmacological data were obtained using the racemate. Weisbach et al.[6] reported routine screening data on (*RS*)-**8** revealing CNS depressant effect in mice, modest ability to elevate the pain threshold in the rat, and weak hypotensive action in the cat. A search of the literature has not revealed that aporphine **8** has been resolved, and effects of aporphine at serotonin receptors have not been determined. Continued interest in comparison of the pharmacological properties of members of enantiomeric pairs stimulated preparation of (+)- and (-)-aporphine (**8**) as a part of our comprehensive study of aporphine derivatives having effects at dopamine and serotonin receptors.

Chemistry. (*RS*)-Aporphine (**8**) was prepared by modification of combinations of routes used by Gadamer et al.[8] and by Weisbach et al.[6] for this compound. (*RS*)-10-Methylaporphine (**7**) was prepared from (*RS*)-11-hydroxy-10-methylaporphine[5] by removal of the aromatic hydroxyl group via hydrogenolysis of its 1-phenyltetrazolyl ether. Alternately, (*RS*)-10-methylaporphine (**7**) could be prepared from 2-nitro-*p*-xylene by the modified literature sequence described in detail the Experimental Section for (*RS*)-**8**. Resolution of racemic **7** and **8** was accomplished by diastereomeric salt formation using (+)- and (-)-di-*p*-toluoyltartaric acids. No attempt was made to determine absolute configurations of the isolated enantiomers.

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Table I. Inhibition by Aporphine Derivatives of Field Stimulation-Induced Contractions in Guinea Pig Ileum

compd no.	ID_{50}, μM (95% confidence limits)[c]	compd no.	ID_{50}, μM (95% confidence limits)[c]
(−)-7[a]	1.2 (0.8–2.1)	(+)-8	5.4 (3.7–8.1)
(+)-7	6.8 (3.9–28.9)	(±)-8-OH DPAT[b]	0.06 (0.01–0.08)
(−)-8	6.7 (3.2–10.8)		

[a] All compounds were tested as their HCl salts. [b] (±)-8-Hydroxy-2-(di-*n*-propylamino)tetralin. [c] Four separate preparations were used for the assay of each compound.

Spectral (IR, NMR, MS) data for all intermediates and final compounds were consistent with the proposed structures.

Pharmacology. Inhibition of field stimulation-induced contractions in the guinea pig ileum is a well-recognized assay procedure for 5-HT_{1A} receptors.[9] DA_2 receptor agonists do not inhibit neurotransmission in this preparation. The ability of the compounds to lower arterial pressure in anesthetized rats was also investigated; both 5-HT_{1a} and DA_2 receptor agonists induce hypotension and bradycardia. As shown in Table I, all four target compounds inhibited contractions induced by field stimulation in the isolated guinea pig ileum.

Compound (−)-7, which was the most active compound in the series for inhibiting ileal neurotransmission, was 0.05 times as active as 8-OH DPAT, and (−)-7 was significantly more active than its (+)-enantiomer. (−)- and (+)-8 were significantly less active than (−)-7, and they showed no stereoselectivity. None of the compounds demonstrated antagonistic properties against the ED_{50} inhibitory concentration (0.06 μM) of 8-hydroxy-2-(di-*n*-propylamino)tetralin ("8-OH DPAT"). Likewise, none of the compounds antagonized the spasmogenic activity of acetylcholine bromide or histamine sulfate or altered the baseline tension (1 g) of the ileum preparation. With intravenous doses up to 50 mg/kg, all target compounds failed to produce consistent alteration of arterial pressure and heart rate. There was no indication of significant DA_2 receptor agonist activity. Likewise, the compounds produced no alteration in cardiovascular responses to epinephrine, acetylcholine, or histamine.

The low dopaminergic and serotonergic activities of both enantiomers of 10-methylaporphine (7) and of aporphine 8 demonstrate the necessity of the combination of a 10-methyl and an 11-hydroxy substituent on the aporphine ring for production of potent interaction at the 5-HT_{1A} receptor. Definition of the role of these ortho substituents at 5-HT_{1A} receptors will require further study, as will the role of the C-10 methyl group in directing the site of pharmacological effect from dopamine receptors to serotonin receptors.

Experimental Section

Pharmacology. Methods: Guinea Pig Ileum. Guinea pig ileum preparations were electrically stimulated by bipolar Pt electrodes, one placed in the lumen and the other adjacent and parallel to a 3-cm strip of ileum. Stimulation parameters were 0.1 Hz, 100 V, and 3-ms pulse duration. Tyrode's solution was the bathing medium. Concentrations of experimental compounds in the bathing medium were increased by 0.48 $\log_{10}$ intervals following stabilization of responses to preceding doses. Concentrations which inhibited contractions by 50% (ID_{50}) were calculated as described by Finney.[10] Four separate preparations were used for the assay of each compound.

Rat Blood Pressure. Rats were anesthetized by ip administration of urethane (900 mg/kg). After induction of anesthesia, the right femoral vein was cannulated for administration of test compounds, and arterial pressure was monitored from the femoral artery. Heart rate and arterial pressure were recorded. Three rats were used for each compound.

Chemistry. Melting points were determined in open glass capillaries on a Thomas-Hoover Uni-Melt apparatus, and are uncorrected. NMR spectra were recorded on a Bruker-IBM NR 80 instrument with Me_4Si as the internal standard. Mass spectra were recorded with a Ribermag R-10-10C mass spectrometer. IR spectra were recorded on a Nicolet 5DXB FT-IR instrument. Optical rotations were determined with a Perkin-Elmer Model 141 digital polarimeter. Preparative chromatography was done either with a Chromatotron apparatus (Harrison Research) using Kieselgel 60PF254 (EM Science) as the stationary phase or by flash chromatography using 150A, 35–75-μm silica gel. 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.

2-Methyl-1-(2-nitrobenzyl)-1,2-dihydroisoquinoline (9). Na (2.45 g, 0.1065 g-atom) was dissolved in 115 mL of anhydrous EtOH, and the resulting solution was divided into two equal portions. 2-Nitrotoluene (redistilled, bp 65 °C, 1.7 mm; 5.31 g, 0.03872 mol) was dissolved in one portion, and this solution was heated to 50 °C. A solution of 6 g (0.0334 mol) of *N*-methylisoquinolinium chloride in the second half of ethanolic NaOEt solution was added in one portion to the 2-nitrotoluene solution. The resulting mixture was heated to 78 °C, allowed to cool to ambient temperature, and stored at 35 °C for several days. Two solids separated: at the top of the liquid was a deep red material, and on the bottom of the vessel was an off-white solid. The deep red solid was chromatographed on a flash column (silica) and was eluted with $CHCl_3$–MeOH (9:1) to yield 5.10 g of a dark red solid. This was recrystallized from anhydrous EtOH to afford 4.04 g (43%) of dark red needles, mp 85 °C (lit.[8] mp 90 °C; lit.[6] mp 95–96 °C).

1-(2-Aminobenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline Dihydrochloride (10). Compound 9 (3.5 g, 0.01249 mol) in 100 mL of anhydrous EtOH and 3 mL of $CHCl_3$ was hydrogenated over 0.14 g of PtO_2 at an initial pressure of 46 psig until the calculated amount of H_2 was absorbed (16 h). The reduction mixture was filtered, and 3 mL of saturated ethanolic HCl was added. The solid which separated was collected on a filter and was recrystallized from absolute EtOH to afford 2.38 g (59%) of white crystals, mp 253–254 °C (lit.[8] mp 247–250 °C; lit.[6] mp 272–273 °C).

(*RS*)-Aporphine Hydrochloride (8). The free base of 10 (1.85 g, 0.0695 mol) in 3 mL of EtOH was added dropwise with stirring to 100 mL of cold (ice–H_2O) 10% H_2SO_4. The resulting mixture was cooled to −9 °C, and 0.5 g (0.0725 mol) of $NaNO_2$ in 15 mL of H_2O was added dropwise with stirring, and the temperature was maintained at −9 °C. After addition was complete, the reaction mixture was stirred in the cold for 25 min, and 1.73 g (0.027 mol) of freshly prepared[11] Cu suspended in 25 mL of H_2O was added. The resulting mixture was stirred and allowed to come to ambient temperature over 23 h. It was then filtered, and the filtrate was cooled in ice–H_2O and was brought to pH 8 (pH paper) with concentrated NH_4OH. This mixture was extracted with three 50-mL portions of $CHCl_3$, and the pooled extracts were dried (Na_2SO_4). Evaporation of the solvent provided a dark brown oil which was chromatographed on silica on a flash column and eluted with $CHCl_3$–MeOH (9:1). The eluate was evaporated under reduced pressure, and the oily residue was taken up in Et_2O. This ethereal solution was treated with excess ethereal HCl, and the resulting solid was recrystallized from absolute EtOH to yield 1.24 g (63%) of a buff powder, mp 255–258 °C (lit.[8] mp above 250 °C; lit.[6] mp 255 °C).

(−)-Aporphine Hydrochloride [(−)-8]. A cold (ice–H_2O) solution of the free base of (*RS*)-8 (3.12 g, 0.0132 mol) in 30 mL of EtOAc was added dropwise to a cold solution of 5.12 g (0.0132 mol) of (+)-2,3-di-*p*-toluoyl-D-tartaric acid in 20 mL of EtOAc. The resulting mixture was stirred for 20 h, and the precipitate which formed was collected on a filter and was washed on the filter with 25 mL of cold EtOAc. The solid was recrystallized repeatedly from absolute EtOH to constant optical rotation, $[\alpha]^{28}_{589} = +40.5°$ (*c* 0.0023, MeOH). A suspension of this salt in 50 mL of H_2O was treated with excess $NaHCO_3$. The resulting

mixture was extracted with two 50-mL portions of $CHCl_3$. The pooled extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure to yield 0.22 g (7%) of a yellow oil. This was treated with ethereal HCl to give a white solid, mp 229–232 °C, $[\alpha]^{28.5}_{589} = -109.4°$ (c 0.0019, MeOH).

(+)-Aporphine Hydrochloride [(+)-8]. The free base of (*RS*)-8 (3.12 g, 0.0132 mol) in 30 mL of EtOAc was treated with 5.12 g (0.0132 mol) of (−)-2,3-di-*p*-toluoyl-L-tartaric acid as described for (−)-8. Recrystallized di-*p*-toluoyltartrate salt (from absolute EtOH) $[\alpha]^{26}_{589} = -43.6°$ (c 0.001 77, MeOH). The resolved material was converted to its HCl salt as described for (−)-8, mp 230–235 °C, $[\alpha]^{28}_{589} = +108.4°$ (c 0.001 66, MeOH).

(*RS*)-10-Methyl-11-(1-phenyltetrazol-5-yl)aporphine Hydrochloride (11). A mixture of 0.320 g (0.001 21 mol) of (*RS*)-11-hydroxy-10-methylaporphine,[5] 0.260 g (0.001 44 mol) of 5-chloro-1-phenyl-1*H*-tetrazole, 1.0 g (0.007 25 mol) of K_2CO_3, and 30 mL of Me_2CO was heated under reflux for 18 h, and then an additional 0.200 g (0.0011 mol) of 5-chloro-1-phenyl-1*H*-tetrazole and 0.50 g (0.003 62 mol) of K_2CO_3 were added. This mixture was heated under reflux for an additional 24 h. The cooled reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was chromatographed on a flash column and eluted with $CHCl_3$ containing 1% MeOH. The resulting orange semisolid was chromatographed on a Chromatotron apparatus and was eluted with $CHCl_3$ containing 1% MeOH. Solvent was removed from the eluate, and the residue was treated with excess ethereal HCl to afford 0.311 g (60%) of a tan solid, mp 242–243 °C dec. For elemental analysis, a small portion was recrystallized twice from EtOH–Et_2O to give a white solid, mp 242–243 °C dec. Anal. ($C_{25}H_{24}ClN_5O$) C, H, N. H_2O (Karl Fischer) 0.53%.

(*RS*)-10-Methylaporphine Hydrochloride (7). A solution of 0.250 g (0.000 575 mol) of 11 in 50 mL of glacial AcOH was hydrogenolyzed at 50 °C for 48 h over 0.125 g of 10% Pd/C at an initial pressure of 50 psig. The cooled reduction mixture was filtered through Celite and the filtrate was evaporated under reduced pressure. The residue was chromatographed on a Chromatotron apparatus and was eluted with hexane–EtOAc (1:1). The residue obtained by evaporation of this eluate was treated with ethereal HCl to produce a finely divided white solid. Two recrystallizations of this from absolute EtOH afforded 0.021 g (13%) of a white crystalline solid, mp 279–282 °C dec (lit.[6] mp 279 °C dec).

(−)-10-Methylaporphine Hydrochloride [(−)-7]. The free base of 7 (0.447 g, 0.001 795 mol) in 4.5 mL of EtOAc was added dropwise to a solution of 0.694 g (0.001 795 mol) of (+)-2,3-di-*p*-toluoyl-D-tartaric acid in 8 mL of EtOAc. The suspension was stirred for 42 h, and the resulting white solid was collected on a filter and was recrystallized twice from EtOAc–EtOH and then twice from EtOH, to constant optical rotation, $[\alpha]^{28}_{589} = +39.2°$ (c 0.018 45, MeOH). The resolved material was converted to the HCl salt and recrystallized from EtOH–Et_2O as described for (−)-8. Yield, 0.040 g (8%) of a white solid, mp 251–254 °C dec, $[\alpha]^{24}_{589} = -112.6°$ (c 0.001 66, MeOH).

(+)-10-Methylaporphine Hydrochloride [(+)-7]. The free base of 7 (2.35 g, 0.0942 mol) was converted into its (−)-2,3-di-*p*-toluoyl-L-tartrate salt as described for (−)-7. This was recrystallized to constant optical rotation from absolute EtOH, $[\alpha]^{23}_{589} = -39.4°$ (c 0.003 675, MeOH). The resolved material was converted to the HCl salt and recrystallized from EtOH–Et_2O as described for (−)-7. Yield, 0.20 g (8%), mp 244–245 °C dec, $[\alpha]^{21}_{589} = +110.6°$ (c 0.002 37, MeOH).

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