Synthesis, Redox Characteristics, and in Vitro Norepinephrine Uptake Inhibiting Properties of 2-(2-Mercapto-4,5-dihydroxyphenyl)ethylamine (6-Mercaptodopamine)

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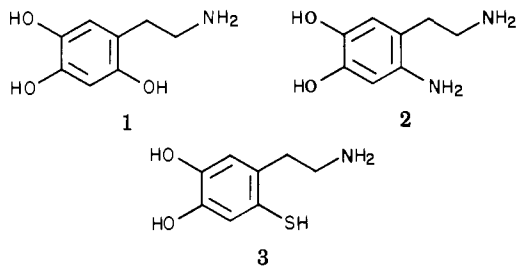
In an attempt to further characterize the structural features of 6-hydroxydopamine analogues that are associated with in vivo neuronal degeneration, the synthesis of 6-mercaptodopamine was undertaken. Although reaction conditions leading to the 1,4 addition of thiols to the model quinone 4-methyl-*o*-benzoquinone were achieved, attempts to obtain 6-thiolated dopamine analogues by this route failed. The synthesis of 6-mercaptodopamine was achieved by the regioselective thiocyanation of *O,O*-dimethyldopamine, followed by bis-*O*-demethylation and reductive cleavage of the *S*-cyano group. Unlike 6-hydroxydopamine, 6-mercaptodopamine was resistant to autoxidation at pH 7.4. Cyclic voltammometric analysis, however, indicated that electrochemically generated oxidation species of 6-mercaptodopamine are unstable and undergo spontaneous reaction, presumably intramolecular cyclization. In vivo tests revealed that 6-mercaptodopamine inhibits the uptake of tritium-labeled norepinephrine by isolated rat heart atria, although to a much lesser extent than 6-hydroxydopamine.

The catecholamine derivatives 6-hydroxydopamine (**1**, 6-OHDA) and 6-aminodopamine (**2**, 6-NH_2DA) have been shown to possess dramatic neurodestructive and enzyme-inhibiting properties.[1] In order to further evaluate the mechanisms of action of this class of compounds, we have initiated studies on the chemical and pharmacological characteristics of the corresponding sulfur analogue 2-(2-mercapto-4,5-dihydroxyphenyl)ethylamine (6-mercaptodopamine, 6-SHDA, **3**). A survey of the literature revealed that the synthesis of 6-SHDA had not been reported.



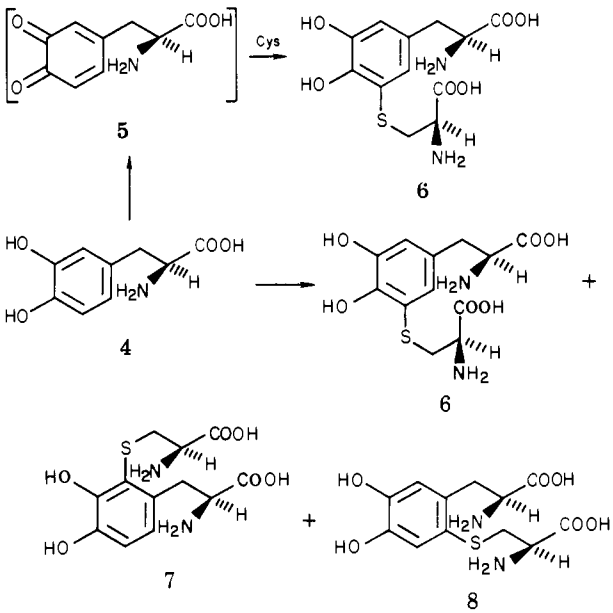
1 **2** **3**

Our current interest in quinone oxidation products of catecholamines[2] led to an examination of *o*-benzoquinones as possible intermediates for the introduction of a thiol-containing moiety into the so-called 6 position of the catechol ring system. The key step required the regiospecific 1,4 addition of an appropriate sulfur nucleophile at the C-5 ring carbon atom of a 4-substituted *o*-benzoquinone.

A review of the literature indicated that under neutral or acidic conditions sulfur nucleophiles add primarily 1,6 to 4-substituted *o*-benzoquinones. For example, reaction of (*S*)-dopaquinone (**5**) generated in situ from (*S*)-Dopa (**4**) with (*S*)-cysteine (Cys) gave the corresponding 5-substituted catechol **6** (Scheme I).[3,4] Incubation of 4 and (*S*)-cysteine in the presence of mushroom tyrosinase at pH 6.8 yielded the 5-, 2-, and 6-(*S,S*)-*S*-cysteinyldihydroxyphenylalanine adducts (**6–8**, respectively) in the ratio of 15:3:trace.[5] The same adducts in approximately the same ratio have been isolated from the tumors and urine of patients with malignant melanomas.[6]

In order to further evaluate the regioselectivity of this type of reaction in the hope of obtaining 1,4-adducts, we have examined the condensation of the readily available 4-methyl-*o*-benzoquinone (**9**)[7] with thioacetic acid. Nucleophilic addition to **9** would be expected to proceed either 1,4, to form the desired model thiol adduct **10**, or 1,6, to generate the isomeric adduct **11**. The results described below establish that the formation of either **10** or **11** may be achieved depending on reaction conditions.

Scheme I



5 **6** **4** **6** **7** **8**

Condensation of **9** and thioacetic acid either in methylene chloride or dilute aqueous HCl gave a product which displayed two meta-oriented aromatic proton signals (doublets, J = 2 Hz) (Scheme II). The spectrum also displayed two methyl singlets which we have interpreted in terms of a mixture of the *S*-acetyl compound **11** and its rearranged *O*-acetyl isomer **12**. On the other hand, the same reactants in neutral or basic solution provided a product whose NMR spectrum displayed two aromatic proton singlets, consistent with the 1,4-addition product **10**. Basic hydrolysis of these reaction products gave the corresponding thiols **13** and **14**. The 2-mercaptotoluene derivative **14** was a solid with identical physicochemical properties to those previously reported for this product which had been prepared by an independent route.[8] The isomeric 5-mercaptotoluene derivative **13** was a distillable liquid which was characterized by NMR and elemental analysis.

These results may be rationalized in terms of the characteristics of the thiol moiety. In water or basic solution, thioacetic acid (pK_a = 3.33) will be essentially completely ionized. Since the thiolate anion is a highly reactive nucleophile, it should attack preferentially at the more electropositive C-5 position of 4-methyl-*o*-benzoquinone (1,4 addition). In an aprotic medium such as methylene chloride or in an acidic aqueous solution, the

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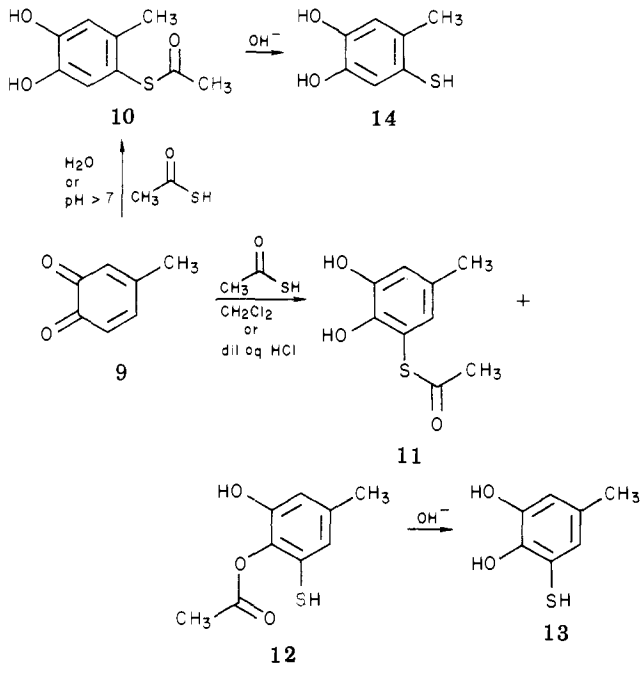
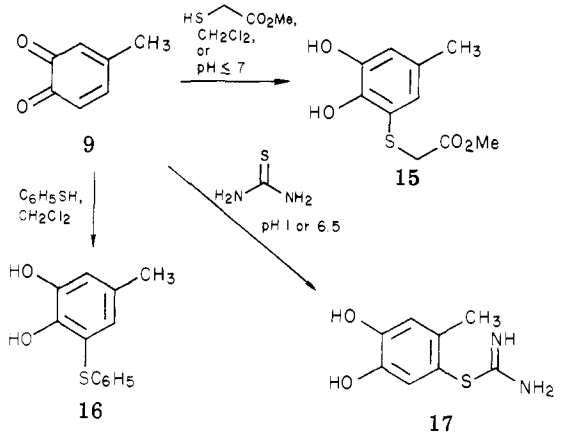
Scheme II



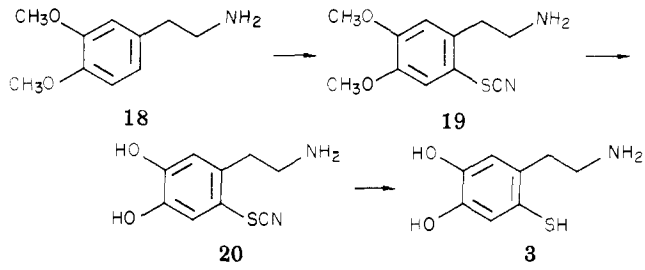
Scheme III



neutral mercaptan is the nucleophile. Since it is less reactive than the thiolate anion, it would be expected to react more selectively at C-6 (1-6 addition), since this position is less sterically hindered and the resulting anion enjoys greater resonance stabilization. Corroborative evidence was obtained with methyl thioglycolate. When this mercaptan was allowed to react with **9** either in methylene chloride, 1 N HCl–methanol, or water–methanol, the 1,6-adduct **15** was obtained in yields of 68, 68, and 80%, respectively (Scheme III). No recognizable product was obtained at pH values greater than 7.0, perhaps because of rapid self-condensation of the ester anion. The reaction with thiophenol and **9** in methylene chloride provided the 1,6-adduct **16** in 68% yield. However, no information could be obtained in aqueous solution due to the insolubility of thiophenol.

Results inconsistent with the above rationale, however, have been obtained with thiourea, which yielded the 1,4-adduct **17** exclusively in near-neutral[8] and acidic solutions. Consequently, inherent reactivity of the nucleophile may be only one factor influencing the regioselectivity of nucleophilic addition to *o*-benzoquinones. Furthermore, our attempts to introduce a protected thiol moiety into dopamine by nucleophilic addition to the in situ generated *o*-quinone failed. Therefore, a new approach

Scheme IV



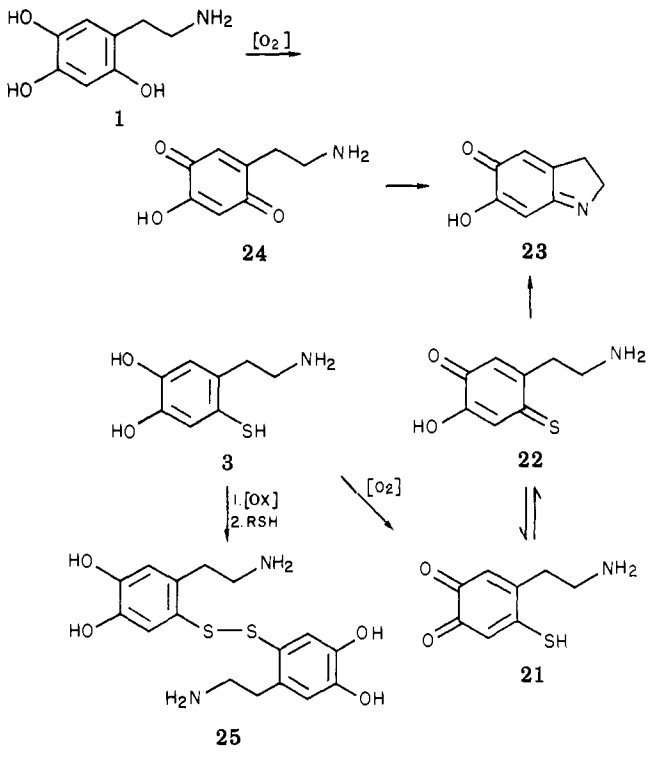
to the synthesis of 6-SHDA was undertaken.

In 1972, NIH workers reported that thiocyanogen chloride was an effective thiocyanation reagent for *m*-tyramine.[9] We attempted the same reaction with dopamine to prepare 6-thiocyanodopamine (**20**) directly. The reaction, however, failed, perhaps because of the poor solubility of dopamine hydrochloride in acetic acid. However, thiocyanation of 3,4-dimethoxyphenylethylamine (**18**) proceeded smoothly to provide 2-thiocyano-4,5-dimethoxyphenylethylamine (**19**), isolated as its HCl salt in 60% recrystallized yield (Scheme IV). Attempts to obtain 6-SHDA by heating **19** in refluxing aqueous HBr unexpectedly gave dopamine, which was identified by NMR and chemical-ionization MS. The mechanism of this interesting desulfurization reaction remains obscure. An alternative approach to **3** was successful. Demethylation of **19** with boron tribromide afforded 6-thiocyanodopamine (**20**) in 73% recrystallized yield. Reductive cleavage of the thiocyano group employing mercaptoethanol,[9] in situ generated NaSH,[9] and $LiAlH_4$[10] has been reported. Brief mention of $NaBH_4$ for this reaction was made by Olsen and Snyder.[11] We have found $NaBH_4$ to be the reagent of choice for the conversion of 6-thiocyanodopamine to 6-SHDA. Treatment of **20** with $NaBH_4$ in ethanol, followed by ion-exchange chromatography and elution with aqueous HCl, provided 6-SHDA (**3**) as its HCl salt in 66% yield. Careful recrystallization of the initially hygroscopic product from isopropyl alcohol/ethyl ether provided a stable, nonhygroscopic solid. It should be mentioned that the reverse approach, that is, reduction of the thiocyano group of **19** to the corresponding mercaptan followed by demethylation, proved less successful.

With 6-SHDA in hand, we turned our attention to a series of studies regarding its chemical and pharmacological characteristics. The ease with which 6-OHDA and 6-NH_2DA undergo air oxidation (at pH 7.4) and the reactivity of the resulting electrophilic species have led to the proposal that the neurotoxicity of these compounds is due to their ability to alkylate nucleophilic functionalities present on macromolecules.[12] By analogy with the proposed pathways for 6-OHDA, 6-SHDA could undergo spontaneous or enzyme-catalyzed oxidation to the corresponding electrophilic *o*-quinone **21** or *p*-thioquinone **22**. Subsequent cyclization would lead to the iminoquinone **23**, the same product obtained from the quinone **24** derived from 6-OHDA.[12,13] Alternatively, 6-SHDA could undergo oxidative coupling to the disulfide **25**.

In order to evaluate the 6-OHDA-like redox properties of 6-SHDA, we examined the ease of oxidation of 6-SHDA relative to 6-OHDA. UV analyses of 6-OHDA, dopamine, 6-SHDA, and 2-mercapto-4,5-dihydroxytoluene (**14**) illustrate the dramatic effect of the 6-hydroxy group of 6-OHDA on the ease of air oxidation. Although a pH 7.4 solution of dopamine was stable, UV analysis of the corresponding solution of 6-OHDA revealed the rapid formation of the zwitterionic quinone **24** (hypsochromic shift from λ_{max} 291 to 268 nm). Subsequent spectra

Scheme V



gradually displayed a bathochromic shift as the quinone **24** underwent intramolecular cyclization to the iminoquinone **23**. The spectrum of 6-SHDA shifted from λ_{max} 295 (observed at pH 5) to 306 nm following pH adjustment to 7.4. Even after standing for 2 h no further change in the spectrum was observed. The model mercaptocatechol **14** displayed nearly identical spectral characteristics to those of 6-SHDA. These results rule out the formation of iminoquinone **23** via oxidative cyclization of 6-SHDA at pH 7.4. Furthermore, pH 7.4 buffered solutions of 6-OHDA quickly turned red, consistent with formation of the red quinone **24**,[13] whereas the corresponding solutions of 6-SHDA remained colorless (Scheme V). Addition of a strong oxidizing agent (e.g., aqueous $NaIO_4$) to the solution of 6-SHDA resulted in the immediate formation of a red-orange color (possibly due to the *o*-quinone **21** or *p*-thioquinone quinone **22**). All of the results taken together would indicate that 6-SHDA does not undergo spontaneous oxidation at pH 7.4 to quinoid products. The spectral shift observed for a solution of 6-SHDA following adjustment of the pH to 7.4 simply may be due to formation of the thiolate anion, although the possible autoxidation to the disulfide dimer **25** clouds this issue.

Although 6-SHDA appears to be stable to autoxidation, the possibility that in vivo oxidation may lead to products similar to those formed from 6-OHDA prompted us to examine the chemical reactivity of electrochemically generated oxidation products of 6-SHDA. We utilized the technique of cyclic voltammetry for this study. As displayed in Figure 1a, at pH 3.5 the model compound 2-mercapto-4,5-dihydroxytoluene (**14**) undergoes simple and reversible oxidation and reduction as denoted by $\uparrow_1$ and $\uparrow_2$, respectively. At pH 3.5, the behavior of 6-SHDA parallels that of the model compound (Figure 1b). Only a simple and reversible redox couple ($\uparrow_3$ and $\uparrow_4$) again is observed. At pH 7.4, however, the cyclic voltammograms of these two compounds differ. The model compound still displays a simple redox couple, but the cyclic voltammogram of 6-SHDA is dramatically altered (Figure 1c).

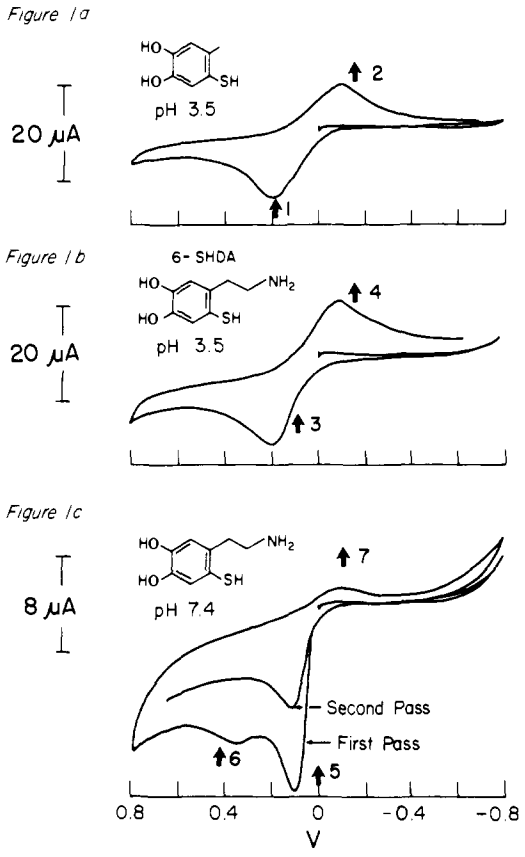


Figure 1. Cyclic voltammograms of 2-mercapto-4,5-dihydroxytoluene at pH 3.5 (a), of 6-mercaptodopamine at pH 3.5 (b), and of 6-mercaptodopamine at pH 7.4 (c). See text for discussion.

Note the loss of the peak at $\uparrow_6$ during the second oxidative scan and the difference in intensity between the oxidative and reductive peaks ($\uparrow_5$ and $\uparrow_7$, respectively). The oxidative peak intensities continue to decrease in successive scans and (although not shown here) the peak intensity at $\uparrow_7$ varies with scan rate, i.e., the slower the scan, the smaller the height of $\uparrow_7$ (peak height is a direct measure of the quantity of the species present). Peak $\uparrow_7$ is associated with the reduction of oxidized species (probably **21** or **22**) back to 6-SHDA. Thus, it would appear that the more time available following oxidation of 6-SHDA and before reduction (back to 6-SHDA), the more extensive the subsequent chemical reaction. Such events are consistent with participation of the aminoethyl side chain in an intramolecular cyclization reaction, similar to that observed with 6-OHDA and related systems.[14] Further characterization of these reaction products will be necessary, however, before any definitive structural assignments can be made.

Based on the analogous structural and redox properties of 6-SHDA and 6-OHDA, we undertook pharmacological studies in order to evaluate the neurotoxicity of 6-SHDA. The percent inhibition of tritiated norepinephrine uptake by heart atria isolated from rats previously administered 6-SHDA (iv) was compared with control and 6-OHDA-treated animals. This assay, which is well established for 6-OHDA,[15,16] provides a direct measure of the extent of neurodegeneration caused by the toxin. Twenty-four hours following intravenous administration of the drug, the rats were sacrificed, and the atria were removed, sectioned, and incubated at pH 7.4 and 37 °C with tritium-labeled norepinephrine. After homogenization and centrifugation, the supernatant fraction was counted for released radioactivity. Figure 2 summarizes the data which reveal

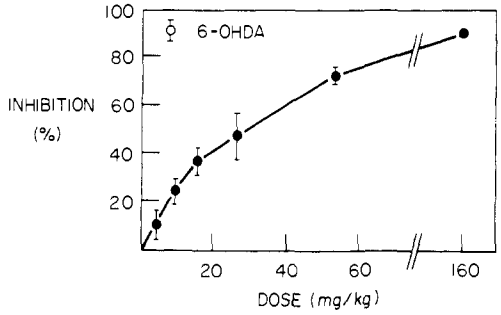


Figure 2. Inhibition of [^{3}H]NE uptake by isolated rat heart atria following in vivo administration of 6-SHDA. Each value (except 160 mg/kg) represents the average (± SD) of twelve determinations. For comparison, 6-OHDA (10 mg/kg only) was also assayed.

that, although considerably less potent than 6-OHDA, 6-SHDA is neurotoxic.

A preliminary attempt was made to determine if 6-SHDA crosses the blood–brain barrier and causes central neuronal damage. The percent inhibition of tritium-labeled norepinephrine uptake by the rat brain hypothalamus following iv drug administration was determined by an adaptation of the atria assay. No significant difference was observed between rats treated with 6-SHDA and controls. The same result was obtained with 6-OHDA. It is well documented[1,17] that administration of 6-OHDA directly into the brain results in extensive damage to the noradrenergic and dopaminergic nerve terminals. It is also known that 6-OHDA cannot effectively cross the blood–brain barrier when administered iv.[1,18] The absence of central neurotoxicity observed for 6-SHDA may also be a consequence of limited access to the brain.

A final pharmacological characterization of 6-SHDA concerned its pressor activity, which was measured in rats by procedures previously described in detail.[2] Table I summarizes the blood-pressure data obtained following iv administration of several catecholamines. Note that 6-SHDA is the least potent in the series studied and over 40 times less active than dopamine.

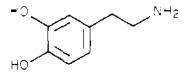
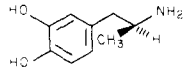
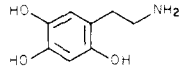
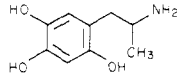
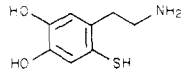
Summary and Conclusion

Our studies show that 6-SHDA is less easily oxidized than 6-OHDA and does not appear to undergo spontaneous oxidation at pH 7.4. In addition, although in vivo administration of 6-SHDA does cause significant inhibition of tritium-labeled norepinephrine uptake by rat atria in vitro, the potency of 6-SHDA is markedly less than that of 6-OHDA. This decrease in potency may be directly related to the higher redox stability of 6-SHDA compared to 6-OHDA. An additional factor may be that the structural perturbation caused by the thiol group is too dramatic to allow for the efficient concentration of 6-SHDA in noradrenergic terminals. The decreased pressor activity of 6-SHDA compared to 6-OHDA is consistent with this hypothesis.

An indication as to the mode of biological activity of 6-SHDA is suggested by the cyclic voltammometric analysis. Electrochemical oxidation of 6-SHDA at pH 7.4 generates a species which appears to react with the side-chain amino group. If formed, in vivo electrophiles such as **23** or **24** could react with nucleophilic moieties on macromolecules and cause neuronal damage. The possibility of disulfide bond formation between 6-SHDA and sulfhydryl groups of macromolecules provides an additional small molecule–macromolecule interaction not available to 6-OHDA or 6-NH_2DA.

The ready availability of synthetic 6-SHDA and its demonstrated neurotoxic activity should provide an impetus for further chemical and pharmacological studies.

Table I. In Vivo Pressor Activity[a]

compound	dose required for increasing systolic BP to 30 mmHg, g
	2.9×10^{-6}
	3.1×10^{-6}
	1.6×10^{-5}
	6.0×10^{-5}
	1.3×10^{-4}

[a] White, male Sprague-Dawley rats were utilized. Drugs were administered iv and blood pressure was monitored according to procedures previously described (see ref 2).

Experimental Section

A. Synthesis. All melting points and boiling points are uncorrected. ^{1}H NMR spectra were determined on either a Varian A-60A or a Perkin-Elmer R-12B 60-MHz spectrometer. Chemical-ionization mass spectrometry (CIMS) was performed on an AEI MS-902 mass spectrometer. Solvents were removed with a Büchi rotary evaporator. Infrared (IR) spectra were determined on a Perkin-Elmer 337 grating spectrophotometer. Microanalyses were performed by the University of California Microanalytical Laboratory, Berkeley, Calif.

Mixture of 3-(Acetylthio)-4,5-dihydroxytoluene (11) and 3-Hydroxy-4-acetoxy-5-mercaptotoluene (12). (a) Methylene Chloride Solvent System. To a solution of 2.0 g (16.14 mmol) of 4-methylcatechol (Aldrich) in 100 mL of water, cooled in an ice bath, was quickly added an ice-cold solution of 3.80 g (17.80 mmol) of sodium metaperiodate in 60 mL of water. The resultant red solution was stirred for 30 s and poured into 60 mL of methylene chloride. The aqueous layer was extracted with an additional 30 mL of methylene chloride, and the combined extract was dried with $MgSO_4$, filtered, and added dropwise over 30 min at room temperature to a solution of 1.28 mL (17.80 mmol) of thioacetic acid in 40 mL of methylene chloride. The yellow solution was stirred for 20 min and evaporated to provide 1.24 g (76%) of a yellowish solid. Recrystallization from chloroform afforded the white crystalline mixture of **11** and **12**: mp 110–118 °C; ^{1}H NMR (Me_2SO-d_6) δ 6.75 (d, J = 2 Hz, Ar H, 1), 6.60 (d, J = 2 Hz, Ar H, 1), 2.33 and 2.25 (s, $SCOCH_3$ and $OCOCH_3$, 3), 2.15 (s, $ArCH_3$, 3); IR ($CHCl_3$) 3530, 1770 cm^{-1}; CIMS (isobutane) MH^+ 119. Anal. ($C_9H_{10}O_3S$) C, H, S.

(b) Aqueous HCl Solvent System. To a solution of 0.264 mL (3.7 mmol) of thioacetic acid in 30 mL of 1 N aqueous HCl was added dropwise a solution of 450 mg (3.7 mmol) of 4-methyl-*o*-benzoquinone (**9**)[7] in 36 mL of water. After stirring for 5 min, the solution was extracted with methylene chloride (3 × 30 mL). The combined extract was dried ($MgSO_4$) and evaporated to yield 415 mg (57%) of the mixture of **11** and **12**, which was characterized by NMR.

2-(Acetylthio)-4,5-dihydroxytoluene (10). (a) Water Solvent System. To a solution of 0.59 mL (8.2 mmol) of thioacetic acid in 70 mL of water was added dropwise a solution of 1.0 g (8.2 mmol) of 4-methyl-*o*-benzoquinone (**9**) in 80 mL of water. After stirring for 5 min, the yellow solution was extracted with methylene chloride (3 × 50 mL). The combined extract was dried ($MgSO_4$) and evaporated to give 1.24 g (76%) of a viscous orange oil. Column chromatography (silica gel, 200–325 mesh, ether–petroleum ether) afforded a yellow-white solid residue.

Recrystallization from chloroform–petroleum ether afforded an analytical sample of **10**: mp 107.5–109.5 °C; ^{1}H NMR ($CDCl_3$) δ 6.75 (s, Ar H, 1), 6.62 (s, Ar H, 1), 6.0–6.5 (br m, OH, 2), 2.42 (s, $SCOCH_3$, 3), 2.15 (s, $ArCH_3$, 3); IR ($CHCl_3$) 3540, 3350, 1680, 1600 cm^{-1}; CIMS (isobutane) MH^+ 199. Anal. ($C_9H_{10}O_3S$) C, H, S.

(b) Aqueous Bicarbonate (pH 8.0) Solvent System. To a solution of 0.088 mL (1.23 mmol) of thioacetic acid in 10 mL of 5% aqueous $NaHCO_3$ (deoxygenated) was added dropwise a solution of 150 mg (1.23 mmol) of 4-methyl-*o*-benzoquinone (**9**) in 12 mL of water. After stirring for 5 min, the mixture was acidified with 4 N aqueous HCl and extracted with methylene chloride (2 × 15 mL). The combined extract was dried ($MgSO_4$) and evaporated to provide 140 mg (58%) of crude **10**.

The same reaction run in 0.1 N pH 7.4 phosphate buffer provided a 69% yield of **10**. The products of both runs gave NMR spectra identical with that of the product obtained in part a.

3-Mercapto-4,5-dihydroxytoluene (13). A mixture of 2.56 g (12.9 mmol) of **11** and **12** and 30 mL of deoxygenated 4 N aqueous NaOH was heated under reflux and a nitrogen atmosphere for 20 min. The reaction mixture was cooled, acidified with 35 mL of 4 N aqueous HCl, and extracted with chloroform (3 × 25 mL). The chloroform extract was dried ($MgSO_4$) and evaporated to a red oil (1.71 g). Column chromatography (silica gel, 200–325 mesh, 1:4 ether–petroleum ether) afforded an oil (1.32 g). Further purification by bulb to bulb distillation [oven temperature 90–95 °C (0.15 Torr)] afforded 0.97 g (48%) of **13** as a clear, colorless oil: ^{1}H NMR (Me_2SO-d_6) δ 6.84 (d, J = 2 Hz, Ar H, 1), 6.60 (d, J = 2 Hz, Ar H, 1), 3.54 (m, SH, 1), 2.15 (s, $ArCH_3$ 3); IR ($CHCl_3$) 3600, 3480, 1610 cm^{-1}. High-resolution EIMS Calcd for $C_7H_8O_2S$: M^+ 156.0244. Found: 156.0236. Anal. Calcd for $C_7H_8O_2S$: C, 53.85; H, 5.13; S, 20.51. Found: C, 54.48; H, 5.37; S, 19.93.

2-Mercapto-4,5-dihydroxytoluene (14). Hydrolysis of **10**, in the same manner as described for the mixture of **11** and **12**, provided mercaptan **14**, which was identified by comparison of melting point, mixture melting point, and ^{1}H NMR spectroscopy with 2-mercapto-4,5-dihydroxytoluene prepared by the literature method of Daneke et al.[8]

3-[[(Methoxycarbonyl)methyl]thio]-4,5-dihydroxytoluene (15). (a) Dichloromethane Solvent System. A dichloromethane solution of 4-methyl-*o*-benzoquinone (**9**; 8.07 mmol) was added to a solution of 0.72 mL (8.07 mmol) of methyl thioglycolate in dichloromethane. The solution was then stirred for 30 min and evaporated to a yellow-brown oil, which slowly crystallized. Distillation [bulb to bulb, oven temperature 120–130 °C (0.1 Torr)] afforded 1.255 g (68%) of **15** as a crystalline solid. Two recrystallizations provided an analytical sample: mp 89–90 °C; ^{1}H NMR ($CDCl_3$, 100 MHZ) δ 6.84 (d, J = 2 Hz, Ar H, 1), 6.77 (d, J = 2 Hz, Ar H, 1), 3.75 (s, OCH_3, 3), 3.52 (s, CH_2, 2), 2.22 (s, Ar CH_3, 3); IR ($CHCl_3$) 3540, 3300, 1735 cm^{-1}; CIMS (isobutane) MH^+ 229. Anal. ($C_{10}H_{12}O_4S$) C, H, S.

(b) Aqueous HCl–Methanol Solvent System. To a deoxygenated solution of 0.268 mL (3 mmol) of methyl thioglycolate in 5 mL of 1 N aqueous HCl and 15 mL of methanol maintained under nitrogen was slowly added a solution of 366 mg (3 mmol) of 4-methyl-*o*-benzoquinone (**9**) in 30 mL of deoxygenated water. The resulting solution was stirred for 5 min and extracted with methylene chloride (3 × 20 mL). The methylene chloride layer was dried ($MgSO_4$) and evaporated to yield 467 mg (68%) of **15** as an orange oil which crystallized on standing. The NMR spectrum of the product was identical with that described in part a.

(c) Water–Methanol Solvent System. To a deoxygenated solution of 0.268 mL (3 mmol) of methyl thioglycolate in 5 mL of water and 15 mL of methanol maintained under nitrogen was slowly added a solution of 366 mg (3 mmol) of 4-methyl-*o*-benzoquinone (**9**) in 30 mL of deoxygenated water. The resulting yellow solution was stirred for 5 min and evaporated to yield 548 mg (80%) of solid **15**. The NMR of the product was identical with that described in part a.

3-(Phenylthio)-4,5-dihydroxytoluene (16). A methylene chloride solution of 4-methyl-*o*-benzoquinone (**9**; 8.07 mmol) was added to a methylene chloride solution of thiophenol (0.8 mL, 8.07 mmol). After stirring for 30 min, the solution was evaporated to a light-orange oil. Bulb to bulb distillation [oven temperature 105–115 °C (0.01 Torr)] afforded 1.27 g (68%) of **16** as a yellow oil: ^{1}H NMR (Me_2SO-d_6) δ 9.9 (br m, OH, 2), 7.20 (m, 5 Ar H, 5), 6.65 (d, J = 2 Hz, Ar H, 1), 6.48 (d, J = 2 Hz, Ar H, 1), 2.10 (s, Ar CH_3, 3).

2-(4,5-Dihydroxy-*o*-tolyl)-2-thiopseudourea Hydrochloride (17). (a) Aqueous Solvent System. Catechol **17** was prepared by the literature procedure of Daneke et al.[8]

(b) Aqueous HCl Solvent System. To a solution of 280 mg (3.69 mmol) of thiourea in 30 mL of 1 N aqueous HCl was added dropwise a solution of 450 mg (3.69 mmol) of 4-methyl-*o*-benzoquinone (**9**) in 36 mL of water. The precipitated product was filtered. After washing with water and ethanol, the white solid was air-dried to provide 0.91 g (100%) of the HCl salt of **17**: ^{1}H NMR (Me_2SO-d_6) δ 7.0 (s, Ar H, 1), 6.88 (s, Ar H, 1), 6.0–6.8 (br m OH, 2), 2.20 (s, Ar CH_3, 3).

2-[2-(Cyanothio)-4,5-dimethoxyphenyl]ethylamine Hydrochloride (19). To a solution of 11.75 g (0.166 mol) of chlorine in 271 mL of dry glacial acetic acid, maintained under nitrogen and in an ice bath, was quickly added 15.08 g (0.166 mol) of oven-dried potassium thiocyanate in one batch. The mixture was then stirred at room temperature for 15 min. A solution of 15.0 g (0.083 mol) of 3,4-dimethoxyphenylethylamine (Aldrich) in 45 mL of acetic acid was then added dropwise over 10 min and the mixture was stirred for 30 min. After the addition of 30 mL of cyclohexene (to quench the excess thiocyanogen chloride), the mixture was filtered (to remove the KCl), and the resulting solution was poured into 500 mL of ether. The product, which immediately crystallized, was isolated and recombined with the prior isolated KCl, which also contained some precipitated product. This product mixture was heated in 600 mL of absolute ethanol and the hot ethanolic mixture was filtered. Upon addition of excess ether, crystals appeared. The solid was filtered, washed with ether, and air-dried to provide **19**. The process of extracting the product from the KCl was repeated, resulting in a total yield of 13.71 g (60%) of **19**. Recrystallization from ethanol–ether afforded an analytical sample: mp 212–214 °C; ^{1}H NMR (D_2O) δ 7.35 (s, Ar H, 1), 7.18 (s, Ar H, 1), 4.0 (s, CH_3O, 3), 3.95 (s, CH_3O, 3), 3.40 (m, CH_2CH_2, 4); IR (nujol) 2150, 1264, 1215, 1168, 1040, 1025, 870 cm^{-1}; CIMS (isobutane) MH^+ 239. Anal. ($C_{11}H_{15}ClN_2O_2S$) C, H, N, S.

2-[2-(Cyanothio)-4,5-dihydroxyphenyl]ethylamine Hydrochloride (20). To a slurry of 5.0 g (18.2 mmol) of 2-[2-(cyanothio)-4,5-dimethoxyphenyl]ethylamine hydrochloride (**19**) in 250 mL of methylene chloride under nitrogen at −60 °C was added 8.6 mL (5 equiv, 0.091 mol) of BBr_3. After stirring the mixture at −60 °C for 5 min and at room temperature for 2 h, methanol (60 mL) was added. Evaporation of the solvents afforded a light tan residue. This material was purified by ion-exchange chromatography (70 g of Bio-Rad AG 50W-X2, 50–100 mesh, and 4 N aqueous HCl) to afford 4.03 g of a yellow-white solid. This material was swirled with 100 mL of hot ethanol, cooled, and treated with 250 mL of ether. The solid was filtered, washed with ether, and air-dried to afford 3.25 g (73%) of white solid **20**, mp 184–187 °C. Recrystallization from ethanol–ether improved the melting point: mp 185.5–187.5 °C; ^{1}H NMR (D_2O) δ 7.05 (s, Ar H, 1), 6.94 (s, Ar H, 1), 2.9–3.4 (m, CH_2CH_2, 4); IR (Nujol) 2160 cm^{-1} (–SCN); CIMS (isobutane), perpentafluoropropionyl (PFP) derivative, MH^+ 649. Anal. ($C_9H_{11}ClN_2O_2S{\cdot}H_2O$) C, H, N.

2-(2-Mercapto-4,5-dihydroxyphenyl)ethylamine Hydrochloride (6-Mercaptodopamine Hydrochloride, 6-SHDA·HCl, 3). To a solution of 3.0 g (12.17 mmol) of 1-[2-(cyanothio)-4,5-dihydroxyphenyl]ethylamine hydrochloride (**20**) in 300 mL of absolute ethanol was added, in portions, 2.76 g (73.0 mmol) of $NaBH_4$ while the reaction vessel was cooled in an ice bath. The mixture was stirred at room temperature for 1.5 h. The sodium chloride was filtered and the reaction quenched with 250 mL of 5% HCl. Evaporation afforded a yellow-white solid residue. Ion-exchange chromatography of the residue (50 g of Bio-Rad AG 50W-X2, 50–100 mesh, and elution with 4 N aqueous HCl) was followed by rotary evaporation to provide a solid residue. To this material was added 100 mL of absolute ethanol and the mixture was heated on the steam bath. The white inorganic solid was filtered and the ethanol evaporated to yield 1.77 g (66%) of **3** as a light-yellow glassy solid. The product was hygroscopic. This material was dissolved in hot 2-propanol and ether was added.

A yellowish gum precipitated and was removed. Further addition of ether to the mother liquor resulted in crystallization of a fine, off-white solid. Filtration, ether washing, and air-drying afforded 0.59 g (22%) of nonhygroscopic **3**, mp 170–176 °C. Recrystallization from 2-propanol–ether provided an analytical sample: mp 173–177 °C; ^{1}H NMR (D_2O) δ 7.03 (s, Ar H, 1), 6.88 (s, Ar H, 1), 3.17–3.5 (m, CH_2, 2), 2.8–3.17 (m, CH_2, 2); IR (Nujol) 2525 cm^{-1} (–SH); CIMS (isobutane) MH^+ 186 and, as PFP derivative, MH^+ 770. Anal. ($C_8H_{12}ClNO_2S$) C, H, N, S.

B. Electrochemistry. Britton–Robinson buffers, utilized for all runs, were prepared from double glass-distilled water. All buffer solutions were electrolyzed for 24 h prior to use. Each cell solution was purged of oxygen and maintained under an atmosphere of oxygen-free nitrogen during all electrochemical studies.

A rapid voltage-scanning technique employing a stationary working electrode (carbon paste) in quiet solution and a repetitive, triangular (isosceles) wave potential sweep between it and a reference electrode (saturated calomel) was used. The sweep interval was −0.8 to +0.8 V vs. SCE. The anodic and cathodic currents were recorded as a function of the applied triangular potential sweeps on a X-Y recorder.

C. Animal Studies. In Vitro Norepinephrine Uptake Inhibition Study. Male Sprague–Dawley rats (Simonson Co.) weighing 150 ± 10 g were utilized. The 6-OHDA·HBr was purchased from Aldrich. The appropriate dose of each compound was dissolved in 0.9% sterilized saline containing 0.1% ascorbic acid (to prevent autoxidation prior to delivery) and administered intravenously in a volume of 0.2 mL. Control animals were administered 0.2 mL of vehicle. The rats were sacrificed after 24 h, and the left and right atria were removed and sectioned into 10–15-mg pieces. The hypothalamus was sectioned with a hand-microtome[19] into 10–15-mg pieces of 0.5-mm thickness in a cold room. The incubation medium for the atria and brain sections consisted of NaCl (154 mM), KCl (3.85 mM), $CaCl_2$ (1.5 mM), $MgSO_4$ (0.65 mM), dextrose (10 mM), and ascorbic acid (1.14 mM) and was basified to pH 7.4 with aqueous NaOH. The requisite amount of norepinephrine-7,8-t_2 (New England Nuclear, 20–40 Ci/mmol) was added to the medium to provide a 10^{-8} M solution (0.25 μCi/mL) of the catecholamine. The sections were placed in individual vials, 1-mL aliquots of [^{3}H]norepinephrine containing medium was added, and the samples were incubated under an atmosphere of 95% O_2–5% CO_2 at 37 °C for 30 min in a metabolic shaker. The tissue sections were removed, placed in fresh vials, 1-mL aliquots of [^{3}H]norepinephrine-free medium was added, and the incubation was resumed as before for another 10 min. Each section was homogenized with 0.5 mL of 0.4 N aqueous $HClO_4$, the homogenates were centrifuged to provide pellets, and 0.2-mL aliquots of the supernatants were removed and placed in individual scintillation vials containing 10-mL portions of Aquasol (New England Nuclear). The solutions were then counted for released radioactivity.

Catecholamine Pressor Activity Studies. The pressor effects of the amines summarized in Table I were determined as described in a previous report.[2] Male Sprague–Dawley normotensive rats weighing 380 ± 20 g were utilized.

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