dation for much longer than the duration of the incubations.

DNA Degradation by Measurement of "Malonaldehyde" Production. Blm and Fe-Blm complexes were tested for the production of malonaldehyde-like products upon reaction with native DNA, using a modified assay of Burger et al.[19] based on the method of Waravdekar and Saslaw.[33] Highly polymerized chicken erythrocyte DNA (P. L. Biochemicals, Inc., Milwaukee, Wis.) served as the substrate. Aldrich Chemical Co. (Milwaukee, Wis.) supplied 2-thiobarbituric acid (4,6-dihydroxy-2-mercaptopyrimidine) and malonaldehyde bis(dimethyl acetal), used as standard. Reaction mixtures (total volume 2.0 mL) were assembled in polystyrene tubes previously rinsed with 0.25 M phosphate buffer, pH 7.0, and exhaustively with glass-distilled water and contained some of the following additions, in order: 60 mM sodium phosphate buffer, pH 7.00; 2.5×10^{-4} M DNA (as total bases); 2.5×10^{-4} M bleomycin [or Fe(III)-Blm complexes). After a 3- to 5-min preincubation at room temperature, Fe^{2+} (1×10^{-3} M) is added as ferrous ammonium sulfate to some incubations. The incubation is mixed briefly and timed aliquots of 0.2 mL are taken up to 25 min. Aliquots are mixed with 1.0 mL of 2-thiobarbitaric acid reagent (4×10^{-2} M 2-thiobarbituric acid, 1×10^{-3} M EDTA) and heated in capped test tubes for 30 min at 75 °C to develop the color. Malonaldehyde standards are prepared simultaneously. After cooling, the absorbance at 532 nm is measured using a Cary 16 spectrophotometer equipped with 1.0-mL cuvettes. Production of malonaldehyde from DNA reactions is quantitated using authentic malonaldehyde produced by acid hydrolysis of malonaldehyde bis(dimethyl acetal), which when reacted with 2-thiobarbituric acid yields a product having an $\epsilon_{532} = 1.6 \times 10^5$ M^{-1}. The intensely pink-colored products of the reaction between 2-thiobarbituric acid and malonaldehyde and 2-thiobarbituric acid and Blm-Fe^{2+} reactions were indistinguishable by visible absorption spectrophotometry.

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Synthetic and Preliminary Hemodynamic and Whole Animal Toxicity Studies on (*R*,*S*)-, (*R*)-, and (*S*)-2-Methyl-3-(2,4,5-trihydroxyphenyl)alanine

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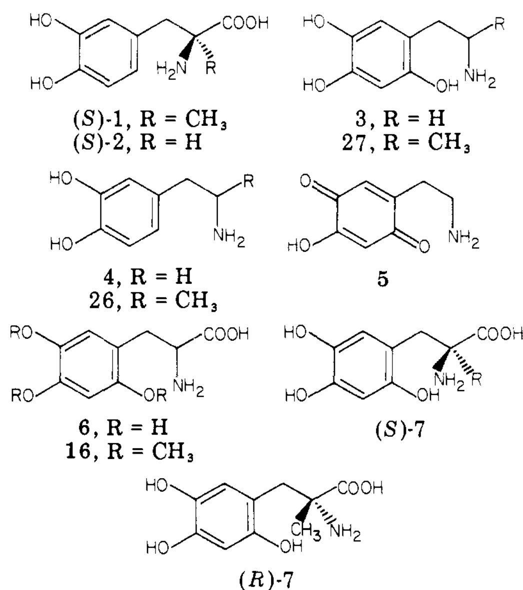
The synthesis, resolution, and absolute configuration assignment of 2-methyl-3-(2,4,5-trihydroxyphenyl)alanine (6-OH-α-Me-Dopa) are reported. Hemodynamic studies in the rat have shown that this structural analogue and potential metabolite of the clinically useful drug (*S*)-α-Me-Dopa possesses weak hypotensive activity which resides in the *R* enantiomer. LD_{50} studies in mice have established that 6-OH-α-Me-Dopa is over four times more toxic than α-Me-Dopa. Chronic exposure to 6-OH-α-Me-Dopa leads to renal and hepatic lesions. The ease of oxidation of this hydroquinone to the electrophilic quinone species may contribute to its enhanced toxicity compared to α-Me-Dopa.

(*S*)-α-Me-Dopa [(*S*)-α-MD, (*S*)-**1**] is a clinically useful



antihypertensive agent.[1] Although this drug is relatively nontoxic,[2] a number of side effects has been reported. These include depression,[3] a Parkinsonian-type syndrome,[4] psychotic behavior,[5] and a positive antiglobulin test sometimes associated with hemolytic anemia.[6] Perhaps the most dramatic and clinically serious potential side effect of (*S*)-α-MD is its hepatotoxicity, which may lead to severe liver damage and death.[7] The well-known susceptibility of catecholamines to autoxidation[8,12] has led to the proposal that the liver abnormalities associated with the use of (*S*)-α-MD may result from the formation of electrophilic oxidation products that covalently bind to biomacromolecules.[9] Evidence to support this hypothesis is the NADPH-dependent binding of tritium-labeled (*S*)-α-MD to hepatic microsomal macromolecules and the isolation of an (*S*)-α-MD glutathione adduct.[9] Similar sulfhydryl adducts of the endogenous catechol amino acid (*S*)-Dopa [(*S*)-**2**] have been characterized in the urine of melanoma patients.[10] Adduct formation presumably

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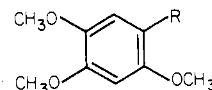
proceeds via attack by the sulfhydryl moiety of glutathione or cysteine on electrophilic oxidation products derived from these molecules.

A structurally related molecule for which extensive biochemical and toxicological data have been reported is the neurotoxin 6-hydroxydopamine (6-OHDA, **3**),[11] a reported chemical[12] and enzymatic[13] product of the neurotransmitter dopamine (**4**). As with (*S*)-α-MD, 6-OHDA forms covalent adducts with biomacromolecules[14] and small-molecule sulfhydryl-containing nucleophiles.[15] These products are likely to be formed via nucleophilic attack on the electrophilic quinone species **5**, which is generated rapidly at pH 7.4 by autoxidation of the *p*-hydroquinone **3**.[16] Similar properties have been reported for 6-OH-Dopa (**6**).[17]

We have undertaken the synthesis of 2-methyl-3-(2,4,5-trihydroxyphenyl)alanine (6-OH-α-Me-Dopa, 6-OH-α-MD, **7**) in order to characterize its physicochemical properties for further metabolic and neurotoxicity studies. The close structural relationship of **7** to α-MD prompted us to investigate its hemodynamic properties. Since only the *S* enantiomer of α-MD possesses antihypertensive activity,[18] we have resolved and established the absolute configuration of **7**. Additionally, the known hepatotoxicity of (*S*)-α-MD[8,12] and the possible metabolic oxidation of (*S*)-α-MD to the potentially reactive 6-OH-α-MD have led us to examine the acute and chromic toxicity of **7** in mice.

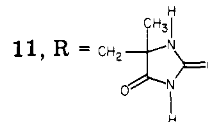
Chemistry. The pathway developed for the preparation of (*RS*)-6-OH-α-MD proceeded via the condensation of 2,4,5-trimethoxybenzaldehyde (**8**) with nitroethane to give 1-(2,4,5-trimethoxyphenyl)-2-nitropropene (**9**).[19] The nitrostyrene **9** was converted to 2,4,5-trimethoxyphenyl-2-propanone (**10**) with Fe/HOAc[20] in 75% yield. The carbonyl moiety of **10** was modified in a Strecker reaction[21] to give the hydantoin **11**, which upon base hydrolysis provided (*RS*)-2-methyl-3-(2,4,5-trimethoxyphenyl)alanine (**12**).

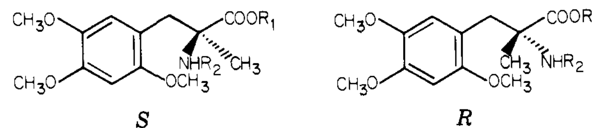
Treatment of **12** with refluxing 48% hydrobromic acid to cleave the methyl ethers led to the isolation of a compound with properties consistent with the lactone **13**. The CIMS obtained following pertrimethylsilylation showed a quasimolecular ion (MH^+) at mass 426, consistent with the tris(tetramethylsilyl) derivative **14**. When **12** was heated in water and the resulting solution chromatographed on AG 50W-X8 cation-exchange resin, the desired amino acid **7** was obtained. Pertrimethylsilylation of this product gave the expected pentakis(tetramethylsilyl) derivative **15** (MH^+ 588). Analogous lactone formation has been observed upon O-demethylation of 1-(2,4,5-trimethoxyphenyl)alanine (**16**), the intermediate to 6-OH-Dopa (**6**).[22]



8, R = CHO
9, R = $CH{=}C(NO_2)CH_3$
10, R = $CH_2C({=}O)CH_3$

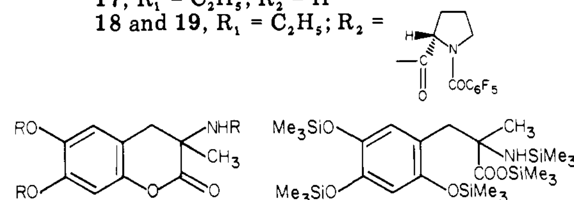
11, R =





S *R*

12, $R_1 = R_2 = H$
17, $R_1 = C_2H_5$; $R_2 = H$
18 and **19**, $R_1 = C_2H_5$; R_2 =



13, R = H
14, R = $Si(CH_3)_3$
15

The resolution of the racemic trimethyl ether **12** was achieved via its ethyl ester **17**, which when treated with 1 equiv of (+)-tartaric acid in 100% ethanol gave crystals that yielded the levorotory amino acid ester. Similarly (−)-tartaric gave a salt of **17** which provided the dextrorotatory product. Estimation of the enantiomeric purity (>90%) of the resolved amino ester was achieved by GLC analysis of the diasteromeric amides **18** and **19** prepared by derivatization with (*S*)-*N*-[(pentafluorobenzoyl)prolyl]-1-imidazolide (PFBPI).[23] Treatment of (−)- and (+)-**17** with refluxing aqueous 48% hydrobromic acid, followed by ion-exchange chromatography, provided the trihydroxyphenyl amino acids (+)- and (−)-**7** as their HCl salts.

The absolute configuration of **7** was established by convergent syntheses starting from (*S*)-α-Me-Dopa ethyl ester [(*S*)-**20**][24] and the enantiomers of **7**. Oxidation of (*S*)-**20** with $K_3Fe(CN)_6$ at pH 7.4 led to the formation of a stable, yellow, crystalline solid originally assigned the aminochrome structure (*S*)-**21** on the basis of its CI mass spectrum (MH^+ 236), UV spectrum [(EtOH) λ_{max} 312 nm (ϵ 23 300) and 412 (1800)], and consistent elemental analysis. Comparison of the ^1H NMR spectrum of this product with that recently reported for the aminochrome **22**,[25] however, clearly ruled out structure (*S*)-**21**. The two methylene proton signals of **22** appear as well-resolved 8 and 6 line multiplets centered at 3.2 and 2.5 ppm, respectively. No equivalent high-field signals appear in the spectrum of the product obtained from the oxidation of (*S*)-**20**: δ ($CDCl_3$) 7.7 (very br s, 1 H, exchanges with D_2O), 6.78 (s, 1 H), 6.40 (br, 1 H, variable s, exchanges with D_2O),

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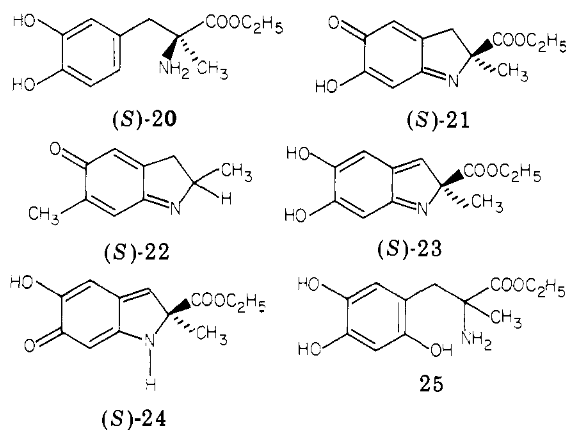

6.29 (s, 1 H), 5.75 (s, 1 H), 4.25 (q, 2 H, CH_3CH_2), 1.69 (s, 3 H, CH_3), 1.3 (t, 3 H, CH_3CH_2). The three nonexchangeable low-field signals establish that this product must contain a double bond in the five-membered ring. Based on these data, only two structures are possible, namely, (*S*)-**23** and (*S*)-**24**. The presence of the very broad one-proton signal centered at 7.7 ppm is most consistent with tautomer (*S*)-**24**, since the proton attached to the nitrogen atom would be coupled with the quadropole moment of the nitrogen atom.

Confirmation of this structure assignment was obtained from the ^{13}C NMR spectrum. The lowest field signal appears at δ 179.12. The carbonyl carbon signal for carboxylic acid esters, such as ethyl acetate, are located near 170 ppm.[26] The spectrum of the yellow oxidation product displays a signal at 169.71 ppm, which is assigned to the ester carbonyl carbon atom. Since the spectrum of (*S*)-**23** should not contain any lower field signal, the structure assignment for this product can be made unambiguously as (*S*)-**24**. The signal at 179.12 ppm can be assigned to the quinonemethide carbonyl carbon of (*S*)-**24**.[27] The remainder of the ^{13}C NMR spectrum also is consistent with this structure assignment (see Experimental Section).

Oxidation of the ethyl ester **25** of 6-OH-α-MD led to the same quinonemethide. An aqueous ethanolic solution of **25** derived from (-)-**7**·HCl rapidly became deep red when treated with sodium ethoxide in the presence of air. Initially the reaction mixture absorbed light maximally at 270 nm. Upon standing, the λ_{max} of this solution shifted to 312 nm, the same absorption maximum observed for (*S*)-**24**. Workup of this reaction mixture provided quinonemethide **24** having the same CD spectrum as that obtained from the oxidation of (*S*)-**20**. The enantiomeric amino ester obtained from (+)-**7**·HCl led to the quinonemethide having the opposite CD spectrum. Based on these data we have assigned the absolute configuration of **7**·HCl as *R* (+) and *S* (-).

Additional evidence supporting this absolute configuration assignment was obtained by comparing the $[\alpha]_D$ values of (+)- and (-)-**7** at different acid concentrations with the corresponding literature values[28] of (*R*)- and (*S*)-α-MD (Lutz and Jirgensons Rule,[29] see Table I). A positive trend in specific rotations with increasing acid strength is observed for both (*S*)-6-OH-α-MD and (*S*)-α-MD, while the opposite trend exists for (*R*)-6-OH-α-MD and (*R*)-α-MD.

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Table I. Variation of $[\alpha]_D$ Values with pH for Various α-Amino Acids

compd	$[\alpha]_D$ (*c* 1) 6 N HCl	1 N HCl	0.1 N HCl	0.00 HCl[b]
(*S*)-α-MD[a]	+4	-0.1	-4	-14
(*R*)-α-MD[a]	-4	+1.5	+5.5	+13
(*R*)-DOPA[a]	+7.5	+9.5	+13	+31.5
(*S*)-6-OH-α-MD	-12.4	-15.0	-18.8	*c*
(*R*)-6-OH-α-MD	+14.4	+17.7	+19.7	*c*

[a] See ref 28. [b] pH 6.5. [c] The trihydroxy compounds were too unstable in the absence of acid for these measurements.

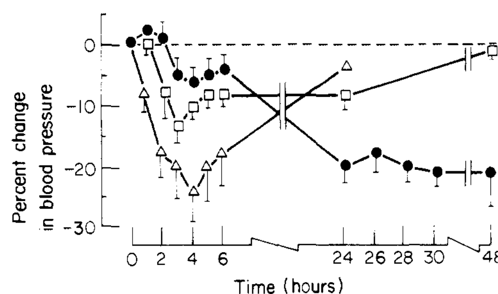

Figure 1. Percent change in blood pressure relative to pretreatment values following ip administration of 400 mg/kg (*RS*)-α-MD (□-□), 200 mg/kg (*S*)-α-MD (△-△), and 100 mg/kg (*RS*)-6-OH-α-MD (●-●) to normotensive rats. The data show the mean plus or minus SD (*n* = 6).

Pharmacology. The possibility that 6-OH-α-MD may possess interesting hemodynamic properties prompted us to compare its effects on blood pressure with those of α-MD using the normotensive rat. Figure 1 summarizes the data obtained following ip administration of (*RS*)-α-MD (400 mg/kg), (*S*)-α-MD (200 mg/kg), and (*RS*)-6-OH-α-MD (100 mg/kg). The 6-hydroxy compound was too toxic to test at higher doses. The blood pressure of saline-treated control animals either increased moderately or remained unchanged relative to pretreatment control values. These studies revealed that racemic **7** is reasonably effective in lowering the blood pressure in this preparation, although its onset of action is slow and its duration of action long relative to both (*RS*)- and (*S*)-α-MD. Differences in the hemodynamic properties of racemic α-MD vs. the clinically used (*S*)-α-MD also were evident. Thus, although the amount of the *S* isomer administered in experiments employing either the racemate or the pure enantiomer was the same, the pharmacologic response elicited with pure *S* enantiomer was considerably more pronounced. It would appear that the presence of the *R* enantiomer interferes with the hypotensive effects of the *S* enantiomer.

The effects of 6-OH-α-MD pretreatment on the hemodynamic effects of (*S*)-α-MD also were examined. Intraperitoneal administration of (*RS*)-6-OH-α-MD (50 mg/kg) 7 days prior to treatment with (*S*)-α-MD led to an enhanced hypotensive effect (Figure 2). When (*RS*)-6-OH-α-MD (3 × 100 μg) was administered intracranially on days 9, 8, and 7 prior to (*S*)-α-MD, no significant change in blood-pressure effects compared to those observed with nonpretreated control animals was observed. Based on these limited data it would appear that the hypotensive activities of α-MD and 6-OH-α-MD are additive but, unlike α-MD, 6-OH-α-MD may act on peripheral receptors only.

The well-established enantioselectivity associated with (*S*)-α-MD's antihypertensive activity[18] prompted us to examine the effects of the enantiomers of 6-OH-α-MD on

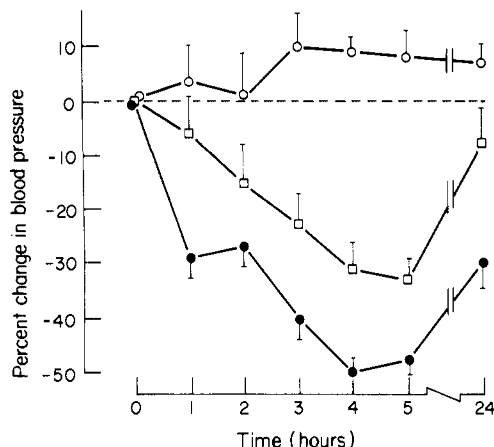

Figure 2. Percent change in blood pressure relative to pretreatment values following ip adminstration of (*S*)-α-MD (400 mg/kg) in nonpretreated animals (□-□) and in animals pretreated with 50 mg/kg (*RS*)-6-OH-α-MD (●-●) 7 days prior to the measurement. The results obtained with saline-treated control animals (○-○) also are plotted. The data shown the mean plus or minus SD (n = 4).

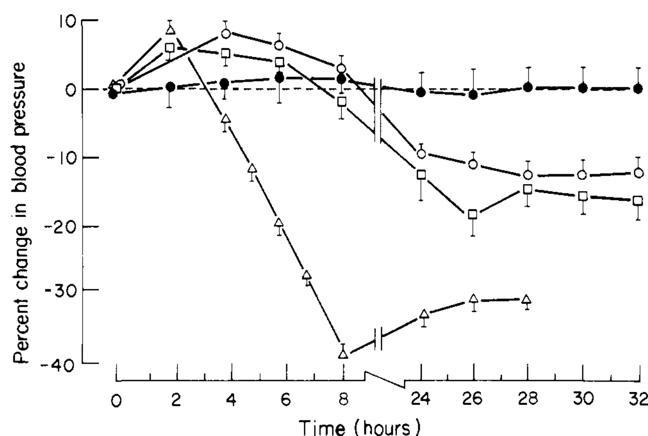

Figure 3. Percent change in blood pressure relative to pretreatment values during constant intravenous infusions [15 (mg/kg)/h] of (*S*)-α-MD (△-△, n = 9), (*S*)-6-OH-α-MD (●-●, n = 12), (*RS*)-6-OH-α-MD (○-○, n = 14), and (*R*)-6-OH-α-MD (□-□, n = 14). The curve for the saline-treated control animals (n = 8) was essentially identical with the curve obtained with (*S*)-6-OH-α-MD.

blood pressure. These studies again employed the normotensive rat but, instead of single ip injections, continuous iv infusions (15 (mg/kg)/h) were examined in an attempt to assess the activities of the isomers under conditions approaching steady state. Surprisingly, the results obtained with animals treated with the *S* enantiomer could not be distinguished from those of the saline-treated control animals (Figure 3). The racemic mixture and *R* enantiomer, however, caused moderate hypotensive responses. Once again, the onset of action was slow compared to that observed with (*S*)-α-MD. At the present time we have no biochemical data to help in the interpretation of these results. The reversed enantioselectivity of the 6-hydroxy compound indicates, however, that its weak hypotensive activity may be mediated by a mechanism(s) different from those suggested for (*S*)-α-MD.[30]

Toxicology. The toxicity of (*RS*)-6-OH-α-MD was compared with various structurally related compounds in

Table II. LD_{50} Values (mg/kg) for Various Catecholamines and Amino Acids in Male White Swiss Mice

		90% confidence level	
compd[a]	LD_{50}	upper	lower
(*S*)-α-MD	1889	2194	1684
(*RS*)-α-MD	1317	1472	1177
(*RS*)-6-OH-α-MD	289	332	224
(*RS*)-α-MeDA	576	662	500
(*RS*)-6-OH-α-MeDA	320	352	291

[a] DA = dopamine.

mice. LD_{50} studies (Table II) were performed with single ip injections. As anticipated, (*RS*)-6-OH-α-MD is considerably more toxic than (*RS*)-α-MD. Death occurred usually 24 h after administration of either drug, suggesting the degeneration of vital peripheral organ function as opposed to disruption of CNS function. The acute toxicity of (*RS*)-6-hydroxy-α-methyldopamine[31] was similar to (*RS*)-6-OH-α-MD in this preparation, although (*RS*)-α-methyldopamine (**26**), the *S* isomer of which is a major metabolite of α-MD,[32] was considerably more toxic than (*RS*)-α-MD. Metabolic studies should provide useful information on the extent to which metabolically formed (*RS*)-6-hydroxy-α-methyldopamine (**27**) may contribute to the acute toxicity of the parent amino acid.

Chronic daily ip administration of sublethal doses of these two amino acids was carried out in order to identify target organ toxicities. Histological examinations revealed no abnormalities in brain or heart tissues. The 6-hydroxy compound at 200 (mg/kg)/day did lead to kidney and liver abnormalities after dosing for 3 days. Liver necrosis appeared as widely scattered minute lesions. Extensive renal tubular necrosis also was observed. Similar, although less, dramatic liver and kidney damage was found with very high doses of (*RS*)-α-MD. This enhanced toxicity of 6-OH-α-MD may be associated with the presence of the *p*-hydroquinone moiety. The greater toxicity of 6-OHDA compared to dopamine[11] is consistent with this view. The possible involvement of metabolites derived from 6-OH-α-MD in the pharmacological and toxicological properties of this compound is under investigation. Should 6-OH-α-MD undergo metabolic conversion to 6-OH-α-MDA, it will be particularly important to assess the neurotoxicity of the parent amino acid.

Experimental Section

Chemistry. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were obtained using a Perkin-Elmer Model 337 spectrophotometer. NMR spectra were recorded on a Varian A-60, Perkin-Elmer R-12B, or Varian FT-80 instrument. Chemical shifts are reported in part per million (ppm) relative to Me_4Si ($CDCl_3$ and Me_2SO-d_6) or DSS (D_2O). CI mass spectra were taken on an Associated Electronics Incorporated Model MS 902 double-focus mass spectrometer equipped with a direct-inlet system and modified for chemical-ionization mass spectrometry. The reagent gas was isobutane at a pressure of 0.5 to 1.0 torr. GLPC were run on a Varian Model 2100 with a U-shaped 2 m × 2 mm Pyrex column. All ultraviolet spectra were taken on a Cary Model 15 instrument using 1-cm quartz cells. Specific rotation measurements were performed on a Perkin-Elmer 141 electronic polarimeter. Microanalyses were performed by the Microanalytical Laboratory, University of California, Berkeley.

1-(2,4,5-Trimethoxyphenyl)-2-propanone (10). A mixture of glacial acetic acid (30 mL) and 20-mesh iron (14 g, 0.24 mol)

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in a 250-mL, three-necked, round-bottom flask equipped with a condenser, heating mantle, and mechanical stirrer was vigorously stirred and heated at reflux until the mixture became grayish-white (about 30 min). A solution of 1-(2,4,5-trimethoxyphenyl)-2-nitropropene (**9**;[19] 6 g, 24 mmol) in glacial acetic acid was added dropwise to this rapidly stirred solution. The reaction mixture was heated under reflux for a total of 3 h. The resulting grayish-dark green mixture was vacuum filtered through a bed of Celite and washed with hot acetic acid. The filtrate was then diluted with 100 mL of water and extracted (3 × 50 mL) with dichloromethane. The combined dichloromethane extract was washed with 5% $NaHCO_3$ and water, dried with $MgSO_4$, and evaporated to give a brown oil. Sublimation at 115–120 °C (0.5 mm) gave 4 g (75%) of the ketone **10**: mp 44–46 °C; NMR ($CDCl_3$) δ 6.72 and 6.58 (2 s, Ar H, 2), 3.95 (2 s, OCH_3, 3), 3.85 (2 CH_3O, 6), 3.62 (s, CH_2, 2), 2.13 (s, CH_3, 3). Anal. ($C_{12}H_{16}O_4$) C, H.

(*RS*)-5-Methyl-5-(2,4,5-trimethoxyphenyl)hydantoin (11). A mixture of ketone **10** (4.8 g, 21.7 mmol), KCN (1.8 g, 28 mmol), and $(NH_4)_2CO_3$ (18.75 g, 190 mmol) in 150 mL of ethanol and 30 mL of water was heated under reflux for 6.5 h. The solid $(NH_4)_2CO_3$ which collected inside the condenser was washed into the reaction vessel periodically with water. Upon cooling, the solid which separated from the reaction mixture was filtered and recrystallized from 100% ethanol to give 5 g (81%) of the desired hydantoin **11**: mp 199–201 °C; IR (KBr) 1700 cm^{-1} (cyclic urea); NMR (Me_2SO) δ 6.73, 6.63 (m, Ar H, 2), 3.76, 3.72, 3.66 (3 s, CH_3O, 9), 2.82 (s, CH_2, 2), 1.29 (s, CH_3, 3). Anal. ($C_{14}H_{18}N_2O_5 \cdot 0.75H_2O$) C, H, N.

(*RS*)-3-(2,4,5-Trimethoxyphenyl)-2-methylalanine (12). Hydantoin **11** (1 g, 36 mmol) was added to a hot, filtered solution of $BaCO_3 \cdot 8H_2O$ (4.5 g, 14.4 mmol) in 37 mL of water. The solution was heated under reflux for 48 h. After the solution cooled, the pH was adjusted to 5.5 with 1 N H_2SO_4 and the precipitated $BaSO_4$ was separated by hot filtration. The white residue obtained after in vacuo concentration of the filtrate was crystallized from absolute ethanol to yield 0.65 g (67%) of the amino acid **12**: mp 230–242 °C; NMR (D_2O) δ 6.75, 6.56 (2 s, Ar H, 2), 4.00 (s, CH_3O, 3), 3.95 (s, 2 CH_3O, 6), 3.13 (s, CH_2, 2), 1.63 (s, CH_3, 3). Anal. ($C_{13}H_{19}NO_5 \cdot 0.5H_2O$) C, H, N.

(*RS*)-3-(2,4,5-Trihydroxyphenyl)-2-methylalanine Hydrochloride [(*RS*)-6-OH-α-MD·HCl, (*RS*)-7·HCl]. The amino acid **12** (0.5 g, 1.85 mmol) was added to freshly distilled 48% HBr and the solution was heated under reflux and a nitrogen atmosphere for 4 h. After cooling to room temperature, the solution was concentrated under vacuum to yield a brown residue (0.4 g, 70.0%), tentatively identified as lactone **13**: CIMS of tris(trimethylsilyl) derivative (**14**), MH^+ 426. The residue (10.2 g, 0.63 mmol) was dissolved in 50 mL of water, heated on a steam bath under a nitrogen atmosphere for 1 h, and concentrated to 2–3 mL on a rotatory evaporator. This concentrate was chromatographed on 1 g (net weight) of Bio-Rad analytical grade cation-exchange resin (AG 50W-X8, 200–400 mesh, H^+ form). The column was washed with water until the eluent was halogen free ($AgNO_3$ test) and then the product was eluted with 2 N HCl into a tinted, round-bottom flask flushed with nitrogen. The eluent was lyophilized to yield the white HCl salt (0.13 g, 75%) of **7**: UV λ_{max} (pH 4) 292 nm (ε 4660); UV λ_{max} (pH 7.4) 269 nm (ε 8560); NMR (D_2O) δ 6.74, 6.58 (2 s, Ar H, 2), 3.5 (s, CH_2, 2), 1.68 (s, CH_3, 3); CIMS as tetrakis(pentafluoropropionyl ethyl ester) derivative, MH^+ 840; as pentakis(trimethylsilyl) derivative (**15**), MH^+ 588. Anal. ($C_{10}H_{14}NO_5Cl \cdot 0.25H_2O$) C, H, N.

(*RS*)-Ethyl 2-Amino-2-methyl-3-(2,4,5-trimethoxyphenyl)propanoate (17). A solution of **12** (13.8 g, 51.5 mmol) in 100 mL of anhydrous 1 N ethanolic HCl was heated under reflux and a nitrogen atmosphere with stirring in a soxhlet apparatus containing a reservoir of Type 3A molecular sieves, 8–12 mesh, MX 158-06, for 12 h. When the solution cooled to 5 °C, the ethyl ester hydrochloride (12.4 g, 73%) separated: mp 212–215 °C; NMR (D_2O) δ 6.82 (s, Ar H, 2), 4.33 (q, CH_3CH_2O, 2), 3.94, 3.88, 3.84 (3 s, OCH_3, 9), 3.18 (s, CH_2, 2), 1.69 (s, CH_3, 3), 1.28 (t, CH_2CH_3, 3).

The ethyl ester amine hydrochloride (10 g, 30.0 mmol) was dissolved in 0.5 N NaOH, and the resulting solution was extracted three times with petroleum ether. The combined extract was dried with $MgSO_4$ and concentrated to a colorless oil (6.2 g, 77%). The oil slowly solifified to a translucent solid, melting at 38–40 °C: NMR ($CDCl_3$) δ 6.7, 6.52 (2 s, Ar H, 2), 4.17 (q, CH_3CH_2, 2), 3.87, 3.80, 3.77 (3 s, OCH_3, 9), 2.95 (d, CH_2, 2), 1.68 (s, NH_2, 2), 1.36 (s, CH_3, 3), 1.25 (t, 3, CH_2CH_3). Anal. ($C_{15}H_{23}NO_5$) C, H, N.

Resolution of 6-OH-α-Me-Dopa. Racemic **17** (6.2 g, 21 mmol) in 75 mL of warm absolute ethanol was added to a warm solution of (+)-tartaric acid (3.3 g, 22 mmol) in 75 mL of absolute ethanol, and the resulting solution was immediately filtered. Crystallization, initially at room temperature (16 h), was completed at 5 °C for 12 h. The salt (4.6 g, 97%; mp 157–160 °C) was recrystallized from absolute ethanol to give an analytical sample, mp 157–159 °C. Anal. ($C_{19}H_{29}NO_{11}$) C, H, N.

The residue obtained from the above combined filtrates in 100 mL of 0.5 N NaOH was extracted with petroleum ether. After drying ($MgSO_4$) and removing solvent, 1.4 g (4.8 mmol) of the amino ester **17** was recovered. When treated with (–)-tartaric acid (0.75 g, 4.83 mmol) in a total of 40 mL of warm absolute ethanol, 0.3 g (19%) of the salt was obtained. Recrystallization from absolute ethanol gave material melting at 155–158 °C.

The above tartrate salts were converted to the corresponding free amino esters by extraction into petroleum ether from 0.5 N NaOH solutions. The melting points (41–42 °C) and NMR spectra [($CDCl_3$) δ 6.71 and 6.53 (2 s, Ar H, 2), 4.16 (q, CH_3CH_2, 2), 3.87, 3.82, 3.79 (3 s, OCH_3, 9), 2.96 (d, CH_2, 2), 2.27 or 1.66 (br s, NH_2, 2), 1.38 (s, CH_3, 3), 1.26 (t, CH_3CH_2, 3)] were essentially identical.

The enantiomeric purities of (+)- and (–)-**17** were estimated by GC analysis of the corresponding amide derivatives obtained by treating the free bases (1 mg each) in 0.1 mL of benzene with 2 mg of (*S*)-*N*-[(pentafluorobenzoyl)prolyl] (PFBP) 1-imidazolide. Each solution was concentrated under a stream of nitrogen gas on the steam bath to about 0.05 mL before analysis on an OV 25 column at 260 °C. The retention time of the PFBP amide from (–)-**17** was 61 min and from (+)-**17** was 68 min. In both instances, enantiomeric purity was estimated at greater than 90%.

(*R*)-(+)-6-OH-α-Me-Dopa·HCl [(*R*)-7]. Levorotatory **17** (0.5 g, 1.8 mmol) in 10 mL of freshly distilled 48% hydrobromic acid containing 1 mL of glacial acetic acid was heated under a nitrogen atmosphere for 3 h. After the solvent was removed in vacuo the brownish residue was chromatographed on a 50W-X8 ion-exchange resin, H^+ form. The resin was washed with doubly distilled water and when the eluent was halogen free the column was eluted with 2 N HCl. The solvent was removed under vacuum to to give a white glassy material (0.35 g, 48%). The product was further heated in 10 mL of distilled water under an atmosphere of nitrogen for 15 min on a steam bath. The TLC (2-propanol–HOAc–H_2O, 4:1:1; R_f 0.67), NMR, and CIMS (PFP ethyl ester derivative) were identical with (*RS*)-**7**: $[\alpha]^{25}_D$ +19.7° (*c* 1, 0.1 M aqueous HCl).

(*S*)-(–)-6-OH-α-Me-Dopa·HCl [(*S*)-(–)-7·HCl]. Dextrorotatory **17** (1.47 g, 4.95 mmol) was O-demethylated and hydrolyzed in the same manner as (–)-**17** and gave 0.750 g (57.6%) of the enantiomeric hydrochloride salt. The TLC, NMR, and CIMS (Me_3Si ethyl ester) properties were identical with (*RS*)-**7** and (*R*)-**7**: $[\alpha]^{25}_D$ –18.8° (*c* 1, 0.1 M aqueous HCl).

Quinonemethide (*S*)-24 from (*S*)-α-Me-Dopa Ethyl Ester [(*S*)-20]. To a 500-mL, three-necked, round-bottom flask containing 100 mL of water were added 3 g of K_3FeCN_6, 1 g of $KHCO_3$, and 50 mL of benzene. The solution, cooled in an ice bath, was stirred for approximately 10 min before 1 g (3.63 mmol) of (*S*)-Me-Dopate [(*S*)-**20**] in 50 mL of water (pH 7.4, 1 N phosphate buffer) was added dropwise. The reaction mixture slowly turned red. The benzene layer was removed and replaced with fresh solvent five times during the 5-h reaction period. The aqueous solution was extracted further with benzene (3 × 25 mL) and the combined, dried ($MgSO_4$) extracts gave a total of 0.22 g (28%) of crude product. The crude isolate was crystallized from benzene/hexane to yield 0.12 g (15%) of pure product: mp 130–133 °C; UV (EtOH) λ_{max} 422 nm (ε 3650), 312 (36 000), 322 sh (33 200); UV (EtOH–NaOEt) λ_{max} 346 nm (ε 39 700), 334 (40 300), 243 (16 400); UV (EtOH–HCl) 318 (ε 17 500); H NMR ($CDCl_3$) δ 7.7 (very br s, exchanges with D_2O, 1 H, N-H), 6.78 (s, 1 H, C=CH), 6.40 (br s, exchanges with D_2O, 1 H, OH), 6.29 (s, 1 H, C=CH), 5.75 (s, 1 H, C=CH), 4.25 (q, 2 H, CH_3CH_2), 1.69 (s, 3 H, CH_3), 1.3 (t, 3 H, (CH_3CH_2); decoupled ^{13}C NMR ($CDCl_3$) δ 179.12 (0.26 relative intensity, O=CC_2), 169.71 [0.16, O=C(O)C], 162.85 (0.23, HOC), 150.86 (0.338, C=*C*–N), 138.03 (0.93, O=C–CH), 132.84 (0.17, C=C–*C*=C), 96.27 and 91.73 (100 and 0.98, two C=*C*H), 73.61 (0.16, *C*–COO), 62.39 (0.25, OCH_2), 22.45 (0.82,

CH_3), 13.81 (CH_2CH_3); CD (25°) (1.08 mg/100 mL of H_2O) $[\theta]_{450}$ 0, $[\theta]_{312}$ −27 800, $[\theta]_{230}$ 33 300, $[\theta]_{200}$ 0. Anal. ($C_{12}H_{13}NO_4$) C, H, N.

Ethyl 3-(2,4,5-Trihydroxyphenyl)-2-methyl-2-aminopropanoate Hydrochloride [(*RS*)-25·HCl]. (*RS*)-6-OH-α-Me-Dopa·HCl (22 mg, 0.08 mmol) in 30 mL of 3.1 N ethanolic HCl was heated under reflux for 48 h in a three-necked, round-bottom flask equipped with a Dean–Stark trap, condenser, and nitrogen bubbler. The solvent was evaporated in vacuo to provide a glass. This material in absolute ethanol (3 mL) was chromatographed on a cation-exchange resin (Dowex 50W-X8, H^+ form, prewashed with absolute ethanol). The resin was washed with additional ethanol (20 mL) and the product finally eluted with 3 N ethanolic HCl. Removal of the solvent gave the salt as a colorless solid (18 mg, 75%): mp 210–213 °C (dec); ^{1}H NMR (D_2O) δ 6.7 and 6.62 (2 s, 2, Ar H), 4.3 (q, 2, OCH_2CH_3), 3.15 (s, 2, Ar CH_2), 1.74 (s, 3, CH_3), 1.29 (t, 3, OCH_2CH_3). CIMS following treatment with pentafluoropropionic (PFP) anhydride displayed quasimolecular ions for the tetrakis(PFP) (MH^+ 840), tris(PFP) (MH^+ 694), and bis(PFP) (MH^+ 548) derivatives. Anal. ($C_{12}H_{18}NO_5Cl \cdot 0.75H_2O$) C, H, N.

Quinonemethide (*S*)-24 and (*R*)-24 Derived from (−)-6-OH-α-Me-Dopa·HCl [(−)-7·HCl] and (+)-6-OH-α-Me-Dopa. The ethyl ester of (−)-6-OH-α-Me-Dopa·HCl was prepared as described above for (*RS*)-7 and characterized by CIMS and ^{1}H NMR. A solution of the ethyl ester (60 mg, 0.18 mmol) in 50 mL of ethanol and 4 mL of water upon treatment with 8–10 drops of sodium ethoxide (4 g/50 mL) turned a deep red color: UV λ_{max} (EtOH) 290 to 270 nm. The solution was stirred for an additional 20 h in air, during which time the λ_{max} shifted to 312 nm. The volume of the solution was reduced to 10 mL and, after adding 50 mL of water, the product was extracted with 3 × 50 mL of chloroform. The combined chloroform extract was dried ($MgSO_4$) and concentrated to an off-red colored solid weighing 175 mg (42%). Crystals of (*S*)-24 obtained from benzene/hexane melted at 125–127 °C: the UV, ^{1}H NMR, CIMS, and CD characteristics were essentially identical with (*S*)-24 obtained from (*S*)-α-Me-Dopa ethyl ester; CD (25°) (1.12 mg/100 mL of H_2O) $[\theta]_{450}$ 0, $[\theta]_{312}$ +28 000, $[\theta]_{230}$ −30 000, $[\theta]_{200}$ 0. Similarly, the quinonemethide obtained from (+)-6-OH-α-Me-Dopa·HCl provided essentially identical UV, NMR, and CIMS. The CD spectrum was the mirror image of that obtained with (*S*)-24: CD (25°) (1.00 mg/100 mL of H_2O) $[\theta]_{450}$ 0, $[\theta]_{312}$ +28 000, $[\theta]_{230}$ −30 000, $[\theta]_{200}$ 0.

Biology. LD_{50} Determinations. The procedures used to determine the LD_{50} values followed those outlined by Litchfield and Wilcox. Solutions of the test compounds were prepared in normal saline just prior to use. The concentrations were adjusted so that the injection volume (0.2 mL) remained constant over the dose range studied. Male, white Swiss mice (25 ± 5 g) in groups of six were injected ip with a 1-cm^3 tuberculin syringe (27-gauge needle). The compounds were given between the hours of 4:00 and 5:00 p.m. and the affects were observed every 24 h. The mice were allowed food and water ad libidium. The percent deaths, based on the number of mice out of a set of six that died within a 48-h period, were plotted against log dose. The 90% upper and lower confidence levels of the LD_{50} values were calculated by extrapolating from a plot of the LD_{50}, LD_{84}, and LD_{16} values.

Chronic Toxicity Studies. Groups of six mice for each dose studied were given daily ip injections of either (*RS*)-α-MD or (*RS*)-6-OH-α-MD between 4:00 and 5:00 p.m. Two animals from each set of six were sacrificed by decapitation at days 5, 7, and 9. The organs (brain, heart, liver, and kidneys) were removed surgically and placed in a bouins solution. The organs were then prepared for histopathologic examination.

Hemodynamic Studies. Male Sprague–Dawley rats weighing 300 ± 30 g were purchased from Simson Co. and housed in the vivarium with food and water provided ad libidium. Cannulation of the left carotid artery, right jugular vein, and/or right cerebral lateral ventricle was performed under sodium pentobarbital anesthesia for the purpose of delivering compounds and measuring blood pressure. The arterial and venous cannulae were prevented from clogging by flushing every 2 days with 0.2 mL of heparinized saline solution. After surgery, the rats were kept warm with heat lamps until recovery, which required 3 h. The experiments generally began 3 days after surgery. Only those animals in good health were used.

The blood pressure was measured by a Grass instrument Model 5 polygraph attached to a Statham P23Dc transducer to which the carotid arterial cannula was attached. Calibration of the instrument was performed routinely during an experiment using external and internal standards.

For acute responses, only the left carotid artery was cannulated. The experiments involved either ip pretreatment with (*RS*)-6-OH-α-MD (50 mg/kg) followed 7 days later with (*S*)-α-MD (400 mg/kg, ip) or just ip administration of (*RS*)-6-OH-α-MD (100 mg/kg), (*RS*)-α-MD (400 mg/kg), or (*S*)-α-MD (200 mg/kg). Compounds wer dissolved in normal saline at concentrations such that each injection volume was 0.5 mL. Blood pressure measurements were taken every hour for 6 h after drug administration and again 24 h later to estimate recovery. Mean arterial pressure was calculated from the systolic and diastolic pressure. The data are presented as percent change from values measured before drug administration. Saline control animals were included in all experiments.

For continuous infusion studies, both the left carotid artery and right jugular vein were cannulated. Compounds were dissolved in normal saline containing 1% $Na_2S_2O_5$ as an antioxidant. The solutions were sterilized by Millipore filtration (22 μm). The solutions were infused at a constant flow rate of 0.85 mL/h and concentrations were adjusted to deliver 15 (mg/kg)/h. The rats were housed in restraining cages and were provided food and water. The saline solutions were delivered by a Harvard continuous-infusion pump.

Administration of (*RS*)-6-OH-α-MD into the right cerebral lateral ventricle was achieved via a chronically implanted icv cannula and a 50-μL syringe attached to a 27-gauge needle. Only the right carotid artery was cannulated in these studies. The volume administered was 10 μL and contained 100 μg of drug.

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