trated. The residue was chromatographed (benzene; 50% $CHCl_3$–benzene) to give an oil: yield 4.7 g (97%); TLC R_f 0.27 (50% $CHCl_3$–benzene). A mixture of this oil (4.7 g, 10.7 mmol) and anisole (21.6 g, 200 mmol) was cooled to 0 °C, and trifluoroacetic acid (60 mL) was added dropwise over 60 min. After 60 min, the mixture was concentrated, and the residue was dissolved in ether and extracted with saturated $NaHCO_3$. The aqueous layer was washed with Et_2O, acidified, and worked up (Et_2O) to give 3.0 g of oil. The ^1H NMR of this material shows two sharp peaks for the *S*-acetylmethyl groups, indicating a mixture of isomers. Fractional crystallization (isopropyl ether–hexane) gave isomer A (**23a**): yield 700 mg (24%); mp 103–108 °C. Anal. ($C_{13}H_{20}O_4S$) C, H, S. The mother liquors were taken to dryness to give 1.1 g of oil, very much enriched in isomer B, as judged by ^1H NMR. This crude isomer B was used without further purification.

***trans*-2-[3-(Acetylthio)-1-oxopropyl]cyclopentanecarboxylic Acid (41a).** Using the procedure described above for **23a,b**, enone **20** was converted to acid **22**, obtained as an oil, which was used without further purification in the synthesis of **43**: TLC R_f 0.53 ($CHCl_3$–MeOH, 9:1).

Ketone Analogues 32 (Table I) and 41–46 (Table II). ***trans*-2-(3-Mercapto-2-methyl-1-oxopropyl)cyclohexanecarboxylic Acid (Isomer A, 46a).** Acid **23a** (isomer A; 680 mg, 2.5 mmol) was stirred with 1.5 mL of concentrated NH_4OH and 1.5 mL of water for 30 min, and the mixture was cooled, acidified, and worked up (EtOAc). The residue was chromatographed (2% MeOH–$CHCl_3$) and recrystallized (isopropyl ether) to give acid **46a**: yield 230 mg (40%); mp 87–90 °C; TLC R_f 0.43 (10% MeOH–$CHCl_3$). Anal. ($C_{11}H_{18}O_3S$) C, H, S.

By the method described above, the following ketone analogues were prepared.

***trans*-2-(3-Mercapto-2-methyl-1-oxopropyl)cyclohexanecarboxylic Acid (isomer B, 46b)**: mp 91–93 °C (diisopropyl ether), 22% yield from crude **23b** (isomer B) described above. Anal. ($C_{11}H_{18}O_3S$) C, H, S.

***trans*-2-(3-Mercapto-1-oxopropyl)cyclohexanecarboxylic acid (45)**: mp 71–74 °C (diisopropyl ether–hexane); 64% yield from **16d**. Anal. ($C_{10}H_{16}O_3S$) C, H, S.

***cis*-2-(3-Mercapto-1-oxopropyl)cyclohexanecarboxylic acid (44)**: mp 82.5–84.5 °C (diisopropyl ether); 72% yield from **16c**. Anal. ($C_{10}H_{16}O_3S$) C, H, S.

***cis*-2-(3-Mercapto-1-oxopropyl)cyclobutanecarboxylic acid (41)**: 66% yield as an oil from **16b**; TLC R_f 0.43 ($CHCl_3$–MeOH, 9:1); dicyclohexylamine salt, mp 129–132 °C (EtOAc). Anal. ($C_8H_{13}O_3S \cdot C_{12}H_{23}N$), C, H, N, S.

6-Mercapto-4-oxohexanoic acid (32): mp 40–42 °C (Et_2O–hexane); 58% yield from **16a**. Anal. ($C_6H_{10}O_3S$) C, H, S.

***trans*-2-(3-Mercapto-1-oxopropyl)cyclopentanecarboxylic acid (43)**: 45% yield as an oil from **22**; TLC R_f 0.45 ($CHCl_3$–MeOH, 9:1); dicyclohexylamine salt, mp 126–128 °C (diisopropyl ether). Anal. ($C_9H_{14}O_3S \cdot C_{12}H_{23}N$) C, H, N, S.

***trans*-2-(3-Mercapto-1-oxopropyl)cyclobutanecarboxylic acid (42)**. Acid **16b** (460 mg, 2 mmol) and NaOMe (324 mg, 6 mmol) were stirred for 2 h at 0 °C in 10 mL of MeOH, and the mixture was acidified, concentrated, and worked up (EtOAc). The residue was purified on a preparative TLC plate (silica gel, 10% MeOH–$CHCl_3$, R_f 0.55) and converted to a DCHA salt (ether): yield 250 mg (34%); mp 140–144 °C. Anal. ($C_8H_{12}O_3S \cdot C_{12}H_{23}N$) C, H, N, S.

4-Hydroxy-6-mercaptohexanoic Acid (34). Sodium borohydride (11.4 g, 300 mmol) was added portionwise over 3 h to a suspension of **16a** (12.24 g, 60 mmol) in 120 mL of H_2O at 0 °C. The mixture was stirred for 60 min, acidified by the slow addition of 2 N HCl, and let stand overnight. Workup (EtOAc) gave an oil, which was mainly the lactone derivative of hydroxy acid **32** as judged by IR. The oil was dissolved in benzene, a small amount of *p*-toluenesulfonic acid was added, and the mixture was heated under reflux in a nitrogen atmosphere and with a water trap for 1.5 h. After the mixture was cooled, a small amount of insoluble material was removed by filtration, and the solvent was removed in vacuo, leaving an oil, which was chromatographed ($CHCl_3$) to give 7.4 g (84%) of colorless oil. Distillation of a 2-g sample gave 1.2 g (51%) of 4,5-dihydro-5-(2-mercaptoethyl)-2(3*H*)-furanone, bp 118–120 °C (0.6 mm). The above lactone (2.0 g, 13.7 mmol) in 20 mL of 1 N NaOH was heated under reflux for 2 h, and the mixture was cooled and then acidified with dilute acetic acid. Workup afforded an oil (1.8 g, 80%), TLC R_f 0.10 (5% MeOH–$CHCl_3$), which was converted to the DCHA salt (EtOAc): yield 1.7 g; mp 123–126 °C. Anal. ($C_{18}H_{35}O_3NS$) C, H, N, S.

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Catechol *O*-Methyltransferase. 10. 5-Substituted 3-Hydroxy-4-methoxybenzoic Acids (Isovanillic Acids) and 5-Substituted 3-Hydroxy-4-methoxybenzaldehydes (Isovanillins) as Potential Inhibitors

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A series of 5-substituted 3-hydroxy-4-methoxybenzoic acids (isovanillic acids) and -benzaldehydes (isovanillins) have been synthesized and evaluated as inhibitors of rat liver catechol *O*-methyltransferase. The compounds exhibited either noncompetitive or competitive patterns of inhibition when 3,4-dihydroxybenzoic acid was the variable substrate. The benzaldehydes were significantly more potent inhibitors than the corresponding benzoic acids, and electron-withdrawing substituents in the 5 position greatly enhanced their inhibitory activity.

The extraneuronal inactivation of catecholamines and the detoxification of many xenobiotic catechols are dependent upon the enzyme catechol *O*-methyltransferase (COMT, EC 2.1.1.6). COMT is a soluble, magnesium-requiring enzyme which catalyzes the transfer of a methyl group from *S*-adenosylmethionine (SAM) to a catechol substrate, resulting in the formation of the meta- and para-O-methylated products.[1]

Inhibition of COMT as a means of controlling endogenous catecholamine metabolism or preventing the inactivation of exogenously administered catechols has been the subject of considerable research interest.[2] Many of the COMT inhibitors identified to date have potent inhibitory effects in vitro, but in general they are less effective in vivo. Their reduced effectiveness in vivo appears to result from a number of factors, including poor bioa-

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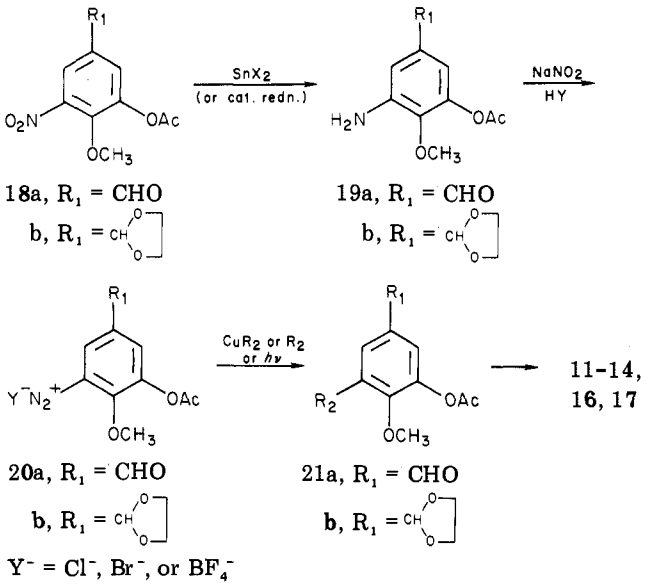
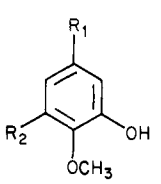
Scheme I



vailability, rapid metabolism, and undesirable distribution and excretion profiles. Effective inhibition of COMT in vivo can only be achieved by repeated dosing with substantial amounts of the drug.[2]

In 1970, Nikodejevic et al.[3] described the in vitro and in vivo COMT inhibitory activity of 3,4-dimethoxy-5-hydroxybenzoic acid (**2**), 3,5-dihydroxy-4-methoxybenzoic acid, and a series of structurally related compounds. Several of these benzoic acids were nontoxic and showed COMT inhibitory activity in vivo. However, the inhibitory effects were of short duration and were achieved only with high doses.

These observations prompted our laboratory to undertake the synthesis of a series of 5-substituted 3-hydroxy-4-methoxybenzoic acids (**1–9**) and -benzaldehydes (**10–17**).



	R_1	R_2		R_1	R_2
1	CO_2H	H	10	CHO	H
2	CO_2H	OCH_3	11	CHO	F
3	CO_2H	Cl	12	CHO	Cl
4	CO_2H	Br	13	CHO	Br
5	CO_2H	I	14	CHO	I
6	CO_2H	NO_2	15	CHO	NO_2
7	CO_2H	NH_2	16	CHO	OH
8	CO_2H	$N(CH_3)_2$	17	CHO	SC(S)OEt
9	CO_2H	CN			

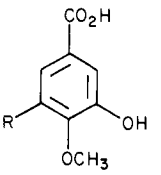
In this way we hoped to define the structure–activity relationship for the COMT inhibitory properties of these compounds. The synthesis and biological evaluation of compounds **1–17** are the subjects of this paper.

Results and Discussion

Chemistry. Since 3-acetoxy-4-methoxy-5-nitrobenzoic acid and -benzaldehyde were readily available by known methods,[4,5] these compounds served as the starting points for the synthesis of the target compounds **3–9** and **11–17**. In the benzaldehyde series (Scheme I), the chlorinated and brominated compounds **12** and **13** were prepared by reduction of the nitro compound **18a** with the appropriate tin halide to **19a** and then treatment of **19a** with sodium nitrite and the appropriate hydrohalous acid to yield **20a**. Compound **20a** was treated with the appropriate cuprous halide to yield **21a** (R = Cl or Br), which was hydrolyzed to the desired compound **12** or **13**.[6] In the preparation of the iodo compound **14**, the nitrobenzaldehyde **18a** was reduced with stannous chloride to **19a**. Amine **19a** was diazotized with sodium nitrite and HCl to **20a**, which was then treated with KI to yield **21a** (R_2 = I).[7] Hydrolysis of **21a** afforded **14**. Where the tin halide reduction method was not appropriate, the nitroaldehyde **18a** was converted to the corresponding acetal **18b** with ethylene glycol. The nitro compound **18b** was then catalytically reduced with PtO_2 to **19b**.[8] The fluorinated compound **11** and the hydroxylated compound **16** were obtained by diazotization of **19b** in HBF_4, followed by photolysis.[9] The xanthate derivative **17** was prepared by diazotization of **19b** in HCl, followed by neutralization and treatment with potassium xanthate.[10]

The halogenated acids **3–5** were readily obtained by Ag_2O oxidation of the corresponding aldehydes **12–14**, respectively.[11] The amino compound **7** was prepared by catalytic reduction (PtO_2) of the known nitro compound **6**. The dimethylated amine **8** was prepared from the amino compound **7** by alternate additions of formaldehyde, sodium cyanoborohydride, and acetic acid.[12] The cyano

Table I. Kinetic Parameters for 5-Substituted 3-Hydroxy-4-methoxybenzoic Acid as Inhibitors of COMT



no.	R	inhibn pattern[a]	kinetic inhibn constants,[b] μM K_{is}	K_{ii}
1	H	NA		
2	OCH_3	NC	999 ± 191	521 ± 148
3	Cl	C	394 ± 75	
4	Br	C	340 ± 47	
5	I	NA		
6	NO_2	NC	50.2 ± 9.1	247 ± 155
7	NH_2	NA		
8	$N(CH_3)_2$	NA		
9	CN	NC	589 ± 211	571 ± 222

[a] NA = no inhibitory activity of COMT when tested at a concentration of 1 mM; C = competitive kinetics; NC = noncompetitive kinetics. [b] Assay mixtures contained 3,4-dihydroxybenzoic acid as the variable substrate (0.02–0.20 mM), SAM (0.2 mM), pH 7.6 phosphate buffer (0.1 M), Mg^{2+} (1 mM), and DTT (4 mM).

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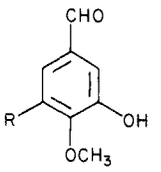
Table II. Kinetic Parameters for 5-Substituted 3-Methoxy-4-hydroxybenzaldehydes as Inhibitors of COMT



no.	R	inhibn pattern[a]	kinetic inhibn constants,[b] μM K_{is}	K_{ii}
10	H	NA		
11	F	NC	56.9 ± 10.2	48.4 ± 11.5
12	Cl	NC	31.6 ± 7.2	10.8 ± 3.0
13	Br	NC	31.8 ± 7.0	16.5 ± 5.3
14	I	NC	111.3 ± 30.2	56.6 ± 21.9
15	NO_2	C	0.70 ± 0.02	
16	OH	NC	9.99 ± 4.8	16.1 ± 11
17	SC(=S)OEt	NC	885 ± 407	272 ± 72

[a] NA = no inhibitory activity of COMT when tested at a concentration of 1 mM; C = competitive kinetics; NC = noncompetitive kinetics. [b] Assay conditions were identical with those described in Table I.

compound **9** was prepared by diazotization of amine **7**, followed by reaction of the diazonium intermediate with cuprous cyanide.[13] The methoxy acid **2** was a byproduct of the monomethylation of gallic acid using dimethyl sulfate.[14]

Biology. The 5-substituted 3-hydroxy-4-methoxybenzoic acids and -benzaldehydes synthesized in this study were evaluated as inhibitors of rat liver COMT. In Tables I and II are shown the kinetic parameters and the kinetic inhibition constants for compounds **1–17** as inhibitors of COMT. The benzoic acid derivatives **1–9** exhibited either noncompetitive or competitive kinetic patterns when 3,4-dihydroxybenzoic acid was the variable substrate. The benzaldehyde derivatives were, in general, noncompetitive inhibitors, except for the nitro derivative **15**, which exhibited competitive kinetics. In earlier studies on structurally related compounds, Nikodejevic et al.[3] had reported that 3,5-dihydroxy-4-methoxybenzoic acid was a noncompetitive inhibitor of COMT. In the current study, the benzaldehydes **10–17** were significantly more potent inhibitors of COMT than the corresponding benzoic acid derivatives **1–9**. Electron-withdrawing substituents in the 5 position of the benzaldehydes greatly enhanced the inhibitory activity, with the most potent member of the series being the nitro derivative **15** (K_{is} = 0.70 ± 0.02 μM).

In general, the inhibitory activities of compounds **1–17** toward COMT were disappointing, except for the activity of the nitrobenzaldehyde derivative **15**. In vivo studies will be necessary to determine the potential of the more active benzaldehyde derivatives as inhibitors of COMT.

However, one potential problem with phenolic compounds in general as inhibitors of COMT is that in vivo they might undergo rapid conjugation by either sulfation or glucuronidation. Preliminary results from our laboratory (Borchardt and Pinnick, unpublished data), in fact, indicate that several classes of COMT inhibitors, including the benzaldehyde derivatives **10–17**, are excellent substrates for phenol sulfotransferase. This metabolism by sulfation probably accounts for the transient in vivo effects of the known phenolic COMT inhibitors.[2,3] Considering the fact that these COMT inhibitors can also interact with phenol sulfotransferase raises a serious question of whether the transient pharmacological or physiological effects, which have been reported with COMT inhibitors in vivo,[2] results from their effects on O-methylation or their effects on sulfation. O-Methylation and sulfation appear to be parallel pathways for the metabolism of catecholamines. Specific inhibitors for COMT that do not alter sulfation will need to be identified in order to properly assess the true physiological role of COMT.

One very interesting compound that emerged from these studies was 5-hydroxy-3-mercapto-4-methoxybenzoic acid.[17] This analogue appears to be an affinity-labeling reagent for COMT, because of its ability to form a ligand–protein disulfide bond. This affinity-labeling mechanism is similar to that proposed by Lutz et al.[19] for the inactivation of COMT by 3-mercaptotyramine. Therefore, more selective and effective COMT inhibitors may only arise based on information concerning the nature of the enzyme's active site and its mechanism of catalysis.

Experimental Section

Materials. *S*-Adenosyl-L-(*methyl*-^{14}C)methionine (SAM-$^{14}CH_3$, New England Nuclear Corp., 50–60 mCi/mmol) was diluted to a concentration of 8.8 μCi/mL and stored at −20 °F. SAM chloride (Sigma Chemical Co.) was stored as a 10 mM aqueous stock solution at the same temperature. 3,4-Dihydroxybenzoic acid and isovanillin (**10**) were obtained from Aldrich Chemical Co., and dithiothreitol (DTT) and isovanillic acid (**1**) were obtained from Sigma Chemical Co. 3-Hydroxy-4-methoxy-5-nitrobenzoic acid (**6**) and -benzaldehyde (**15**) were prepared by known literature procedures.[4,5] The methoxy acid **2** was isolated as a byproduct of the monomethylation reaction of gallic acid using dimethyl sulfate.[14]

COMT Purification and Assay. COMT was purified from rat liver (male, Sprague–Dawley, 180–200 g) using a modification[15,16] of the procedure described by Nikodejevic et al.[3] The enzyme preparation used in the kinetic experiments was purified through the calcium phosphate step, resulting in a 48-fold increase in specific activity as compared to the crude supernatant. This enzyme preparation had a specific activity of 49.6 nmol of product (mg of protein)$^{-1}$ min^{-1} using 3,4-dihydroxybenzoic acid as the substrate. The enzyme activity was determined using SAM-$^{14}CH_3$ and 3,4-dihydroxybenzoic acid as substrates according to a previously described radiochemical assay.[15] Upon prolonged storage, some loss of enzyme activity was detected. Therefore, prior to use, COMT was routinely reactivated by preincubation for 40 min at 37 °C in phosphate buffer (pH 7.6) containing 4 mM DTT.

The kinetic inhibition patterns and the corresponding kinetic inhibition constants (K_{is}, K_{ii}) were determined using 3,4-dihydroxybenzoic acid as the variable substrate (0.02–0.20 mM). The incubation mixtures also contained SAM (0.2 mM), 0.05 μCi of SAM-$^{14}CH_3$, Mg^{2+} (1 mM), and DTT (4 mM) in pH 7.6 phosphate buffer (100 mM). Data were analyzed by making plots of the reciprocal velocities vs. reciprocals of the substrate concentrations. In all cases, linear relationships were observed. These data were fitted by computer to the appropriate equations, and the inhibition constants calculated.[20]

Chemical Methods. Melting points were obtained on a Thomas-Hoover Uni-melt apparatus and are uncorrected. IR data were recorded on a Beckman IR-33 spectrophotometer, and NMR

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data were recorded on a Perkin-Elmer R-24B spectrophotometer. Scintillation counting was done on Beckman LS-3133T and LS-7500 scintillation counters. TLC were run on Analtech silica gel GHLF (250 μm) plates, and the spots were detected by visual inspection under UV light. Mass spectra were recorded on a Varian MAT CH-5 spectrophotometer interfaced with a Digital PEP-8 computer.

3-Chloro-5-hydroxy-4-methoxybenzaldehyde (12). A 7.0 g (36 mmol) sample of stannous chloride was dissolved in 9.25 mL of concentrated HCl and cooled to 0–5 °C (N_2). Then, 2.0 g of 3-hydroxy-4-methoxy-5-nitrobenzaldehyde (15)[4] was added to the mixture in one portion with stirring. The ice bath was removed, and the temperature was allowed to rise, reaching a maximum of about 65 °C. The mixture was again cooled to 0–2 °C and checked for completeness of reaction by TLC on silica gel (cyclohexane–EtOAc, 2:3). A 23% sodium nitrite solution was then added dropwise while maintaining the temperature at 0–5 °C. When approximately 2.3 mL of the nitrite solution had been added, the mixture became positive to starch–iodine paper. This procedure required approximately 1.5 h. Meanwhile, a freshly prepared cuprous chloride solution was made by first dissolving 2.9 g (12 mmol) of cupric sulfate and 2.5 g (43 mmol) of sodium chloride in 9.2 mL of H_2O. This mixture was combined with a separately prepared solution of 6.3 g (33 mmol) of sodium metabisulfite and 0.415 g of sodium hydroxide (10 mmol) in 4.6 mL of H_2O. The resulting cuprous chloride solution was warmed to 75 °C, and the diazonium mixture was added slowly to it with stirring. The composite was then allowed to cool to room temperature, and an additional 13 mL of concentrated HCl was added. While the solution was standing overnight, some material precipitated. The mixture was extracted thoroughly with Et_2O, and these combined extracts were filtered and dried over $MgSO_4$. Filtration and evaporation in vacuo gave an oil, which was taken up in 10 mL of EtOH and diluted with 300 mL of boiling H_2O. A gelatinous precipitate was filtered, and the supernatant solution was allowed to cool. The resulting two crops of crystals were collected and purified on a silica gel column (cyclohexane–EtOAc). Recrystallization from hot H_2O gave 0.35 g (18.3%) of product: mp 114–117 °C; IR (KBr) 3430 (OH), 3080, 3060, 3020, 2960, 2890 and 2860 (CH), 1700 (C=O, conjugated), 1620, 1590, 1550, 1540, and 1510 (Ar) cm^{-1}; NMR ($CDCl_3$) δ 10.00 (s, 1, CHO), 8.00 and 7.75 (2 d, J = 2 Hz, 1 each, Ar H's), 6.40 (s, 1, OH), 4.05 (s, 3 OCH_3). Anal. ($C_8H_7O_3Cl$) C, H, Cl.

3-Bromo-5-hydroxy-4-methoxybenzaldehyde (13). In a three-neck flask charged with nitrogen was placed 3.7 g (31 mmol) in tin metal and 15 mL (130 mmol) of 48% HBr. The mixture was heated with stirring until all the tin had dissolved and then cooled to 10 °C. To this was added 2.39 g (10 mmol) of 3-acetoxy-4-methoxy-5-nitrobenzaldehyde (**18a**) in one portion. The temperature of the reaction spontaneously warmed to 65 °C and then subsided. After 40 min, a TLC (silica gel, 2:3 cyclohexane–EtOAc) showed the reduction of the nitro group to be complete; so the mixture was cooled to −2 °C. A 30% solution of sodium nitrite was slowly added so as to maintain a temperature of 0–3 °C until a positive starch–iodine test was achieved (ca. 2.5–2.7 mL of solution).

Meanwhile, a freshly prepared cuprous bromide solution was made by first dissolving 2.83 g (11.4 mmol) of cupric sulfate and 4.22 g (41 mmol) of sodium bromide in 9.1 mL of hot (75 °C) water. To this was added a solution of 0.625 g (3.29 mmol) of sodium metabisulfite ($Na_2S_2O_5$) and 0.415 g (10.4 mmol) of NaOH in 4.6 mL of H_2O. The resulting dark mixture was then swirled while the above diazo bromide solution was added in several portions. An additional 5 mL of HBr was used to complete the transfer of reagents. After stirring at room temperature overnight, the mixture was extracted with Et_2O, and then the Et_2O was extracted with brine, saturated sodium bicarbonate, and brine. Drying over $MgSO_4$ and evaporation of the solvent at reduced pressure yielded 1.80 g (78%) of a crude brown oil.

Column chromatography of this crude product (silica gel, cyclohexane–EtOAc) gave a major fraction, which was shown by NMR to consist of a mixture of the 5-acetyl and 5-hydroxy compounds. Therefore, the sample was hydrolyzed using a 2% NaOH solution on a 2.5 mL/100 mg of sample basis. The sample was boiled under N_2 until it just dissolved, and then it was acidified with 5% HCl. The aqueous mixture was extracted with Et_2O, and the Et_2O was extracted with saturated $NaHCO_3$ and brine. The Et_2O was evaporated, and the off-white solid was recrystallized from EtOH–H_2O to give 809 mg (35%) of pure sample: mp 117–118 °C; IR (KBr) 3500–3100 (OH), 2950 (saturated CH), 2870, 2780 (aldehyde CH), 1690 (C=O, conjugated), 1600, 1570, 1485, 1450 (Ar=C) cm^{-1}; NMR ($CDCl_3$) δ 9.85 (s, 1, CHO), 7.62 (d, J = 4 Hz, 1, H-6), 7.40 (d, J = 4 Hz, 1, H-2), 6.15 (s, 1, OH), 4.00 (s, 3, OCH_3). Anal. ($C_8H_7O_3Br$) C, H, Br.

5-Hydroxy-3-iodo-4-methoxybenzaldehyde (14). A slurry of 7.0 g (31.1 mmol) of stannous chloride in 9.2 mL of concentrated HCl was stirred at 0–5 °C under nitrogen while 2.39 g (10 mmol) of 3-acetoxy-4-methoxy-5-nitrobenzaldeyde (**18a**) was added in one portion. Heat from the resulting reaction caused the internal temperature to rise to about 80 °C. The reaction subsided, and the mixture was stirred for 40 min. When a TLC (silica gel, 2:3 cyclohexane–EtOAc) showed this reaction to be complete, the solution was cooled to 0 °C, and 2.9 mL of a solution containing 0.725 g (10.5 mmol) of sodium nitrite was added gradually below the surface of the liquid at a rate so as to maintain the temperature at 0–2 °C. At this point the mixture gave a positive starch–iodine test, showing completion of the diazotization. This mixture was then poured into a solution of 1.75 g (10.5 mmol) of KI in 5.0 mL of water and allowed to stand overnight. The mixture was warmed slightly to ensure removal of nitrogen and then extracted with Et_2O. The Et_2O was washed with 5% sodium thiosulfate, saturated $NaHCO_3$, and brine. After the solution was dried over $MgSO_4$, the Et_2O was evaporated to dryness, resulting in 1.82 g (65%) of a residue, which was shown by NMR to still contain 10% of the acetate compound. Therefore, the material was taken up in 25 mL of 2% NaOH (under N_2) and heated to reflux until all the solid had dissolved (ca. 30 min). Acidification, extraction with Et_2O, and evaporation gave 1.82 g of a residue, which was applied to a silica gel column (30 g) in a small amount of EtOAc. A gradient of cyclohexane–EtOAc was used, beginning with 100% cyclohexane and finishing with 70% cyclohexane–EtOAc. Recrystallizations of the appropriate fractions from 5% EtOH–H_2O gave a total of 1.37 g (49%): mp 129–31 °C; IR (KBr) 3400–3000 (phenol OH), 2940 (saturated CH), 2850 and 2760 (aldehyde CH), 1685 (conjugated C=O), and 1590, 1550, 1480 (aromatic C=C) cm^{-1}; NMR ($CDCl_3$ δ 9.90 (s, 1, CHO), 7.87 (d, J = 3 Hz, 1, H-6), 7.45 (d, J = 3 Hz, 1, H-2), 6.05 (s, 1, OH), 4.00 (s, 3, OCH_3). Anal. ($C_8H_7O_3I$) C, H, I.

2-(3-Acetoxy-4-methoxy-5-nitrophenyl)dioxolane (18b). A 6.0 g (25 mmol) sample of 3-acetoxy-4-methoxy-5-nitrobenzaldehyde (**18a**) was dissolved in 600 mL of toluene and, together with 0.60 g of *p*-toluenesulfonic acid monohydrate and 3.0 mL of ethylene glycol, was refluxed overnight with a Dean-Stark trap. The reflux mixture was allowed to cool and was treated with a small amount of anhydrous $NaHCO_3$. The organic layer was washed with aqueous $NaHCO_3$ and brine and dried over Na_2SO_4. The material was evaporated under reduced pressure to give 7.41 g (>100%) of a product suitable for further transformation: NMR ($CDCl_3$–Me_4Si) δ 7.55 and 7.95 (2 d, J = 3 Hz, 1 each, Ar), 5.95 (s, 1, Ar CH), 4.10 [s, 4, $(OCH_2)_2$], 4.05 (s, 3, OCH_3), 2.50 (s, 3, OAc).

3-Fluoro-5-hydroxy-4-methoxybenzaldehyde (11) and 3,5-Dihydroxy-4-methoxybenzaldehyde (16). 2-(3-Acetoxy-4-methoxy-5-nitrophenyl)dioxolane (1.2 g, 5 mmol) was dissolved in 300 mL of EtOH and, together with 300 mg of PtO_2, was hydrogenated at 45 psi for 2 h. This sample was filtered under N_2, evaporated at reduced pressure, and used immediately. The freshly prepared sample of 2-(3-acetoxy-5-amino-4-methoxyphenyl)dioxolane (1.2 g, 5 mmol) was dissolved in cold HBF_4 (48%, 75 mL) and gradually treated with 400 mg of $NaNO_2$ in 3.0 mL of H_2O. The solution was maintained at 5 °C during the addition and was stored in the cold overnight to ensure complete formation of the stable diazonium fluoroborate. At the end of this period, starch–iodine, paper, when treated with the reaction mixture, turned blue. The solution was then diluted to 100 mL with more cold HBF_4, chilled, and photolyzed through a pyrex filter in a quartz vessel using a Hanovia lamp (450 W, medium pressure Hg). Aliquots were removed at 20 and 30 min and reacted with alkaline β-naphthol, giving no pink color, indicating completion of the reaction.[9] The sample was diluted with an equal volume of cold H_2O, cooled, and extracted with CH_2Cl_2. The CH_2Cl_2 was backwashed with saturated $NaHCO_3$, which, in turn, was acidified

and extracted with CH_2Cl_2. The organic solutions were washed with brine, dried over anhydrous $MgSO_4$, and evaporated at reduced pressure to give 450 mg of product consisting of four spots on TLC (silica gel, 3:2 EtOAc–cyclohexane), including the acetylated derivatives of **11** and **16**. Acidification of the $NaHCO_3$ solution and extraction with Et_2O gave an additional 160 mg of material, which was shown by NMR to be a mixture of nonacetylated products **11** and **16**. The acetylated products of **11** and **16** were separated by chromatography on preparative plates (silica gel, 3:2 EtOAc–cyclohexane). The final products **11** and **16** were obtained by treating the appropriate chromatographed fractions containing the separate acetylated products with 2% NaOH under N_2 and heating to reflux for 1 h. Acidification, Et_2O extraction, drying, and evaporation gave the fluorinated phenol **11** and the dihydroxy product **16**. Compound **11** was purified by sublimation (50 °C, 0.02 mm) to give 95 mg (11%) of a product: mp 97–98.5 °C; IR (KBr) 3400–3100 (OH), 2840 (CHO), 1680 (C=O), 1610, 1570, 1500, 1450 cm^{-1}; NMR (acetone-d_6) δ 9.1 (2 s, 1, CHO), 8.1 (br, 0.7, OH), 6.5 (d of d, $J_{2,F}$ = 12 Hz, $J_{2,6}$ = 3 Hz, 1, Ar H_2), 6.45 (d, $J_{6,2}$ = 3 Hz, 1, Ar H_6), 3.3 (2 s, 3, OCH_3). MS peak matching: calcd, 170.03787; found, 170.03746. Anal. Calcd for $C_8H_7O_3F$: C, 56.44; H, 4.14; F, 11.16. Found: C, 56.20; H, 4.10; F, 12.23.

Compound **16** was isolated as described above and was purified by recrystallization (EtOAc–cyclohexane) to give a single-spot material which melted at 143–148 °C (lit mp 139 °C).[18] Spectral data were consistent with the desired compound: NMR (acetone-d_6), δ 9.65 (s, 1, CHO), 8.5–7.9 (br, 2, OH), 6.85 (s, 2, Ar H), 3.80 (s, 3, OCH_3).

5-(*O*-Ethylthiocarboxy)-3-hydroxy-4-methoxybenzaldehyde (17). A sample of 2-(3-acetoxy-5-amino-4-methoxyphenyl)dioxolane (**18b**) prepared from 1.13 g (4.0 mmol) of the corresponding nitro compound (**18a**) was taken up in 6.0 mL of 5% H_2SO_4 (12 mequiv of H^+) and cooled to 5 °C. To this mixture was slowly added 320 mg (4.4 mequiv) of $NaNO_2$ in 1 mL of H_2O while maintaining the temperature below 10 °C. When the diazotization was complete, 250 mg (4 mequiv) of Na_2CO_3 was added in portions until the pH was ~4. Meanwhile, a separate solution of 680 mg (4.25 mequiv) of potassium ethyl xanthate in 1.5 mL of H_2O was warmed to 40 °C under N_2. After all of the cold diazo mixture had been filtered slowly into the warm xanthate solution, the combined mixture was heated at this temperature for 30 min and then allowed to stand for several hours. The aqueous solution was extracted with EtOAc, and the organic layers were backwashed with brine and dried over $MgSO_4$. Evaporation gave 0.67 g (53%) of crude product which was purified by column chromatography (silica gel, 4:1 cyclohexane–EtOAc) to give 250 mg of pure material. Recrystallization of this fraction from benzene gave an analytically pure product: mp 114–115.5 °C; IR (KBr) 3500–3100 (OH), 3020 (Ar CH), 2840 and 2730 (CHO), 1695 (C=O), 1600 and 1570 (Ar, C=C) cm^{-1}; NMR ($CDCl_3$) δ 9.90 (s, 1, CHO), 7.60 (s, 2, Ar H), 6.10 (s, 1, OH), 4.65 (q, 2, OCH_2), 4.02 (s, 3, OCH_3), 1.37 (t, 3, CH_2CH_3). Anal. ($C_{11}H_{12}O_4S_2$) C, H, S.

General Procedure for the Oxidation of Aromatic Aldehydes to Acids. A 1 M stock solution of $AgNO_3$ was prepared by dissolving 8.50 g of $AgNO_3$ in 50 mL of water. A second stock solution was prepared by dissolving 2.2 g of sodium hydroxide in water and diluting to 20 mL.

To prepare enough silver oxide for 2.0 mmol of aldehyde, 2.1 mL of the stock $AgNO_3$ was stirred with 0.8 mL of the NaOH. The resulting precipitate was washed well with water and then suspended in 4.0 mL of water. To this was added 400 mg of NaOH, the mixture was heated to 55 °C, and 2.0 mmol of the appropriate starting aldehyde was added in one portion. After this addition, the mixture was stirred for 15 min. The metallic silver was filtered and washed well with hot water. The combined filtrates were bubbled with SO_2 and then treated with 1–2 mL of 6 N HCl until the pH was 1. In most cases, the solid acid crystallized on standing.

3-Chloro-5-hydroxy-4-methylbenzoic Acid (3). 3-Chloro-5-hydroxy-4-methoxybenzaldehyde (**12**) was oxidized with $AgNO_3$ as described above. On acidification of the final aqueous solution, the product precipitated, yielding 279 mg 69%). The product was purified by recrystallization (benzene or EtOAc–cyclohexane) and by sublimation to yield 210 mg (50% overall) of a pure product: mp 204–205 °C; IR (KBr) 3430 (OH), 3200–2300 (CH and COOH), 1680 (conjugated C=O), 1605, 1570, 1480, 1450 (Ar C=C); NMR (acetone-d_6) δ 7.45 (s, 2, Ar H), 3.90 (OCH_3). Anal. Calcd for $C_8H_7O_4Cl$: C, 47.43; H, 3.48; Cl, 17.50. Found: C, 47.70; H, 3.50; Cl, 18.11.

3-Bromo-5-hydroxy-4-methylbenzoic Acid (4). 3-Bromo-5-hydroxy-4-methoxybenzaldehyde (**13**) was oxidized with $AgNO_3$ as described above. When the final solution was acidified, 246 mg (67%) of product crystallized out. This crude material, which showed unreacted aldehyde (TLC, silica gel, 20% EtOH–$CHCl_3$), was taken up in EtOAc–cyclohexane and extracted with saturated $NaHCO_3$ solution. This bicarbonate solution was washed with Et_2O and reacidified to give 184 mg of crystals, mp 200–202 °C. Sublimation (140 °C, 0.02–0.05 mm) gave 165 mg (43%): mp 201–202 °C; IR (KBr) 3440 (OH), 3200–2400 (COOH and CH), 1680 (conjugated C=O), 1610, 1570, 1480, 1450 (Ar C=C) cm^{-1}; NMR (acetone-d_6) δ 7.70 and 7.55 (2 d, J = 2 Hz, 1 each, Ar H), 4.0 (s, 3, OCH_3). Anal. ($C_8H_7O_4Br$) C, H, Br.

5-Hydroxy-3-iodo-4-methoxybenzoic Acid (5). 5-Hydroxy-3-iodo-4-methoxybenzaldehyde (**14**) was oxidized with $AgNO_3$ using 1.5 mmol of starting material. On acidification, the reaction mixture gave 398 mg (90%) of a crude product, mp 165–186 °C. The solid was taken up in saturated aqueous $NaHCO_3$, the solution was washed with Et_2O, the bicarbonate was acidified, and the crystals were collected. The material recovered from the bicarbonate solution was sublimed (130–140 °C, 0.02–0.05 mm) to give 119 mg (26%) of pure material: mp 207–208 °C; IR (KBr) 3360 (OH), 3200–2400 (COOH and CH), 1700 (C=O), 1570, 1490, 1450, 1420 (Ar C=C) cm^{-1}; NMR (acetone-d_6) δ 7.90 and 7.55 (2 d, J = 2 Hz, 1 each, Ar H's), 3.85 (s, 3, OCH_3). Anal. ($C_8H_7O_4I$) C, H, I.

5-Amino-3-hydroxy-4-methoxybenzoic Acid (7). 3-Hydroxy-4-methoxy-5-nitrobenzoic acid (**6**; 500 mg, 2.35 mmol) was dissolved in 100 mL of absolute EtOH and hydrogenated with 100 mg of PtO_2 at 40 psi for 1.75 h. After filtration, the product **7** could be converted to the HCl salt by treatment with HCl gas or to the sulfate salt by treatment with dilute H_2SO_4. **7**·HCl: mp 266–268 °C; NMR (Me_2SO-d_6, D_2O) δ 7.40 (2 d, 2, Ar), 3.80 (s, 3, OCH_3). Anal. ($C_8H_{10}NO_4Cl$) C, H, N. **7**·SO_4: mp 214–215 °C.

5-(Dimethylamino)-3-hydroxy-4-methoxybenzoic Acid (8). 3-Hydroxy-4-methoxy-5-nitrobenzoic acid (**6**; 534 mg, 2.5 mmol) was treated with 125 mg of PtO_2 in 125 mL of EtOH and hydrogenated at 45 psi in a Parr shaker for 1.5 h. The reaction mixture was filtered, and the volume was reduced in vacuo to 30 mL. The intermediate amine **7** was then methylated in steps by alternate additions of 0.25 mL of formaldehyde at times 0, 45, 75, 105, and 135 min and 140 mg of $NaBH_3CN$ at times 15, 60, 90, 120, and 150 min. The total amounts added were 1.25 mL of formaldehyde and 700 mg of $NaBH_3CN$. After each addition of $NaBH_3CN$, the pH of the reaction mixture was checked and adjusted if necessary to pH 5–7 using HOAc. After a total reaction time of 3.5 h, the reaction mixture was evaporated to dryness. The resulting solid was dissolved in a minimum amount of H_2O, adjusted to pH 5–6, and then applied to a Dowex 2 column (X-8, Cl form, 100–200 mesh, 25-mL bed volume). The column was washed thoroughly with water and then with 1 N HCl. The UV-positive fractions eluting between pH 1 and 3 were evaporated to dryness to give 509 mg (96%) of a white solid, which decomposed at 235–239 °C. Crystallization (H_2O) of 100 mg of the crude material gave 53 mg (53% recovery) of purified material: mp 237–238 °C; NMR (D_2O) δ 7.75 and 7.50 (2 d, J = 1.5 Hz, 2, Ar H's), 4.0 (s, 3, OCH_3), 3.28 [s, 6, $N(CH_3)_2$]; IR (KBr) 3800–2800 [COOH and $NH^+(CH_3)_2$], 1725 (C=O), 1610, 1530 cm^{-1}. Anal. ($C_{10}H_{14}NO_4Cl$) C, H, N.

5-Cyano-3-hydroxy-4-methoxybenzoic Acid (9). CuCN was freshly prepared as follows: To a solution of $CuSO_4$·$5H_2O$ (450 mg, 1.8 mmol) in 1.5 mL of H_2O warmed to 50 °C was added dropwise a solution of $Na_2S_2O_3$ (175 mg, 0.9 mmol) in 1.2 mL of H_2O. To this mixture was added a solution of NaCN (90 mg, 1.8 mmol) in 1.2 mL of H_2O. The reaction mixture was stirred for 10 min and then filtered, and the white precipitate was then dissolved in a cold (5 °C) solution of NaCN (175 mg, 3.6 mmol) in 1.0 mL of H_2O.

Separately, the diazotization reaction was carried out as follows: A 422-mg sample (1.5 mmol) of 5-amino-3-hydroxy-4-methoxybenzoic acid sulfate was dissolved in H_2O (8 mL) and EtOH (2 mL) and cooled to 0–5 °C. To this cooled solution was added

dropwise a $NaNO_2$ solution (280 mg, 4 mmol in 2.0 mL of H_2O). This diazonium mixture was then filtered and added to the cold (0–5 °C) CuCN solution prepared above. The reaction mixture was heated in a 50 °C bath with stirring for 1 h and then stirred at room temperature overnight. The mixture was concentrated in vacuo and mixed with EtOH. The residue was washed thoroughly with EtOH, the EtOH was acidified with dilute H_2SO_4, and the resulting solids were extracted again with EtOH. The total combined EtOH extracts were evaporated to give 220 mg (76%) of a crude solid: mp 192–195 °C. The crude solid was purified by preparative TLC (1000 μm, Woehlm silica gel, 90% $CHCl_3$ saturated with HCO_2H, 10% EtOH) and then sublimed (120–140 °C, 0.3 mm) to give the product: mp 225–227 °C (~50% total recovery from purification steps); NMR (acetone-d_6) δ 7.90–7.80 (2 d, 2, Ar), 4.10 (s, 3, OCH_3), no other absorptions observed; IR (KBr) 3250 (OH), 3200–2400 (COOH), 2235 (C≡N), 1740 (C=O), 1690, 1580, 1500, 1430 (Ar) cm^{-1}. Anal. ($C_9H_7NO_4$) C, H, N.

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Catechol *O*-Methyltransferase. 12. Affinity Labeling the Active Site with the Oxidation Products of 5,6-Dihydroxyindole

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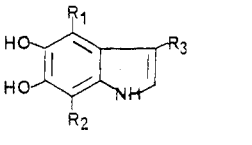
5,6-Dihydroxyindole (5,6-DHI) and a series of 4- and/or 7-methylated analogues of 5,6-DHI have been synthesized and evaluated for their ability to inactivate purified rat liver catechol *O*-methyltransferase (COMT). The inactivation of COMT by these agents could be prevented by excluding oxygen from the incubation mixtures, indicating the necessity for their oxidation to the corresponding aminochromes. Substrate protection studies and kinetic studies suggested that the loss of enzyme activity resulted from the modification of a crucial amino acid residue at the active site of COMT through reaction with the quinoid oxidation products. The COMT inhibitory activity of the 4- and/or 7-methylated analogues of 5,6-DHI argue against a mechanism involving a 1,4 Michael addition reaction at positions 4 or 7 on the aminochrome. Considering the number of potential electrophilic centers on the basic aminochrome structure, the site of the reaction might change depending on the aromatic substitution pattern. The preferred pathway of reaction may be determined in part by the juxtaposition of the protein nucleophile to the possible sites of attack on the electrophilic ligand but also in part on the reactivity of the electrophilic site which might change with substitution on the aromatic ring.

The extraneuronal inactivation of catecholamines and the detoxification of many xenobiotic catechols are dependent upon the enzyme catechol *O*-methyltransferase (COMT, EC 2.1.1.6). COMT is a soluble, magnesium-requiring enzyme which catalyzes the transfer of a methyl group from *S*-adenosylmethionine (SAM) to a catechol substrate, resulting in the formation of the meta- and para-O-methylated products.[1]

Until recently, limited information has been available concerning the nature of the amino acid residues present at the active site of COMT. Through the use of functional-group reagents[2,3] and affinity-labeling reagents,[2,4-9] our laboratory has been able to show that COMT has two nucleophilic residues at its active site; both groups are essential for enzymatic activity. The mechanisms by which these amino acid residues react with the functional-group reagent *N*-ethylmaleimide[3] and the affinity-labeling reagents, *N*-haloacetyl derivatives of 3,5-dimethoxy-4-hydroxyphenylethylamine and 3,4-dimethoxy-5-hydroxyphenylethylamine,[4-6] appears fairly straightforward because of the single electrophilic center present in each of these ligands. In contrast, the reactivity of the protein nucleophiles with the oxidation products of 6-hydroxydopamine[7,9] and 6-aminodopamine[8] is less clearly defined because of the multitude of electrophilic species generated upon air-oxidation of these hydroquinones and the various reactive centers present in each electrophilic species.

Chart I



	R_1	R_2	R_3
1	CH_3	CH_3	H
2	CH_3	H	H
3	H	CH_3	H
4	H	H	H
5	H	H	$CH_2CH_2NH_2$

Our earlier studies[7] suggested that one of the oxidation products of 6-hydroxydopamine which produced inactivation of COMT was aminochrome II which is generated by air-oxidation of 5,6-dihydroxyindole (DHI). In an attempt to better define the involvement of aminochrome II in the COMT inactivation process and to elucidate the nature of the chemical reaction that occurs between this electrophile and the protein nucleophile, a series of 4- and/or 7-methylated analogues of DHI have been prepared and their ability to inactivate COMT has been determined. The compounds of particular interest were 4,7-Me_2-5,6-

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