Synthesis and Pharmacology of Hydroxylated Metabolites of Methylphenidate

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threo-dl-p-Hydroxymethylphenidate and *erythro-dl-p*-hydroxymethylphenidate (**5a** and **5b**) and the deesterified products, *threo-dl-* and *erythro-dl-p*-hydroxyritalinic acid (**6a** and **6b**), were synthesized. The effects of the intracerebroventricular administration of these compounds on the locomotor activity of rats was determined and compared to that of the respective racemates of methylphenidate (**1a** and **1b**) and ritalinic acid (**2a** and **2b**) as a relative index of in vivo dopaminergic activity. The maximal locomotor response was significantly greater for **5a** than for **5b**, **1a**, or **1b**. These findings suggest that metabolite **5a** may play a role in the pharmacology of **1a**. The intracerebroventricular administration of acids **2a**, **2b**, **6a**, and **6b** all produced a small increase in locomotor activity relative to their methyl esters which was not appreciably affected by stereochemistry or para-hydroxylation.

The central stimulant *threo-dl*-methylphenidate (**1a**, Ritalin), the drug of choice in the treatment of the hyperkinetic syndrome in children,[1] undergoes extensive biotransformation. A major portion (60–81%) of an oral dose in man is excreted in the urine as the deesterified metabolite, *threo-dl*-ritalinic acid (**2a**). Oxidative metabolism of **1a** occurs at the 6 position of the piperidine ring to yield the lactam **3**. Bartlett and Egger[2] found that the deesterified urinary metabolite **4** accounted for 5–12% of the administered dose of **1a** in man. Whereas **1a** increases locomotion in animals, the lactam **3** is devoid of locomotor-facilitating activity upon intracerebroventricular administration.[3] Pharmacological properties of **2a** have not been reported.

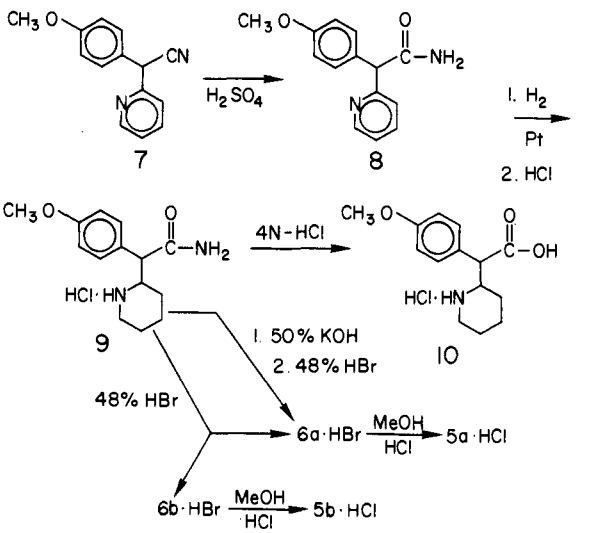
threo-dl erythro-dl

1a, $R_1 = CH_3$; $R_2 = R_3 = H$ **1b**, $R_1 = CH_3$; $R_2 = H$
2a, $R_1 = R_2 = R_3 = H$ **2b**, $R_1 = R_2 = H$
3, $R_1 = CH_3$; $R_2 = H$; $R_3 = O$ **5b**, $R_1 = CH_3$; $R_2 = OH$
4, $R_1 = R_2 = H$; $R_3 = O$ **6b**, $R_1 = H$; $R_2 = OH$
5a, $R_1 = CH_3$; $R_2 = OH$; $R_3 = H$
6a, $R_1 = R_3 = H$; $R_2 = OH$

Evidence for the metabolic hydroxylation of **1a** was first presented by Bernhard et al.[4] in 1959 and Sheppard and co-workers[5] in 1960. The actual hydroxylated metabolites were later identified as *threo-dl-p*-hydroxymethylphenidate (**5a**) and *threo-dl-p*-hydroxyritalinic acid (**6a**) or their glucuronide conjugates.[6] Small amounts of these para-hydroxylated metabolites were detected in the urine of humans given **1a**. Compound **5a** was reported to be the major metabolite in rat brain, and the total hydroxylated metabolites in urine accounted for 45% of an administered dose in this species.[3]

A group at Regis Chemical Co. has synthesized a diasteromeric mixture of **5a,b**·difumarate by a circuitous pathway.[7] However, this compound was found to be almost exclusively the pharmacologically less active racemate, *erythro*-**5b**, as defined by GC analysis.

Scheme I



Faraj et al.[8] reported a chemical synthesis of an isomeric mixture of **5a,b** and a similar mixture of **6a,b**. The intracerebroventricular administration of **5a,b** (30:70 threo–erythro mixture) to mice facilitated locomotor activity, though less than the parent compound, **1a**. A preponderance of the erythro racemate of the hydroxy metabolite may have accounted for the low locomotor activity as compared with the parent compound.[8] Described herein is a synthesis of the hydroxylated metabolites of **1a**, their structural verification, and the isolation of the threo and erythro racemates. In addition to the chemistry, the pharmacological actions of the separate racemates, **5a** and **5b**, and their corresponding deesterified products, **6a** and **6b**, were evaluated and compared to the effects of the nonhydroxylated analogues.

Chemistry. The hydroxylated metabolites of **1a** were synthesized via the route illustrated in Scheme I. Nitrile **7**[9] was partially hydrolyzed to produce the acetamide **8** using concentrated H_2SO_4 at room temperature.[10] Complete hydrolysis of **7** to the pyridine-2-acetic acid was avoided, since this molecular arrangement readily decarboxylates.[11] Compound **8** was hydrogenated to produce

(1) J. G. Millichap, *Ann. N.Y. Acad. Sci.*, **205**, 321 (1973).
(2) M. F. Bartlett and H. P. Egger, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 31, 537 (1972).
(3) B. A. Faraj, Z. H. Israili, J. M. Perel, M. L. Jenkins, S. G. Holtzman, S. A. Cucinell, and P. G. Dayton, *J. Pharmacol. Exp. Ther.*, 191, 535 (1974).
(4) K. Bernhard, J. Bühler, and M. H. Bickel, *Helv. Chim. Acta*, **42**, 802 (1959).
(5) H. Sheppard, W. H. Tsien, W. Rodegker, and A. J. Plummer, *Toxicol. Appl. Pharmacol.*, **2**, 353 (1960).
(6) B. A. Faraj and M. L. Jenkins, *Pharmacologist*, **15**, 155 (1973).
(7) A. Zirnis, Regis Chemical Co., Morton Grove, IL, private communications.
(8) B. A. Faraj, Z. H. Israili, J. M. Perel, M. L. Jenkins, S. G. Holtzman, S. A. Cucinell, and P. J. Dayton, NAPS document no. 02465; ASIS/NAPS, c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163.
(9) A. Karim, R. E. Ranney, and S. Kraychy, *J. Pharm. Sci.*, **61**, 888 (1972).
(10) L. Panizzon, *Helv. Chim. Acta*, **27**, 1748 (1944).
(11) P. A. J. Janssen, *J. Am. Chem. Soc.*, **76**, 6192 (1954).

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a threo–erythro mixture (ca. 20:80) of **9** using Adam's catalyst in glacial HOAc.[10] When **9**·HCl was hydrolyzed with aqueous HCl, *p*-methoxyritalinic acid (**10**) hydrochloride was obtained.[12] Compound **9**·HCl was refluxed in 48% HBr to produce **6a,b**·HBr. This diastereomeric mixture was particularly amenable to fractional recrystallization (unlike **9**·HCl), providing **6a**·HBr and **6b**·HBr. Treatment of these amino acids with MeOH–HCl yielded the threo and erythro racemates, **5a**·HCl and **5b**·HCl, respectively. The threo racemate **6a**·HBr, being the lower percentage component of **6a,b**·HBr, proved difficult to obtain in adequate quantities through fractional crystallization. Column chromatographic separation on silica gel of precursor **9**·HCl only succeeded in providing pure *erythro*-**9**.

A KOH-mediated epimerization has been reported for obtaining the threo amino acid **2a** from **2a,b**.[13] These conditions were applied to amino acid **6a,b**. However, problems were encountered in the product isolation of the epimerized product **6a**. The nonmethoxylated analogue of **9** has also been used in the literature as an epimerization substrate to obtain its pure threo racemate from an erythro–threo mixture.[14] Thus, a KOH epimerization of diastereomeric **9** was conducted and provided nearly pure *threo*-**9**.[13] All traces of the erythro stereochemistry were eliminated in the subsequent crystallizations involved in obtaining **5a**·HCl from this epimerized product.

The spectral and physical properties of **5a,b** and **6a,b** reported here differ from those published by Faraj et al.[3,8] even when similar erythro–threo mixtures are compared.

The aromatic protons in the NMR of the compounds synthesized in the present work and by Regis Chemical Co. appear as pairs of doublets, supportive of the 1,4-disubsituted phenyl ring. The relatively low frequency of the carbonyl absorption (1690 cm^{-1}) in the IR spectrum of **6a,b**·HBr in the present study and **6a,b** derived from **5a,b**·difumarate supplied by Regis Chemical Co. is consistent with intermolecular hydrogen bonding of the carboxyl carbonyl to a phenolic hydroxyl.

The erythro and threo stereochemistry of the various compounds in the present study was assigned on the basis of several investigative approaches. The stereochemical outcome of the catalytic reduction step appears to be governed by the conformation assumed by the substrate during reduction. Intramolecular hydrogen bonding between the carbonyl oxygen and the protonated nitrogen of the pyridine ring yields a favorable six-membered ring. In this conformation, the phenyl ring is positioned so as to impede hydrogenation of the pyridine ring on the face leading to a threo diastereomer. Consistent with this reasoning, a 20:80 threo–erythro mixture of **9** was obtained (GC). Further, in the present study, when phenylacetonitrile was used instead of *p*-methoxyphenylacetonitrile in the synthetic scheme,[10] the threo/erythro ratio of the resulting piperidyl amide (nonmethoxylated **9**) exhibited the same 20:80 ratio found for the para-methoxylated system. The original synthesis[10] of **1a,b** produced a 20:80 threo–erythro mixture[15] under conditions identical with those used in this laboratory. When the nonmethoxylated *threo-erythro*-piperidyl amide was hydrolyzed to provide

(12) This compound is presently being used as an internal standard for a serum **2a** assay which will be described in a forthcoming paper.
(13) R. Rometsch, U.S. Patent 2957880; *Chem. Abstr.*, **55**, 9433e (1961).
(14) Ciba Ltd., British Patent 878167; *Chem. Abstr.*, **58**, 509g (1963).
(15) I. Weisz and A. Dudás, *Monatsh. Chem.*, 91, 840 (1960).

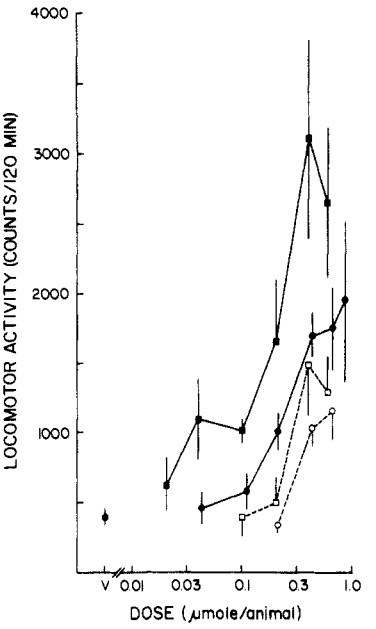

Figure 1. Effects of **1a**·HCl (closed circles), **1b**·HCl (open circles), **5a**·HCl (closed squares), and **5b**·HCl (open squares) on the locomotor activity of rats. The locomotor response to the vehicle is indicated by "v". Symbols represent the mean of three to six separate determinations, and vertical lines represent 1 SEM.

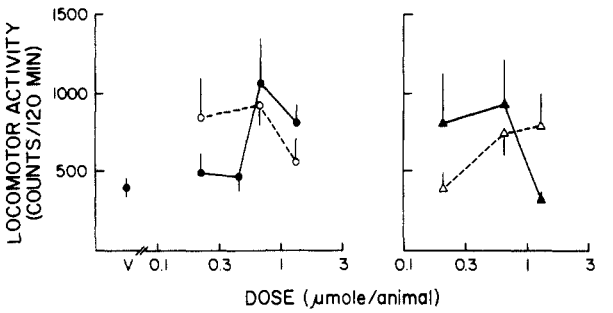

Figure 2. The left side of the figure depicts the effects of **2a**·HBr (closed circles) and **2b**·HBr (open circles) on the locomotor activity of rats. The right side of the figure depicts the effects of **6a**·HBr (closed triangles) and **6b**·HBr (open triangles) on locomotor activity. The locomotor response to the vehicle is indicated by "v". Symbols represent the mean of four to six separate determinations, and vertical lines represent 1 SEM.

2a,b, the lower percentage racemate cochromatographed (GC) with authentic **2a** (Ciba-Geigy). To further establish the correct assignment of stereochemistry, rats were administered **1a**·HCl (Ciba-Geigy), and GC–MS analysis of their amino acid urinary metabolites demonstrated the presence of only the threo racemates, **2a** and **6a**. Furthermore, **5a**·HCl was considerably more active in facilitating locomotor activity in rats than was **5b**·HCl (Figure 1). This is consistent with the corresponding activities of the nonhydroxylated analogues, **1a** and **1b**.[13] Finally, *erythro*-**9** was converted to *threo*-**9** by treatment with 50% KOH at reflux. The same erythro to threo KOH epimerization of nonmethoxylated *erythro*-**9** is known to occur.[13,14]

Results

The intracerebroventricular administration of **1a** and **1b** produced a concentration-dependent increase in locomotor activity (Figure 1), with the threo racemate producing a significantly greater maximal response than the erythro racemate. Furthermore, both **5a** and **5b** increased locomotor activity (Figure 1), with the maximal locomotor

response to **5a** being significantly greater than that found for **5b** or the corresponding nonhydroxylated racemates, **1a** and **1b**.

Both **2a** and **2b** produced an approximate doubling of base-line rates of locomotor activity when administered by an intracerebroventricular route, but their maximal effect was considerably less than that for **1a** (Figure 2). Both **6a** and **6b** similarly increased locomotor activity. No significant differences were noted between the maximal responses to racemates **6a** and **6b** or their nonhydroxylated congeners, **2a** or **2b**.

Discussion

The pharmacological actions of **1a** are generally attributed to cellular actions on dopamine-containing neurons, resulting in an increased synaptic transmission. This action has been well documented for the locomotor stimulant effects of **1a**,[16,17] and this behavior was utilized to estimate the relative dopaminergic stimulating properties of the compounds in the present study. The compounds were administered intracerebroventricularly (lateral ventricle) to circumvent their peripheral metabolism and to minimize differences in transport across the blood–brain barrier. The findings of the present study corroborate reports concerning the relationship between the pharmacological activity of **1a** and **1b** and their stereochemistry;[15] i.e., the locomotor stimulating activity resides predominantly in the threo racemate, **1a**. This study has extended such a relationship to include compounds **5a** and **5b**. The present study also demonstrated that **5a** was considerably more active than **1a** in this in vivo test of dopaminergic activity (Figure 1). These results suggest that the metabolite **5a** may play a role in the pharmacology of **1a**.

Amphetamine, a central stimulant also used in the treatment of childhood hyperkinesis, is readily para-hydroxylated by rodents and, to a lesser extent, by humans.[18] Furthermore, *p*-hydroxyamphetamine, like *p*-hydroxymethylphenidate, has been demonstrated to stimulate the locomotor activity of rats to a greater extent than its nonhydroxylated form when each compound was administered by the intracerebroventricular route.[19] Whether or not the mechanism of action mediating the locomotor response to **5a** is equivalent to that of **1a** can not be deduced from the present study. In this regard, it is of interest that the duration of locomotor stimulation is longer for **5a** than for **1a** (Figure 3). This same temporal relationship has been reported for *p*-hydroxyamphetamine relative to amphetamine.[19] It is also not known whether the differential activity of these compounds generalizes to other behavioral tests attributed to dopaminergic mechanisms, e.g., stereotyped behavior. Both lines of investigation are currently under study in this laboratory.

The intracerebroventricular administration of the deesterified compounds **2a**, **2b**, **6a**, or **6b** all exhibited weak pharmacological activity (Figure 2), but unlike their esters, the extent of the locomotor increase was not appreciably affected by stereochemistry of para-hydroxylation. The relatively large plasma concentrations of **2a** that were reported following **1a** administration[20] and the low brain/plasma distribution ratio of **2a**[3] suggests that **2a** may mediate some of the peripheral side effects[21] associated with **1a** therapy. This is appealing to speculate, since the administration of **1b** produces hypertensive effects and an LD_{50} value equal to that of **1a**[22] while exhibiting only a minimal degree of central stimulation.[13,15] These equipotent effects of the two racemates, **1a** and **1b**, may in fact be due to the equipotencies of their principal metabolites, **2a** and **2b**, respectively.

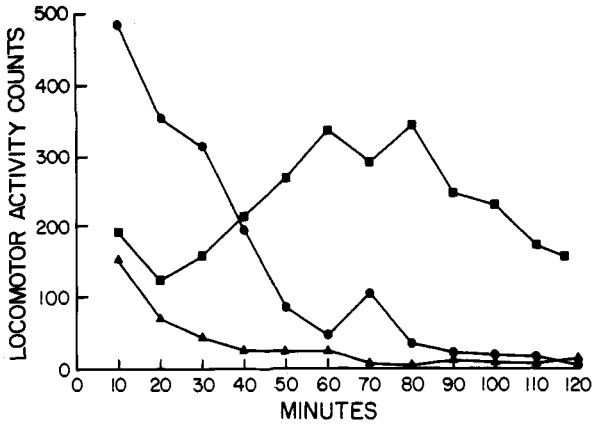

Figure 3. Time course of the effects of **1a**·HCl (0.43 μmol/animal, circles), **5a**·HCl (0.4 μmol/animal, squares), and the vehicle (10 μL of isotonic saline, triangles) on the locomotor activity of rats.

Experimental Section

Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Infrared spectra were recorded on a Perkin-Elmer Model 297 spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Associates T-100 spectrometer. Electron-impact mass spectra were obtained on a Finnigan Model 3300 mass spectrometer at 70 eV. A Varian series 2400 gas chromatograph (6 ft × $^1/_8$ in., 1.5% OV-101 on 100–120 Chrom GHP, 180 °C, carrier gas He, 30 mL/min; air 150 mL/min; H_2 3.1 mL/min) with thermionic (nitrogen/phosphorus) specific detection was used for GC determinations of relative percentages of the erythro and the later eluting threo racemates.

Compounds **1b** and **2b** were obtained by standard methods from *erythro-dl*-2-(2′-piperidyl)acetamide hydrochloride, which in turn was prepared according to the method of Panizzon,[10] followed by fractional crystallization to remove the lower percentage threo racemate.

***dl*-2-(4-Methoxyphenyl)-2-(2′-pyridyl)acetamide (8).** Finely ground compound **7**[9] (1.26 g, 5.6 mmol) was added in portions to 2.4 mL of stirring concentrated H_2SO_4 over a period of 20 min. Stirring was continued for 12 h at room temperature, and then the reaction vessel was placed on ice. Ice and 15% NaOH solution were added alternately to the reaction mixture to obtain a pH of 8. The product was extracted with $CHCl_3$ (3 × 35 mL), and the combined $CHCl_3$ extracts were evaporated under reduced pressure to obtain a brown oil (0.839 g, 61%), which resisted attempts at recrystallization. This oil was used in the next step without further purification. When the alkalinized reaction mixture was refrigerated overnight without extraction, the product was obtained in a sparing yield as white crystals: mp 102–105 °C; IR (KBr) 3132 (NH), 1650 (C=O) cm^{-1}; NMR (Me_2SO-d_6; Me_4Si) δ 3.75 (s, 3 H, OCH_3), 5.02 (s, 1 H, CHCO), 6.88 (d, 2 H, 2,6-Ph H, J = 9 Hz), 7.02–7.40 (m, 5 H, 3,5-Ph H, pyr H, $CONH_2$), 7.6–7.8 (m, 2 H, pyr H), 8.49 (d, 1 H, 3′-pyr H, J = 5 Hz); mass spectrum, m/e (relative intensity) 242 (7.8) (M^+), 199 (100) (M^+ – CONH), 185 (52), 167 (43), 154 (40).

***erythro-dl*- and *threo-dl*-2-(4-Methoxyphenyl)-2-(2′-piperidyl)acetamide Hydrochloride (9·HCl).** Compound **8** (1.1

(16) J. E. Thornburg and K. E. Moore, *Neuropharmacology*, 12, 853 (1973).
(17) G. R. Breese, B. R. Cooper, and A. S. Hollister, *Psychopharmacology*, 44, 5 (1975).
(18) L. G. Dring, R. L. Smith, and R. T. Williams, *Biochem. J.*, 116, 425 (1970).
(19) W. A. Taylor and F. Sulser, *J. Pharmacol. Exp. Ther.*, 185, 620 (1973).
(20) S. J. Soldin, B. N. Hill, Y. M. Chan, J. W. Swanson, and J. G. Hill, *Clin. Chem.*, 25, 51 (1979).
(21) W. R. Martin, J. W. Sloan, J. D. Sapria, and D. R. Jasinki, *Clin. Pharmacol. Ther.*, 12, 245 (1971).
(22) L. Szporny and P. Görög, *Biochem. Pharmacol.*, 8, 263 (1961).

g, 4.5 mmol) was dissolved in 15 mL of glacial HOAc, and 50 mg of PtO_2 was added. The mixture was placed under a slight positive pressure of hydrogen and stirred until the hydrogen uptake ceased (ca. 30 h). The reaction mixture was filtered and evaporated under reduced pressure to an oil. The oil was dissolved in MeOH, treated with Norite, and filtered, and then an excess of Et_2O–HCl was added. A white solid was obtained upon evaporation of the solvent under reduced pressure, yielding granules (0.78 g, 72%) from 95% EtOH–Et_2O: mp 222–223 °C; IR (KBr) 1675 (C=O), 1400 (CN) cm^{-1}; NMR (Me_2SO-d_6; Me_4Si) δ 1.3–1.98 (m, 6 H, $CH_2CH_2CH_2$), 2.6–3.18 (m, 2 H, NCH_2), 3.44–3.64 (m, 1 H, NCH), 3.64–3.84 (m, 1 H, CHCO), 3.73 (s, 3 H, OCH_3), 6.92 (d, 2 H, 3,5-Ph H, J = 9 Hz), 7.36 (d, 2 H, 2,6-Ph H, J = 9 Hz); mass spectrum, m/e (relative intensity) 204 (0.4) ($M^+ - CONH_2$), 165 (5), 148 (3), 121 (21) (MeO – tropylium), 84 (100) ($C_5H_{10}N^+$). Anal. ($C_{14}H_{21}N_2O_2Cl$) C, H, N.

Approximately 0.1 mg of **9**·HCl, obtained before recrystallization, was derivatized with trifluoroacetic anhydride (50 μL in a screw-cap vial, 100 °C, 15 min) and subjected to GC analysis: 20:80 threo–erythro; retention time: 10 and 13.7 min, respectively.

***erythro-dl*- and *threo-dl*-2-(4-Methoxyphenyl)-2-(2′-piperidyl)acetic Acid Hydrochloride (10·HCl).**[12] Compound **9**·HCl (0.224 g, 0.8 mmol) was dissolved in 10 mL of 4 N HCl and heated at reflux for 6 h. The solution was then evaporated to dryness under reduced pressure, and the white residue was recrystallized from 95% EtOH-Et_2O to afford 0.16 g (57%) of **12**·HCl: mp 215–216 °C; IR (KBr) 2960 (NH_3^+), 1690 (C=O) cm^{-1}; NMR (Me_2SO-d_6; Me_4Si) δ 1.5–1.95 (m, 6 H, $CH_2CH_2CH_2$), 3.79 (s, 3 H, OCH_3), 3.93 (d, 1 H, $CHCO_2$, J = 10 Hz), 6.98 (d, 2 H, 3,5-Ph H, J = 9 Hz), 7.36 (d, 2 H, 2,6-Ph H, J = 9 Hz); mass spectrum, m/e (relative intensity) 205 (2.6) ($M^+ - CO_2$), 121 (30) (MeO – tropylium), 84 (100) ($C_5H_{10}N^+$).

***threo-dl*-2-(4-Methoxyphenyl)-2-(2′-piperidyl)acetamide (*threo*-9).** Compound **9**·HCl (1.0 g, 3.4 mmol) was refluxed in 50% KOH for 4 days or until an aliquot contained no more than 5% of the erythro isomer (GC). The mixture was cooled and filtered, and the filter cake was washed with portions of water. Crystallization from EtOAc provided glistening granules of *threo*-11 (95+% threo, 0.45 g, 53%): mp 181–182 °C; IR (KBr) 1678 (C=O).

***threo-dl*-2-(4-Hydroxyphenyl)-2-(2′-piperidyl)acetic Acid Hydrobromide (6a·HBr).** Compound *threo*-**9** (0.41 g, 1.65 mmol) was refluxed in 4 mL of 48% HBr under nitrogen for 4 h. After the solution was cooled and refrigerated overnight, white crystals of **6a** were collected on a filter and dried to constant weight (95+% threo, 0.43 g, 94%): mp 270–272 °C dec; IR (KBr) 1705 (C=O), 1510 (NH_2^+) cm^{-1}; NMR (D_2O; TMP) δ 1.32–2.04 (m, 6 H, $CH_2CH_2CH_2$), 2.89–3.48 (m, 2 H, NCH_2), 3.48–3.75 (m, 1 H, NCH), 3.88 (d, 1 H, $CHCO_2$, J = 9 Hz), 6.99 (d, 2 H, 3,5-Ph H, J = 9 Hz), 7.22 (d, 2 H, 2,6-Ph H, J = 9 Hz).

Erythro-*dl* and Threo-*dl* Enrichment of 2-(4-Hydroxyphenyl)-2-(2′-piperidyl)acetic Acid Hydrobromide (6a·HBr and 6b·HBr) by Fractional Crystallization. Compound **9**·HCl (1.0 g, 3.4 mmol) was dissolved in 15 mL of 48% HBr and heated at reflux under nitrogen with stirring for 4 h. The volume of the reaction was then reduced by approximately one-half, and this was refrigerated overnight to provide white granules of **6a,b**·HBr (0.52 g, 48%): mp 258–259 °C dec; IR (KBr) 1690 (C=O), 1510 (NH_2^+) cm^{-1}; NMR (D_2O; TMP) δ 1.35–2.3 (m, 6 H, $CH_2CH_2CH_2$), 2.9–3.49 (m, 2 H, NCH_2), 3.58–3.78 (m, 1 H, NCH), 3.84 (d, 1 H, $CHCO_2$, J = 8 Hz), 6.97 (d, 2 H, 3,5-Ph H, J = 9 Hz), 7.32 (d, 2 H, 2,6-Ph H, J = 9 Hz); mass spectrum, m/e (relative intensity) 191 (1.3) ($M^+ - CO_2$), 107 (11) (HO – tropylium), 84 (100) ($C_5H_{10}N^+$). Anal. ($C_{13}H_{18}NO_3Br$) C, H, N.

This procedure provides **6a,b**·HBr in an ca. 10:90 ratio in the first crystal crop as determined by GC analysis of derivatized **6a,b** (ca. 0.1 mg of **6a,b**·HBr, 20 μL of pentafluoropropanol, 50 μL of trifluoroacetic anhydride in a screw-cap vial, 100 °C, 15 min); retention time 4.2 and 5.3 min, respectively. Two recrystallizations from MeOH–Et_2O permitted the obtainment of nearly pure (99%) **6b**·HBr.

Upon reducing the volume of the first crystal crop filtrate, followed by refrigeration overnight, a second crystal crop of **6a,b**·HBr (0.165 g, 15%) was obtained which contained ~95% the threo racemate and was used without further purification for pharmacological evaluation.

Methyl *erythro-dl*-2-(4-Hydroxyphenyl)-2-(2′-piperidyl)acetate Hydrochloride (5b·HCl). The first crystal crop of **6a,b**·HBr (0.15 g, 0.47 mmol) was dissolved in 10 mL of MeOH–HCl. The solution was brought to reflux and stirred for 12 h, then the solvent was evaporated under reduced pressure, and the residue was recrystallized from acetone–Et_2O to obtain **5a,b**·HCl (0.128 g, 94%) containing 8% of the threo racemate (GC). A further recrystallization from MeOH–Et_2O yielded pure **5b**·HCl: mp 224–225 °C; IR (KBr) 2940 (NH_2^+), 1720 (C=O), 1590 (NH_2^+) cm^{-1}; NMR (Me_2SO-d_6; Me_4Si) δ 1.4–1.95 (m, 6 H, $CH_2CH_2CH_2$), 2.72–3.22 (m, 2 H, NCH_2), 3.62 (s, 3 H, OCH_3), 3.63–3.72 (m, 1 H, NCH), 3.99 (d, 1 H, $CHCO_2$, J = 10 Hz), 6.81 (d, 2 H, 3,5-Ph H, J = 9 Hz), 7.2 (d, 2 H, 2,6-Ph H, J = 9 Hz), 9.74 (s, 1 H, PhOH); mass spectrum, m/e (relative intensity) 221 (0.4) ($M^+ - CO$), 207 (0.4) ($M^+ - CO_2$), 188 (1.2), 166 (1.2), 107 (8) (HO – tropylium), 84 (100) ($C_5H_{10}N^+$); GC retention time (F_3Ac) 8.4 min. Anal. ($C_{14}H_{20}NO_3Cl$) C, H, N.

Methyl *threo-dl*-2-(4-Hydroxyphenyl)-2-(2′-piperidyl)acetate Hydrochloride (5a·HCl). Compound **6a,b**·HBr (95+% threo, 0.06 g, 0.19 mmol) was esterified according to the preceding procedure, affording pure **5a**·HCl (0.04 g, 73%) from acetone–Et_2O: mp 224–226 °C; IR (KBr) 2940 (NH_2^+), 1720 (C=O), 1580 (NH_2^+) cm^{-1}; NMR (Me_2SO-d_6; Me_4Si) δ 1.20–1.86 (m, 6 H, $CH_2CH_2CH_2$), 2.75–3.10 (m, 2 H, NCH_2), 3.66 (s, 3 H, CO_2CH_3), 3.70–3.88 (m, 1 H, NCH), 4.01 (d, 1 H, $CHCO_2$, J = 10 Hz), 6.80 (d, 2 H, 3,5-Ph H, J = 9 Hz), 7.05 (d, 2 H, 2,6-Ph H, J = 9 Hz), 9.74 (s, 1 H, PhOH); mass spectrum, m/e (relative intensity) 221 (0.38) ($M^+ - CO$), 207 (0.45) ($M^+ - CO_2$), 188 (1.2), 107 (6.5) (HO – tropylium), 84 (100) ($C_5H_{10}N^+$); GC retention time (F_3Ac) 7 min. Anal. ($C_{14}H_{20}NO_3Cl$) C, H, H.

Pharmacology Methods. Animals used in these experiments were male rats of the Sprague–Dawley strain (Charles River Laboratories, Wilmington, MA) weighing 220–300 g. Animals were anesthetized with chloral hydrate (400 mg/kg, ip) and guide cannulae (22 gauge) stereotaxically implanted into the right lateral ventricle at the following coordinates: 1 mm posterior to bregma, 1.1 mm lateral to midline, and 2.7 mm ventral to the dura using the stereotaxic atlas of Konig and Klippel[23] as a guide. A minimum of 1 week passed before behavioral testing was initiated. Locomotor activity was recorded in "doughnut-shaped" cages with six photocell sensors equally spaced around a 9-cm-wide runway.[17] Cages were located in sound-attenuating chambers illuminated by a 7-W bulb. Rats were "habituated" to the activity cages for 1 h prior to drug administration. Counts from light-beam interruptions were recorded at 10-min intervals for 120 min following drug treatment.

The compounds were dissolved in sterile water and concentrations were adjusted to permit the administration of a constant volume (10 μL) at each dose. Internal cannulae (28 gauge) were placed into the guide cannulae, and drug injections were made using a 10-μL syringe attached to the internal cannula via polyethylene tubing. The compounds to be tested were injected at an approximate rate of 5 μL/min in 0.25-μL increments. The internal cannula was left in place for 1 min following the injection of a given compound to permit diffusion of the compounds from the site of injection. Animals were placed in the locomotor apparatus immediately following drug administration. Animals were used no more than twice, with at least 1 week between drug tests. The locomotor response to each compound was compared to the number of counts recorded following the intracerebroventricular administration of 10 μL of sterile water.

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