DMF); pc R_f 0.54 (BAW), R_f 0.65 (BAWP); tlc (silica gel) R_f 0.13 (BAW), R_f 0.43 (BAWP), R_f 0.09 (BPW); E(Glu) 1.35 (pH 1.9). Amino acid ratios found were: Sar, 1.09; Arg, 0.954; Val, 1.00; Tyr, 0.91; Ile, 2.04; His, 0.956; Pro, 0.935. *Anal.* ($C_{46}H_{73}N_{13}O_{10}\cdot 2H_2O$) C, H, N.

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Sulfur Analogs of Dopamine and Norepinephrine. Inhibition of Catechol-*O*-methyltransferase

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3-Mercaptotyrosine, 3-mercaptotyramine, *N*-acetyl-3-mercaptotyramine, 2-mercapto-5-hydroxyphenethylamine, and 2-mercapto-5-hydroxyphenethanolamine have been synthesized from phenolic precursors by reaction with thiocyanogen chloride followed either by direct reduction to the desired mercapto derivative with hydrosulfide ion or mercaptoethanol, or by acid hydrolysis of the cyclization product, a 2-imino-1,3-benzoxathiole, to a 2-oxo-1,3-benzoxathiole, which is further hydrolyzed or hydrazinolyzed to the desired *o*-mercaptophenol, a relatively stable compound. 3-Mercaptotyramine, the mercapto analog of dopamine, is not a substrate for catechol-*O*-methyltransferase, but irreversibly inhibits the enzyme, presumably by formation of a disulfide bridge to a reactive mercapto group in the active site. Studies with 3-mercaptotyramine in the anesthetized dog indicate only minimal effects on blood pressure and cardiac contractility, and little if any effect on renal blood flow. This mercapto analog of dopamine appears to be a very weak, indirectly acting, sympathomimetic amine without significant agonist or antagonist activity for norepinephrine or dopamine receptors.

Catecholamines, such as epinephrine, norepinephrine, and dopamine, are important neurohormones whose structure-activity relationships have been extensively studied (for pertinent references see ref 1). Both alterations in the substitution on the terminal nitrogen, and, to a lesser extent, the effects of substituents on the aromatic ring have been investigated.[1] Thus, one of the phenolic groups may be replaced by a methanesulfonamide[2] or a hydroxymethyl group[3] with significant retention of biological activity. Another logical chemical alteration of the catechol moiety would be the replacement of either or both of the phenolic groups by a mercapto group. Such compounds might prove to be site-directed sulfhydryl reagents. Replacement of hydroxyl groups by mercapto groups in biologically active compounds has been reported for the estrogen, hexestrol, the thiophenol isostere of which was synthesized and found to be biologically inactive.[4] A mercapto analog of norepinephrine in which the β-hydroxy group was replaced by a mercapto group was only $^1/_{240}$ as active a pressor agent as norepinephrine.[5]

This report describes the synthesis of analogs of L-dopa, dopamine, and norepinephrine in which the *m*-hydroxy group of the catechol moiety has been replaced by a mercapto group. The effects of the dopamine analog on catechol-*O*-methyltransferase (COMT) and on cardiovascular responses in the anesthetized dog have been investigated.

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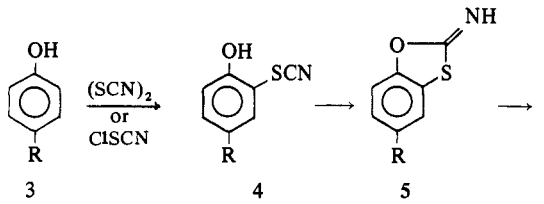
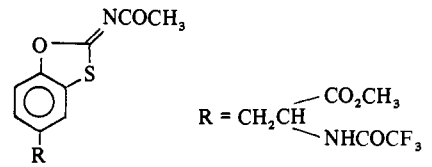
Synthesis. Both thiocyanogen $(SCN)_2$ (1) and thiocyanogen chloride (ClSCN, 2) were used for the introduction of the thiocyano group into the aromatic ring systems of suitable phenols. When *N*-trifluoroacetyl-L-tyrosine methyl ester (3) was treated with 1 generated from cupric thiocyanate,[6] the acetylated 2-imino-1,3-benzoxathiol (6) was isolated in small yield. Thiocyanation must have taken place in the 3 position to give the thiocyano compound 4, which cyclized to 5 under the conditions of the reaction accompanied by acetylation to 6. Attempts to carry out the thiocyanation with cupric thiocyanate under milder conditions were unsuccessful. Thiocyanogen chloride (2), a much more reactive thiocyanating reagent,[7] was examined next.



3 4 5



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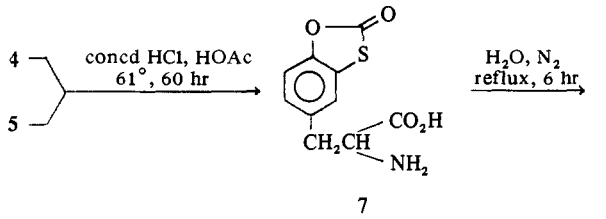
When the tyrosine derivative 3 was treated with excess 2 in acetic acid, the thiocyano derivative 4 was formed in good yield. Compound 4 is a crystalline solid which, although stable for many months at room temperature, rapidly cyclizes to the imino compound 5 in alcoholic pyridine at room temperature. This cyclization was also effected by maintaining 4 at the melting point for a few seconds or by boiling its solution in toluene. The presence of the imino compound 5 in such mixtures was demonstrated by paper electrophoresis. Imino esters, such as the 2-imino-1,3-benzoxathioles, are basic and hence exhibit ionophoretic mobility in acidic buffers. The thermal ring closure of compounds, such as 4, leads to products that tend to be amorphous yellow solids due to the presence of by-products, the nature of which is under investigation.

The ease of cyclization of *o*-thiocyanophenols[8] renders their recrystallization difficult. In unbuffered neutral media, the cyclization to the basic imino compound becomes an autocatalytic process. For successful recrystallization *o*-thiocyano compounds were cautiously precipitated from acidic solution at room temperature.

Acetylation of 5 afforded a product identical with material obtained from the cupric thiocyanate reaction, in confirmation of structure 6. When 5 was heated with a mixture of concentrated hydrochloric and acetic acid, the protecting groups and the imino group were hydrolyzed to give the 2-oxo-1,3-benzoxathiole (7), which was also obtained more directly by treating 4 in the same manner. That 2-oxo-1,3-benzoxathiols are stable to acidic hydrolysis is known.[9] Hydrolysis of 7 with base occurred readily as indicated by color reaction with the Ellman reagent§ and by the liberation of carbon dioxide when the hydrolysis mixtures were acidified. 3-Mercapto-L-tyrosine (8) was slowly formed when a dilute aqueous solution of 7 was

§ 5,5'-Dithiobis(2-nitrobenzoic); see ref 10.



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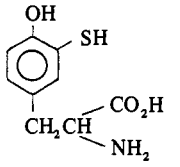

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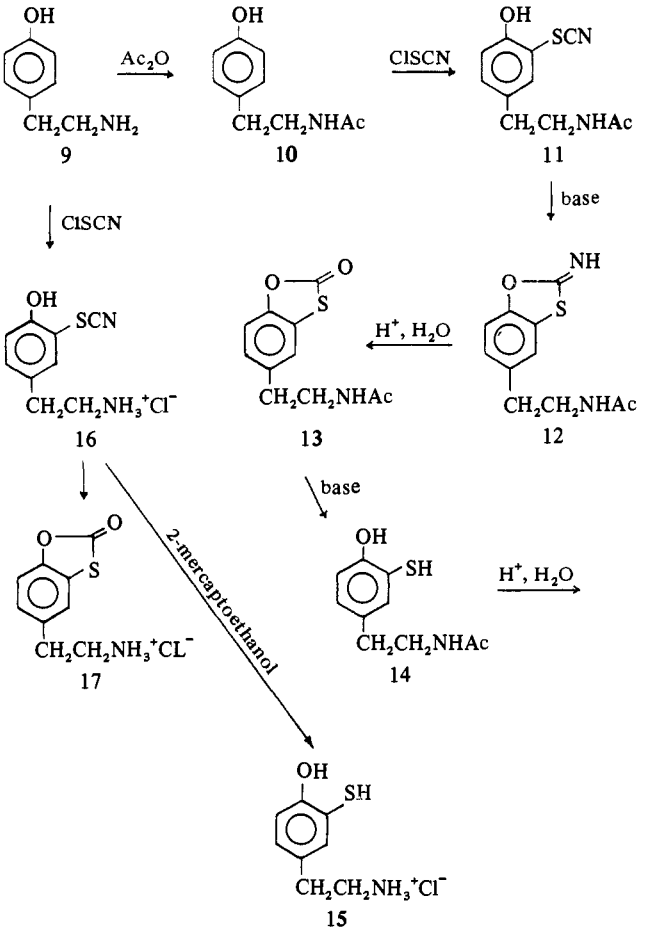
boiled in a nitrogen atmosphere. The hydrochloride of 8 was then isolated by evaporation of the solution. In analogy to L-dopa, 8 is stable in acid solution, and alkaline solutions quickly turn bluish green.

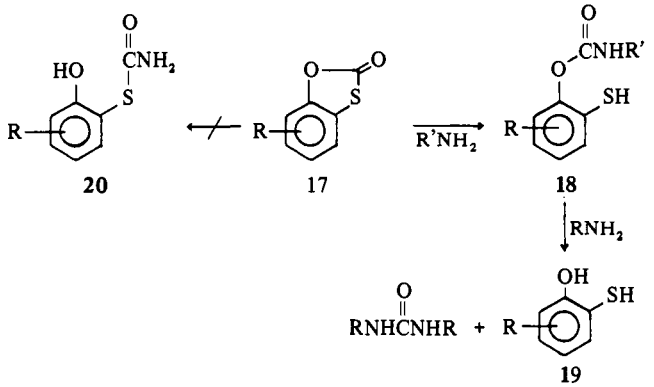
Synthesis of the dopamine analog 15 was first attempted from *N*-acetyltyramine (10). Considerable difficulty was experienced in preparing *N*-acetyltyramine (10) from tyramine (9) because of facile formation of *N,N*-diacetyl and



9 10 11 12 13 14 15 16 17

O,N,N-triacetyl derivatives.[11] Thiocyanation of 10 gave the 3-thiocyano compound 11. In the same manner as 4, 11 was readily cyclized to the imino compound 12 by pyridine at room temperature. Hydrolysis of 12 with hot dilute hydrochloric acid afforded the 2-oxo-1,3-benzoxathiole (13). Compounds of this type were readily detected qualitatively on tlc plates or paper electropherograms by a differential visualization procedure. Duplicate chromatograms were run and the developing solvents evaporated. One chromatogram was ex-

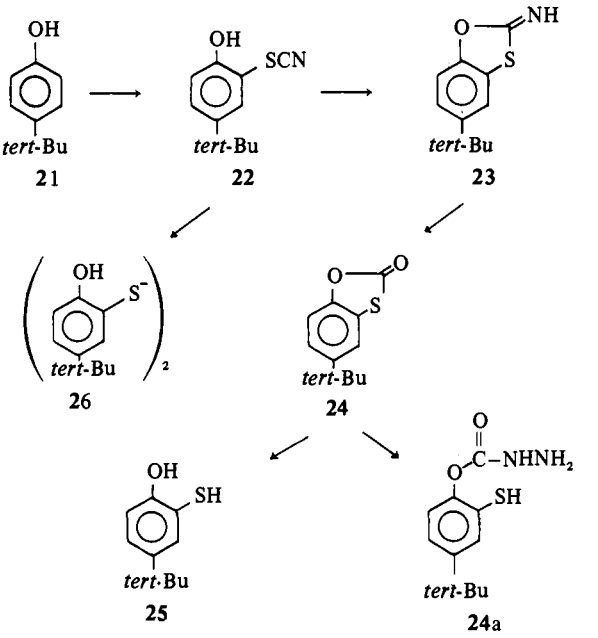
posed to ammonium hydroxide vapors for 15-30 sec. After removal of residual ammonia, both chromatograms were sprayed with Ellman's reagent or with ferric ferricyanide (see Experimental Section). 2-Oxo-1,3-benzoxathioles after treatment with ammonia give a deep yellow color with Ellman's reagent or an intense blue color with ferric ferricyanide. Without this ammonia treatment no color with Ellman's reagent and only a very pale blue color with ferric ferricyanide is observed. These results suggest that 2-oxo-1,3-benzoxathiols are opened by ammonia or by primary or secondary amines to mercaptocarbamates (**18**) and/or mercaptophenols (**19**). Either mercapto compound would give color reactions with the above two reagents. This sequence **17** → **18** is consistent with the easier aminolysis of thiol compared with normal esters.[12] Accordingly, the contention of Ouperoff-Urne[13] that 2-oxo-1,3-benzoxathioles react with primary or secondary amines to give phenolic



thiolcarbamates (**20**) would appear to be incorrect. The isolation and characterization of a mercaptohydrazide (**24a**) similar to **18** is described below.

The treatment of **13** with *excess* hydrazine in the presence of sodium dithionite afforded **14**, a sulfur analog of the sclerotizing insect hormone *N*-acetyldopamine.[14] The hydrochloric-acetic acid mixture which was effective in hydrolyzing the trifluoroacetyl group in the first series (**4** or **5** → **7**) failed in this series. It was, however, possible to thiocyanate directly unprotected phenolic amines, such as **9**, in acidic medium. The thiocyanation product **16** was hydrolyzed with dilute hydrochloric acid to the 2-oxo-1,3-benzoxathiol (**17**) hydrochloride. The dopamine analog, 3-mercaptotyramine (**15**) was readily obtained by hydrazinolysis of **18** at room temperature, a process in which the 2-carbonyl presumably was removed in the form of formhydrazide. The mass spectrum of **15** showed a strong parent peak at *m/e* 169 in addition to peaks of higher *m/e* which point to the presence of traces of the disulfide, which also appear on chromatograms and electropherograms. The disulfides usually had a higher mobility than the corresponding mercaptans on electrophoresis, but had a lower mobility on tlc in the buffer and solvent systems used.

A direct hydrolysis of the thiocyano compound **16** to the dopamine analog **15** was not attempted. Simple aryl thiocyanates are cleaved by alkali to give disulfides. Possible mechanisms for this reaction have been discussed.[15] In the present case it was expected that a simple alkaline treatment would lead to partial if not complete cyclization. Cleavage by a direct reductive process was, therefore, attempted. By reduction with 2-mercaptoethanol in a bicarbonate buffer, **15** was obtained directly from **16** in yields of 55-69%. A strong odor of hydrogen cyanide developed in the reaction mixture. Dithiothreitol was less satisfactory as a reducing



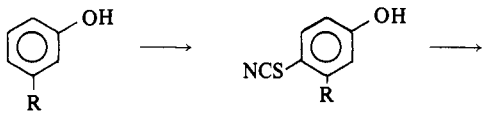
agent. The disulfide corresponding to **15** was prepared by allowing an ammoniacal solution of **15** to stand exposed to air for several days.

Considerable difficulty was experienced in the purification of certain *o*-thiocyanophenols. To investigate this problem a simpler *o*-thiocyanophenol (**22**) was prepared by thiocyanation of *p*-butylphenol (**21**). The thiocyano compound **22** proved to be quite unstable and decomposed readily at room temperature to a mixture of **23**, **24**, **26** (*m/e* 362), and an unidentified polar substance. The remarkably rapid decomposition of **22** compared with *o*-thiocyanophenols which have acylamino or ammonium substituents on the side chain was surprising. It probably reflects that extreme sensitivity of the cyclization reaction to the nucleophilicity of the phenolic oxygen atom.

Treatment of **24** with hydrazine gave the mercaptophenol **25**, a compound mentioned in the literature[16] without preparative details or physical constants and the mercaptohydrazide **24a**, which migrated as a cation on electrophoresis in an acidic buffer, showed carbonyl absorption at 1631 and 1642 cm^{-1}, and gave positive sulfhydryl tests with Ellman's reagent; **24a** gave an immediate purple color with 2,6,-*N*-trichlorobenzoquinonimide,[17] a reagent which holds considerable promise for visualizing mercaptans. Hydrazine must have opened the oxathiolone ring with initial cleavage of the C-S linkage rather than the C-O linkage. Excess hydrazine removes the carbonyl moiety completely.

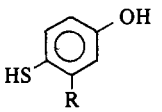
The thiocyanation of some phenolic amines of pharmacological interest bearing meta substituents was also examined. When *m*-tyramine (**27a**) and *m*-octopamine (**27b**) were thiocyanated, the thiocyano group entered the ring para to the hydroxyl group to give **28a** and **28b**, respectively. This assignment is based in part on a similar orientation in the thiocyanation product of *m*-cresol[7a] but mainly on the much greater stability of the thiocyano compounds **28a** and **28b** in comparison with similar *o*-thiocyano compounds. The fact that the thiocyano group of **28a** survives boiling with hydrochloric acid or treatment with ammonium hydroxide places these thiocyanation products in a clearly different class from the ortho compounds.

The mercapto compounds **29a** and **29b** were prepared by treating **28a** and **28b** with a sodium hydrosulfide solution saturated with hydrogen sulfide. The presence of thiocyanate ion in the reaction mixtures was indicated by the



27a, R = $CH_2CH_2NH_2$
b, R = $CH(OH)CH_2NH_2$

28a, R = $CH_2CH_2NH_2$
b, R = $CH(OH)CH_2NH_2$

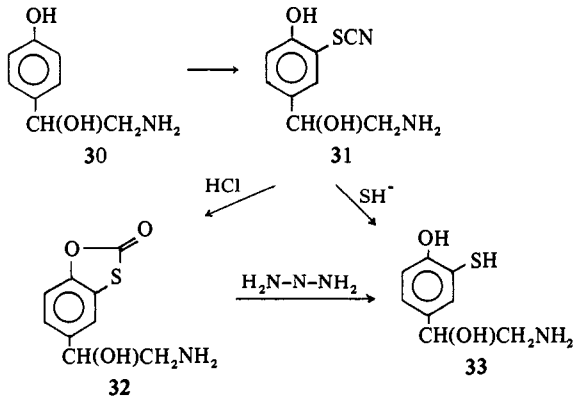


29a, R = $CH_2CH_2NH_2$
b, R = $CH(OH)CH_2NH_2$

deep red color produced by the addition of ferric ions in strong aqueous acid. This suggests the following mechanism.

$ArSCH + HS^- \rightarrow ArSH + SCN^-$

Considerable difficulty was encountered in attempts to isolate the sulfur analog **33** of norepinephrine. The thiocyanation of DL-octopamine (**30**) afforded a crude thiocyano product, presumably **31**, on the basis of the infrared spectrum. Cleavage of **31** both by 2-mercaptoethanol and



by hydrosulfide gave reaction products which on tlc or electrophoresis showed a component positive to ninhydrin and Ellman's reagent. Pure **33** has so far not been isolated from such mixtures. The treatment of **31** with hot aqueous hydrochloric acid afforded crude **32**. Although the characterization of the product has not been completed, the infrared spectrum shows a strong band at 1760 cm^{-1} in common with the other 2-oxo-1,3-benzoxathioles described. Hydrazinolysis of this material afforded solutions whose properties indicated the presence of the sulfur analog of norepinephrine (**33**). Efforts to purify and characterize these products further are in progress.

Biological Activity. As part of an extensive investigation of the biological properties of this type of mercaptophenol, the interaction of 3-mercaptotyramine with catechol-*O*-methyltransferase and its cardiovascular activity in intact animals were determined. Mercaptotyramine, an isostere of dopamine, conceivably might serve as a substrate for COMT and be converted to an *S*- or *O*-methyl derivative. This was not the case and no detectable methylation product was formed in the presence of the enzyme under conditions which were optimal for the O-methylation of dopamine (Table I). However, 3-mercaptotyramine effectively inhibited enzymatic O-methylation of various catechols, such as dopamine, norepinephrine, and 3,4-dihydroxybenzoic acid (Table II). According to kinetic analysis, the inhibition is of the *mixed type, i.e.*, the presence of the inhibitor

Table I. Interaction of 3-Mercaptotyramine with COMT. Lack of Formation of Methylated Products

Substrate	Extractable radioactivity,[a] dpm
None	835
3-Mercaptotyramine	841
Dopamine	93400

[a] Conditions were as described in the Experimental Section except that the concentration of unlabeled *S*-adenosyl-L-methionine was reduced to 0.1 m*M* and the incubation time was 30 min. The reaction with dopamine was essentially complete (93%).

Table II. Inhibition of COMT by 3-Mercaptotyramine

Substrate, 1 m*M*	[I],[a] m*M*	Rate,[b] nmole/min	Inhibition, %
3,4-Dihydroxybenzoic acid		12.8	0
3,4-Dihydroxybenzoic acid	0.04	11.2	13
3,4-Dihydroxybenzoic acid	0.40	6.2	52
3,4-Dihydroxybenzoic acid	1.00	1.8	86
3,4-Dihydroxybenzoic acid	2.00	<0.2	100
+ DTT[c]	2.00	15.8	0
Dopamine		7.6	0
Dopamine	0.4	3.1	60
Dopamine + DTT[c]	0.4	8.4	0

[a] Final concentration of 3-mercaptotyramine. [b] Reaction was initiated by the addition of 0.4 mg of enzyme and the rate determined after 5 min as described in the Experimental Section. [c] Dithiothreitol (DTT) was added prior to enzyme to a final concentration of 2 m*M*.

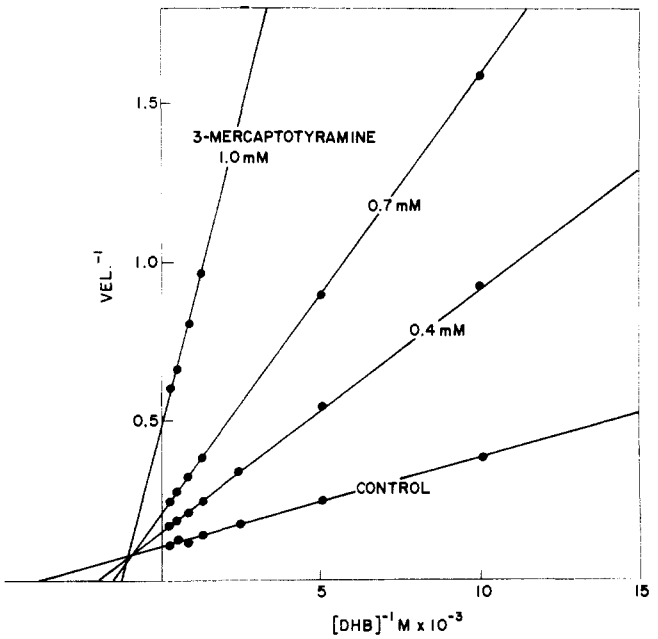


Figure 1. Mixed inhibition; effect of 3-mercaptotyramine on the double reciprocal plot of concentration of substrate, 3,4-dihydroxybenzoic acid [DHB] *vs.* reaction velocity. *S*-Adenosyl-L-methionine concentration (1 m*M*). Velocity = nmoles of product/0.05 ml of COMT per min.

interferes to some extent with the binding of substrate as well as producing a noncompetitive inhibition.[17] The same type of inhibition by 3-mercaptotyramine was shown both with respect to the catechol substrate and with respect to the cosubstrate, *S*-adenosyl-L-methionine (Figures 1, 2). The K_i for the initial competitive reaction calculated from the ratio of the slopes of the double reciprocal plots[18] was 0.6 m*M*. The inhibition was markedly enhanced by preincubation (Figure 3) and was essentially irreversible by dialysis (Figure 4), but could be readily reversed or prevented by di-

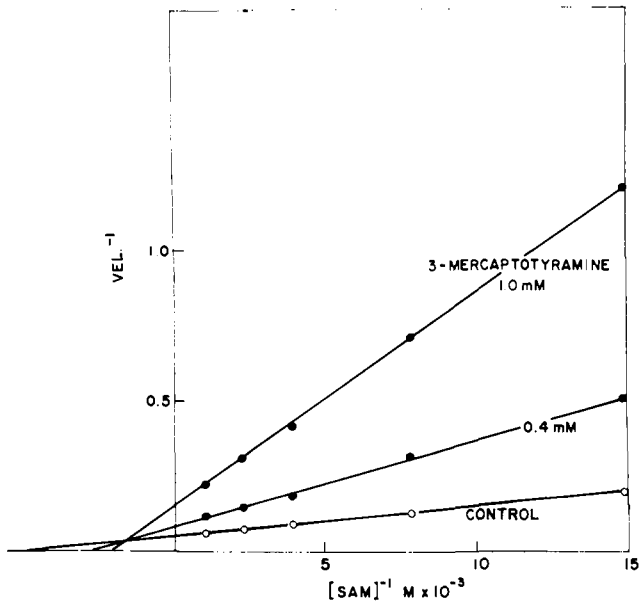


Figure 2. Mixed inhibition; effect of 3-mercaptotyramine on the double reciprocal plot of concentration of *S*-adenosyl-L-methionine [SAM] *vs.* reaction velocity. 3,4-Dihydroxybenzoic acid concentration (2 m*M*). Velocity = nmoles of product/0.05 ml of COMT per min.

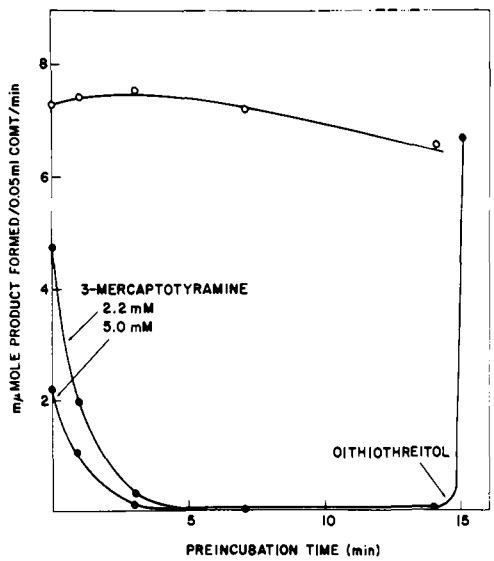


Figure 3. Effect of preincubation with 3-mercaptotyramine (2.2 m*M* and 5.0 m*M*) on the activity of COMT. Dithiothreitol (5 m*M*) was added to inhibited enzyme as indicated by arrow. Assay with dihydroxybenzoic acid, 1 m*M*.

thiothreitol (Table II and Figures 3, 4), a selective reagent for the reduction of disulfides.[19] The present evidence strongly suggests that 3-mercaptotyramine inhibits COMT by reacting with a cysteine residue in or near the active site by the formation of a disulfide bond. The partially competitive nature of the inhibition suggests that the reaction is site directed by the affinity of COMT for mercaptotyramine which resembles the normal substrates. Whether 3-mercaptotyramine and other mercaptophenols are also active as inhibitors of COMT *in vivo* is under investigation.

3-Mercaptotyramine in the anesthetized dog[20] had no effect on mean blood pressure, renal blood flow, or cardiac contractile force after intravenous administration of doses below 80 μg/kg. Slight increases in contractile force were observed at higher doses with a maximal effect at 320 μg/kg.

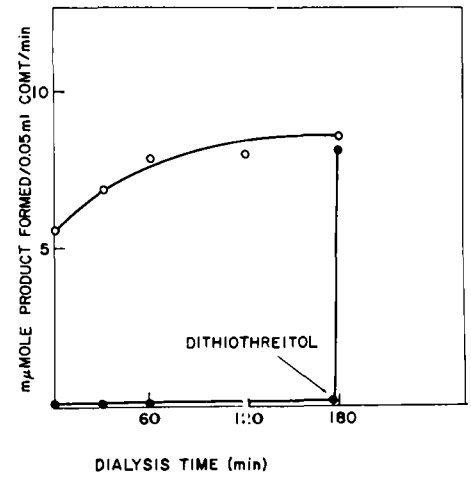


Figure 4. Effect of dialysis on 3-mercaptotyramine-inhibited COMT. Open circles; uninhibited enzyme, closed circles; enzyme preincubated 7 min with 3-mercaptotyramine (2 m*M*). Dithiothreitol (5 m*M*) added as indicated by arrow. Assay with dihydroxybenzoic acid, 1 m*M*.

At this dose there was a small increase in arterial pressure, equivalent to that elicited by the intravenous administration of 15 μg/kg of tyramine. This increase, in the same way as that produced by tyramine, was blocked by the cocaine-like blocking agent, methylphenidate (5 mg/kg). Thus, 3-mercaptotyramine appears to be a weak indirect sympathomimetic agent which owes its activity to release of endogenous norepinephrine. Administration of 3-mercaptotyramine (320 μg/kg) did not significantly alter the subsequent responses (increase in mean arterial pressure and contractile force) to a test dose of norepinephrine (0.75 μg/kg).

Renal blood flow in dogs is very sensitive to dopamine and responds with maximum vasodilation with as little as 20 μg/kg of dopamine.[20] 3-Mercaptotyramine (320 μg/kg) did not block the increase in renal blood flow elicited by dopamine (20 μg/kg). The direct administration of a large dose of 3-mercaptotyramine (1.7 mg) into the renal artery did not elicit renal vasodilation nor did it block the vasodilation to a minimal test dose of dopamine (5 μg/kg).[20] 3-Mercaptotyramine, therefore, appears to have little agonist or antagonist activity toward peripheral receptors of norepinephrine or dopamine. Further studies will be necessary to explore the possible usefulness of this class of mercapto analogs of catecholamines.

Experimental Section

***N*-Trifluoroacetyl-L-tyrosine Methyl Ester (3).** L-Tyrosine methyl ester (Aldrich) (20.6 g, 0.10 mole) was slurried in 40 ml of DMF and stirred magnetically in an ice bath, while 42.95 g of trifluoroacetic anhydride was added at such a rate that the temperature of the mixture did not exceed 20°. After 1 hr, 14 ml of triethylamine (0.10 mole) was added maintaining at <20°. After 30 min, the mixture was poured into 200 ml of ice and H_2O. The precipitated product was filtered, washed with H_2O, and dried to give 16.9 g (58%) of colorless crystals: mp 138–141.5° (lit.[20] mp 137–138.5°). An additional 7.3 g, mp 141.7–143.5°, was recovered from the filtrate. Recrystallization of the first fraction from toluene or aqueous HOAc afforded pure **3**: mp 141–143°.

2-Acetimino-5-(2-methyloxycarbonyl-2-trifluoroacetamidoethyl)-1,3-benzoxathiole (6). A. A mixture of 873 mg of ester **3**, 2.16 g of $Cu(SCN)_2$, and 8 ml of HOAc–Ac_2O was boiled for 7 min. CuSCN was removed by filtration and the filtrate diluted with ice and H_2O to give 720 mg of crude product. Recrystallization from *i*-PrOH afforded colorless crystals: mp 249°; ir (Nujol) 3270, 1750, 1700, 1656 cm^{-1}; mass spectrum parent ion *m/e* 390.

B. The precursor **5** was acetylated to **6** by treating a suspension of 50 mg of **5** in 0.2 ml of EtOAc with 1 drop each of Ac_2O and

pyridine. The solution was seeded and the crystals which separated were filtered after 45 min, washed with EtOAc, and dried. The product, 36 mg (70%), was recrystallized from *n*-BuCl–EtOAc (2:1) to give an analytical sample: mp 252–252.5°. The mass and ir spectra were identical with the spectra of the material prepared by method A. *Anal.* ($C_{15}H_{13}F_3N_2O_5S$) C, H, N.

***N*-Trifluoroacetyl-3-thiocyano-L-tyrosine Methyl Ester (4).** A solution of ClSCN was prepared by stirring 7.76 g (80 mmoles) of finely powdered dry KSCN with a solution of 1.36 g (60 mmoles) of dry $(CaCl_2)Cl_2$ in 295 ml of dry HOAc for 15 min. The phenol **3** (8.73 g, 30 mmoles) was quickly added and stirring continued at room temperature for 30 min. This gave optimal yields. The mixture was poured into 3000 ml of ice and H_2O. After 1 hr, the product was filtered, washed with H_2O, and dried to give 8.44 g (81%) of colorless leaflets. Heating during attempted recrystallizations promoted conversion to a yellow amorphous material, but unrecrystallized material could be obtained that was analytically pure: mp 102–106°; mass spectrum parent peak *m/e* 348; ir (Nujol) 3370, 3304, 2150 (SCN), 1729 (ester), 1700 (amide), 1558, 1410, 1350, 1300, 1212, 1180, 1148, 829, 808 cm^{-1}. *Anal.* ($C_{13}H_{11}F_3N_2O_4S$) C, H, N.

L-5-(2-Trifluoroacetamido-2-methyloxycarbonylethyl)-2-imino-1,3-benzoxathiole (5). A suspension of 500 mg of 4 in 3 ml of *i*-PrOH was treated with *ca.* 0.2 ml of pyridine, and the mixture warmed slightly with swirling. After 15 min, the product was precipitated with cold H_2O, filtered, washed with H_2O, and air-dried to give 440 mg (88%) of **5**. Recrystallization successively from MeOH–H_2O, *tert*-BuOH, and *i*-PrOH afforded analytically pure material: mp 132–134°; mass spectrum parent peak *m/e* 348; ir (Nujol) 3320, 3250, 3185, 1743, 1700, 1650 cm^{-1}. *Anal.* ($C_{13}H_{11}F_3N_2O_4S$) C, H, N.

L-5-(2-Amino-2-carboxyethyl)-1,3-benzoxathiol-2-one Hydrochloride (7). A solution of 2.0 g of 4 in a mixture of 10 ml of HOAc and 10 ml of concd HCl was sealed in an ampule and kept at 61° for 46 hr. Crystallization began during the reaction period and was completed in an ice bath. Filtration afforded off-white crystals (1.09 g, 69%, mp 256–260° dec) which were washed with HOAc and ligroin. The identical compound was obtained by acid hydrolysis of **5**. An analytical sample was prepared by dissolving 95 mg of 7 in 1 ml of 88% formic acid. The solution was filtered and diluted with 2 ml of HOAc to give a white crystalline solid: mp 254–257° dec; ir (Nujol) 2620, 1765, 1740, 1480, 1240, 1228, 1205, 1088, 1020, 830 cm^{-1}; mass spectrum molecular ion *m/e* 239. Some samples having the same mp, chromatographic properties, analytical data, and mass spectrum had apparently crystallized in a polymorphic modification since the ir spectrum differed in some respects. Bands were then observed at 1750, 1742, 1590, 1236, 1190, 1085, 1020, 835, 820, 721 cm^{-1}. The optical purity of this material was not ascertained and it is possible that varying ratios of L and DL forms are responsible for this behavior. When chromatograms or electropherograms were exposed to NH_3 vapors prior to spraying with Ellman's reagent or $Fe[Fe(CH)_6]$, only the exposed material gave a color reaction (yellow or deep blue, respectively). The untreated material gave no color with Ellman reagent and only a faint blue spot with $Fe[Fe(CN)_6]$.

3-Mercapto-L-tyrosine Hydrochloride (8). A stream of N_2 was passed through a solution of 1.06 g (4.0 mmoles) of 7 in 500 ml of H_2O. After CO_2 evolution had virtually ceased (6 hr), the reaction mixture was evaporated *in vacuo*. The solid residue was triturated with $EtCO_2H$, filtered, and washed successively with $EtCO_2H$, EtOAc, and hexane to afford a white solid. Recrystallization of 800 mg from 7 ml of concd HCl afforded a light tan powder: mp 227–230° dec; ir (Nujol) 3435, 2615, 2560, 1735, 1228, 1215, 1043, 813 cm^{-1}; mass spectrum molecular ion, *m/e* 213; uv λ_{max} 290 nm (ϵ 2480); R_f 0.56 on silica gel with *n*-BuOH–H_2O–HOAc (4:1:1).

***N*-Acetyl-3-thiocyanotyramine (11).** Dry finely powdered KSCN (386 mg, 4 mmoles) was added to 24 ml of 280 mg of Cl_2 in dry HOAc, and the mixture stirred for 15 min. *N*-Acetyltyramine (**10**) (358 mg, 2 mmoles) was then added in 1 portion, and the mixture stirred for 23 min. Excess ClSCN was treated with 2 ml of cyclohexene, KCl was removed by filtration, and the filtrate was diluted with petroleum ether to a total volume of 125 ml. The precipitate was triturated, collected on a sintered glass funnel, washed with petroleum ether, and air-dried to give 473 mg of white powder. The solid was triturated with H_2O, filtered, washed, and dried over P_2O_5. It was then dissolved in 98% formic acid without heating, filtered through a sintered glass funnel, and diluted with 5 ml of H_2O, and the mixture cooled in ice to yield 300 mg (63%) of a powder: mp 107° dec. The still impure material was dissolved in 1 ml of concd HCl, and the solution diluted with 3 ml of H_2O to give a white powder: mp 129–131°. The ir spectra (Nujol) of the high and low melting forms were virtually identical with SCN absorption at 2170 cm^{-1}. However, the high melting form also exhibited a weak but sharp shoulder at 2160 cm^{-1}: other bands 3345, 1650, 1570, 1508, 1460, 1290, 1238, 1200, 1055, 830 cm^{-1}; mass spectrum molecular ion at *m/e* 236. An impurity gave a molecular ion at 261. This latter peak, in conjunction with the 2160-cm^{-1} shoulder, suggests the presence of 2-cyanimino-5-(2-acetylaminoethyl)-1,3-benzoxathiole.

Analytical values for carbon were consistently low by 0.4–0.7%.

5-(2-Acetamidoethyl)-2-imino-1,3-benzoxathiole (12). To a slurry of 1.39 g of 11 in 9.3 ml of MeOH was added 0.5 ml of 10% aqueous K_2CO_3, and the mixture stirred at room temp for 5 min. The starting material dissolved within a few seconds and the product crystallized shortly thereafter. The mixture was stirred for 15 min in an ice bath and filtered, and the filter cake was washed with ice water. The air-dried product, which weighed 1.15 g (83%), was dissolved in 6 ml of 1 *M* HCl, decolorizing charcoal was added, and the solution immediately was filtered into 1 ml of ice-cold concd NH_4OH. The solid was collected, washed with cold H_2O, and immediately dried *in vacuo* over P_2O_5 to give an off-white powder: mp 153–158° dec. The analytical sample was prepared by dissolving 800 mg of **12** in DMSO at room temperature and by adding 24 ml of MeCN until colorless crystals appeared: mp 155.5–157° dec; ir (Nujol) 3270, 3220, 3080, 1662, 1640, 1281, 1217, 1231, 1010, 864, 809 cm^{-1}; mass spectrum molecular ion at *m/e* 236. *Anal.* ($C_{11}H_{12}N_2O_2S$) C, H, N.

5-(2-Acetamidoethyl)-1,3-benzoxathiol-2-one (13). The imine **12**, 2.45 g, was dissolved in 40 ml of 1 *M* HCl and filtered with decolorizing charcoal through sintered glass and the filtrate boiled for 5 min. Cooling afforded a solid, which on recrystallization from Me_2CO at 5°, afforded 1.13 g (46%) of colorless needles: mp 131.5–133.5°. Recrystallization from Me_2CO and sublimation at 0.05 Torr afforded an analytical sample: mp 131–133.5°; mass spectrum molecular ion at *m/e* 237; ir (Nujol) 3293, 1757, 1732, 1640, 1565, 1249, 1028, 827, 730 cm^{-1}. *Anal.* ($C_{11}H_{11}NO_3S$) C, H, N.

***N*-Acetyl-3-mercaptotyramine (14).** To a solution of 0.286 g (4.2 mmoles) of hydrazine hydrate and 0.1 g of $Na_2S_2O_4$ in 1 ml of H_2O was added 340 mg of **13** and the mixture warmed slightly to give a clear solution. Filtration and acidification with HOAc afforded a yellow gum which was separated by decantation. Trituration with HOAc afforded a white crystalline solid which was washed with *i*-PrOH and hexane and dried *in vacuo* to give 120 mg (47%) of **14**: mp 194–198.5° dec. An additional 60 mg was obtained from the filtrate. The combined solids were dissolved in 0.8 ml of DMF containing a few milligrams of dithiothreitol, the solution was filtered, and the filtrate was diluted with 2.4 ml of H_2O. The solid, mp 186–190°, which was collected, gave a positive reaction with Ellman reagent: mass spectrum molecular ion at *m/e* 211; ir (Nujol) 3280, 3120, 1630, 1570. 1421, 1281, 1222, 1200, 1062, 1027, 919, 830 cm^{-1}. *Anal.* ($C_{10}H_{13}NO_2S$) C, H, N.

3-Thiocyanotyramine Hydrochloride (16). To 2.87 g (41.2 mmoles) of 0.20 *M* Cl_2 in 205 ml of HOAc was added 4.0 g (41.2 mmoles) of powdered, dry KSCN, and the mixture stirred for 10 min at 17°. A solution of 2.81 g (20.6 mmoles) of tyramine in 20 ml of HOAc was added, and the mixture stirred for 10 min at 17°. KCl was removed by centrifugation, 3 ml of cyclohexene added to the supernatant, and the mixture poured into 300 ml of ether. The KCl residue was washed with EtOAc and centrifuged, and the supernatant combined with the main fraction. The mixture was stirred for 15 min and filtered, and the filter cake washed with Et_2O and dried over concd H_2SO_4 and soda lime. The tan powder weighed 4.19 g (88%) and melted above 200° with decomposition: ir (Nujol) 2155 (SCN), 3200, 3030, 2723, 2540, 1283, 1146, 944, 823, 793 cm^{-1}. It decomposed rapidly in hot solvents and at room temperature it decomposed in HOAc within 16 hr. Some purification could be achieved by dissolving the crude product in formic acid at room temp followed by precipitation with EtOAc. An analytically pure sample was not obtained. The crude material was fully satisfactory for subsequent syntheses.

5-(2-Aminoethyl)-1,3-benzoxathiol-2-one Hydrochloride (17). A solution of 400 mg of **16** in 5 ml of 1 *M* HCl was boiled for 10 min, filtered (Darco, Celite) through sintered glass, and evaporated *in vacuo*. Trituration of the residue with *i*-PrOH afforded a crystalline solid which was filtered and washed with *i*-PrOH, EtOAc, and hexane to give a mixture of product and NH_4Cl. The mixture was dissolved in 5 ml of H_2O and applied to a 1 × 4.5 cm column of Dowex-50 X-8 (H^+ form). Ammonium salts were eluted with 100 ml of 0.2 *M* HCl. A zone near the top of the column which was darker in color was removed and formed into a new column about

4 mm in diameter which was then eluted with 6 *M* HCl. The progress of elution was followed by checking small samples of the eluate with ninhydrin and with NH_3 vapors followed by $Fe[Fe(CN)_6]$. Most of the organic material eluted in the first 40 ml, which was then evaporated. Trituration of the residue with *i*-PrOH and EtOAc followed by filtration afforded 189 mg of white solid: mp 187–193° dec. Recrystallization of the crude reaction product from hot 9 *M* HCl also afforded an NH_4Cl-free material: mp 196–199°. An analytical sample, prepared by recrystallization from $EtCO_2H$, had mp 190–196° dec: mass spectrum molecular ion at *m/e* 195; ir (Nujol) 2600, 1755, 1240, 1128, 1081, 1018, 811 cm^{-1}; R_f 0.55 on silica gel (*n*-BuOH–H_2O–HOAc, 4:1:1). *Anal.* ($C_9H_{12}ClNO_3S$) C, H, N.

3-Mercaptotyramine (15). A. By Hydrazinolysis of 17. To a solution of 0.3 g (1.7 mmoles) of $Na_2S_2O_4$ in 4 ml of H_2O was added 1.07 g (4.34 mmoles) of 17. Hydrazine hydrate, 0.87 g (17.4 mmoles), was added to the resulting slurry, and N_2 bubbled through the solution. After 30 min at room temp, the mixture was cooled in an ice bath for 10 min, and the product filtered and washed with cold deoxygenated H_2O. After being dried *in vacuo* over NaOH and concd H_2SO_4, the tan product weighed 480 mg (62%): mp 253° dec (sample inserted in bath at 250°). The analytical sample was prepared by treating a solution of the product in 1 *M* HCl with a solution which was 1 *M* in NaOH and 0.1 *M* in $NaBH_4$ until the pH rose to 7. The precipitated product was washed with deoxygenated H_2O, *i*-PrOH, and hexane to give an off-white powder: mp 251–253° dec; mass spectrum molecular ion at *m/e* 169; ir (Nujol) 3275, 2660, 2595, 1235, 920, 813, 745. *Anal.* ($C_8H_{11}NOS$) C, H, N.

B. By Reduction of 16 with 2-Mercaptoethanol. To a magnetically stirred solution of 1.72 g (17.2 mmoles) of K_2CO_3 and 167 mg of $Na_2S_2O_4$ in 6.4 ml of H_2O was added 3.95 g of 2-mercaptoethanol, quickly followed by 1.98 g (8.6 mmoles) of **16** hydrochloride at such a rate that loss of material due to foaming is prevented. Stirring was continued for 15 min at room temp and for 15 min at 0°. The product was filtered, washed with cold deoxygenated H_2O, and dried *in vacuo* over concentrated H_2SO_4 and soda lime to give 1.0 g (69%) of tan solid: mp 252–254° (sample inserted in bath at 245°).

3-Mercaptotyramine Hydrochloride. 3-Mercaptotyramine base, 565 mg, was dissolved in 3.0 ml of concd HCl at the boiling point and filtered through sintered glass. The filtrate was kept for 20 min in an ice bath and filtered, and the solid washed with cold concd HCl. The product, dried *in vacuo* over concd H_2SO_4 and soda lime, weighed 520 mg (79%): mp 185–188° dec. Recrystallized in the same manner, it melted at 187–188.3°: uv λ_{max} 288 nm (ϵ 5160), in 0.1 *M* HCl. *Anal.* ($C_8H_{12}ClNOS$) C, H, N.

Disulfide of 3-Mercaptotyramine. A solution of 60 mg of **15** in 2 ml of concd NH_4OH was filtered and the filtrate allowed to stand in air for 2 days. Filtration afforded 33 mg of light yellow needles: mp 191.5–193° dec; ir (Nujol) 3555, 1582, 1395, 1270, 880, 820, and 800 cm^{-1}; mass spectrum parent peak *m/e* 336. Its electrophoretic mobility of Whatman No. 1 paper with 5 *M* HOAc was slightly greater than that of **15**. *Anal.* ($C_{18}H_{20}N_2O_2S_2$) C, H, N.

4-*tert*-Butyl-3-thiocyanatophenol (22). *p-tert*-Butylphenol (1.5 g) was added with stirring to a solution of **2** prepared by adding 2.14 g (22 mmoles) of dry KSCN to 89 ml of dry HOAc containing 1.4 g of Cl_2. The mixture was kept at 15–18° for 25 min, treated with 2 ml of allyl alcohol to destroy excess **2**, and poured into 2 l. of ice and H_2O. After 20 min, the crystalline solid was filtered, and the filter cake washed with ice H_2O. The solid was triturated with H_2O, filtered, and quickly dissolved in 15 ml of HOAc. A 15-ml slurry of ice and H_2O was added, and the precipitated solid was filtered and washed with cold 50% HOAc. It was quickly placed *in vacuo* (0.1 mm) for 1 hr over P_2O_5. The pale yellow solid was then stored at Dry Ice temp. At room temp, a sample of this material liquified in 2–3 hr, to give a mixture of products which was shown to include **23**, **24**, and **26** (see text). A sample dissolved in HOAc and precipitated with concd HCl gave pale yellow crystals: mp mp 72–73° (with no preheating); ir (Nujol) 3255 (OH), 2180 cm^{-1} (SCN); mass spectrum molecular ion at *m/e* 207. The rapid decomposition of this material precluded the preparation of an analytical sample.

5-*tert*-Butyl-1,3-benzoxathiol-2-one (24). To a solution of 600 mg (2.9 mmoles) of **22** in 3 ml of HOAc acid was added 1.5 ml of 2 *N* HCl, and the mixture was refluxed for 20 min. The solvent was evaporated, the residue taken up in EtOAc, and the NH_4Cl removed by filtration. Evaporation of the filtrate followed by cooling yielded yellow crystals: 530 mg (88%). Recrystallization at low temp from hexane followed by repeated sublimation at 90° (0.05 mm) afforded pure material: mp 37–39°; ir (CCl_4) 2965, 2905, 2870, 1765, 1726 cm^{-1}; mass spectrum parent peak *m/e* 208. *Anal.* ($C_{11}H_{12}SO_2$) C, H, S.

5-*tert*-Butyl-1,3-benzoxathiol-2-imine (23). **22** (310 mg) was dissolved in a mixture of 3 ml of MeOH and 1 ml of pyridine and allowed to stand for 10 min. Evaporation of the MeOH, followed by addition of H_2O and filtration afforded a white crystalline solid: 310 mg (100%); mp 98–103.5°. Recrystallization from C_6H_6–hexane afforded an analytical sample: mp 106–108.5°; ir (Nujol) 3290, 1655 cm^{-1}; mass spectrum molecular ion at *m/e* 207. *Anal.* ($C_{11}H_{13}NOS$) C, H, N.

The hydrochloride salt obtained as light yellow crystals from HOAc melted at 210° dec: ir (Nujol) 2720, 2570 and 1680 cm^{-1}; mass spectrum molecular ion at *m/e* 207. *Anal.* ($C_{11}H_{14}ClNOS$) C, H, Cl.

4-*tert*-Butyl-3-mercaptophenol (25). A solution of 530 mg (2.55 mmoles) of **24** in 3 ml of MeOH was added to a solution of 0.1 g of $Na_2S_2O_4$ and 383 mg (7.65 mmoles) of hydrazine hydrate in 1 ml of H_2O under nitrogen. After a few minutes, excess H_2O was added and the oil which separated was extracted into two 5-ml portions of Et_2O. The combined extract was shaken with 5- and 1-ml portions of 1 *M* NaOH which was 0.1 *M* in $NaBH_4$. The yellow aqueous extract was then acidified with HCl and extracted with Et_2O; the extract was dried ($CaCl_2$) and evaporated to give 240 mg of cloudy oil. Distillation at 70° (0.25 Torr) afforded 200 mg (43%) of a colorless oil; $n^{18}D$ 1.5655; mass spectrum molecular ion at *m/e* 182; ir (CCl_4) 3460, 2960, 1492 cm^{-1}. *Anal.* ($C_{10}H_{14}OS$) C, H, S.

4-*tert*-Butyl-3-mercaptophenyl Carbazate (24a). The residue from the above distillation was dissolved in 4 ml of hot MeCN and filtered, and the filtrate evaporated. The treatment was repeated. Trituration of the residue with EtOAc gave a white powder: mp 152–154°; ir (Nujol) 3292, 3195, 1643, 1632 cm^{-1}; mass spectrum molecular ion at *m/e* 240. *Anal.* ($C_{11}H_{16}N_2O_2S$) C, H, N.

5-Hydroxy-2-thiocyanatophenethylamine Hydrochloride (28a). Dry powdered KSCN (2.11 g) was added to a solution of 1.54 g of Cl_2 in 34 ml of dry HOAc, followed by stirring for 10 min and removal of KCl by centrifugation. The supernatant was decanted onto 1.74 g of *m*-tyramine hydrochloride (**27a**) and the mixture stirred for 10 min. The mixture was then cooled until the HOAc began to freeze, and the solid was filtered and washed with HOAc and Et_2O to give 1.94 g (84%) of white solid: mp 194–200°. Recrystallization from hot 9 *M* HCl gave 1.52 g of white crystals: mp 201–203.5°; ir (Nujol) 3200, 2160, 1772, 878 cm^{-1}; mass spectrum molecular ion at *m/e* 218. *Anal.* ($C_9H_{11}ClN_2OS$) C, H, N.

DL-6-Thiocyanatonorphenylephrine Hydrochloride (5-Hydroxy-2-thiocyanatophenylethanolamine Hydrochloride) (28b). A solution of **2** from 4.76 g of Cl_2 and 6.5 g of KSCN in 100 ml of HOAc was freed from KCl by centrifugation. DL-*m*-Octopamine hydrochloride (**27b**, 4.5 g) was added as a fine powder and the mixture stirred for 2.5 hr. The solid was centrifuged, washed twice with EtOAc by decantation, and filtered, and the filter cake was washed with EtOAc and hexane to give 4.4 g (85%) of white solid: mp 183–193° dec. The product was dissolved in 6 ml of hot H_2O and filtered, and the filtrate diluted with 6 ml of concd HCl. Cooling afforded 3.33 g of crystals: mp 203–206° dec; ir (Nujol) 3260, 3190, 2150, 1569, 1215, 1165, 1048, 991, 885, 815 cm^{-1}; mass spectrum molecular ion at *m/e* 210. An analytical sample, mp 205–207°, was prepared by the recrystallization of similar material from 9 *M* HCl. *Anal.* ($C_9H_{11}ClN_2O_2S$) C, H, N.

Salts of 6-Mercapto-*m*-tyramine (5-Hydroxy-2-mercaptophenethylamine) (29a). H_2S was bubbled through 6.7 ml (6.5 mmoles) of 0.97 *M* NaOH for 20 min. The hydrochloride **28a**, 690 mg (3 mmoles), was added in small portions during 10 min and H_2O addition was continued for 1 hr. Dithiothreitol, 66 mg, was added to remove residual yellow color. After 15 min the reaction mixture was poured into a solution of 762 mg (3 mmoles) of picric acid in 3 ml of MeOH. The mixture was kept in an ice bath for 45 min and filtered, and the solid washed with cold deoxygenated H_2O and quickly dried over concd H_2SO_4 at 0.15 mm to give 1.02 g (85%) of yellow crystals of the picrate: mp 151.5–152°. Recrystallization of similar material from H_2O afforded an analytical sample: mp 155–156° dec (no preheating, *in vacuo*). *Anal.* ($C_{14}H_{14}N_4O_8S$) C, H, N.

Treatment of a slurry of the picrate in EtOAc with gaseous HCl afforded a nearly white hydrochloride: mp 169°; ir (Nujol) 3320, 2750, 2700, 2600, 2545, 2480, 2453 cm^{-1}; mass spectrum molecular ion at *m/e* 169.

A slurry of 1.01 g of the above picrate in 25 ml of EtOAc was treated with 875 mg of *p*-toluenesulfonic acid monohydrate and the mixture stirred for 1 hr. Filtration afforded pale yellow crystals of the salt: mp 170°. Recrystallization from 1:10 *i*-PrOH–EtOAc gave 700 mg of white salt: mp 170.5–171.5°. *Anal.* ($C_{15}H_{19}NO_4S_2$) C, H, N.

Salts of DL-6-Mercaptonorphenylephrine (5-Hydroxy-2-mercaptophenylethanolamine) (29b). H_2S was bubbled through 2.0 ml (2 mmoles) of 1 *M* LiOH for 10 min. The hydrochloride **28b** (122 mg, 50 mmoles) was added at such a rate that foaming over did not occur. H_2S addition was continued for 30 min at which time 68 mg of dithiothreitol was added. Gas addition was continued for an additional 30 min. Picric acid (508 mg) was added and the mixture filtered. On standing, 151 mg (73%) of the yellow picrate separated: mp 171–173° dec. *Anal.* ($C_{14}H_{14}N_4O_9S$) C, H, N.

The picrate (102 mg) was slurried with 1 ml of EtOAc and *p*-toluenesulfonic acid monohydrate was added until the yellow color of the picrate was discharged. Filtration afforded 51 mg (57%) of the *p*-toluenesulfonate salt, which after recrystallization from *i*-PrOH-EtOAc–hexane melted at 202–204°. *Anal.* ($C_{15}H_{19}NO_5S_2$) C, H, N.

DL-3-Thiocyanatooctopamine Hydrochloride (DL-4-Hydroxy-3-thiocyanatophenylethanolamine Hydrochloride) (31). KSCN (1.77 g, 18.2 mmoles) was added to 40 ml of dry HOAc containing 1.30 g of Cl_2, the mixture was stirred for 10 min and centrifuged, and the supernatant added to 0.95 g of DL-octopamine hydrochloride and the mixture stirred for 1 hr. The mixture was then diluted with Et_2O to a volume of 200 ml, stirred for 10 min, and centrifuged, the precipitate covered with 200 ml of fresh Et_2O and centrifuged, and the precipitate washed with EtOAc and hexane. The white solid weighed 1.35 g (109%). It was extremely hygroscopic and melted broadly above 100°. Infrared bands were found at 3150, 2160, 1606, 1225, 1050, and 830 cm^{-1} (Nujol). A satisfactory method of purification of this material has not yet been found.

DL-5-(2-Amino-1-hydroxyethyl)benzoxathiol-2-one Hydrochloride (32). A solution of 674 mg of crude thiocyanato compound **31** was refluxed for 30 min in 2.5 ml of 1 *M* HCl and then evaporated to near dryness *in vacuo*. The residue was extracted with 4 ml of hot *i*-PrOH and then with 2 ml of hot HOAc. The extracts were combined and evaporated to a gum. Trituration with *i*-PrOH afforded a filterable solid, 238 mg, which, when thoroughly dried, melted at 139–146° dec. The filtrate, on standing overnight, deposited a few milligrams of small white crystals, mp 147–152° dec, electrophoretically indistinguishable from the first fraction. Thin-layer chromatography on silica gel with *n*-BuOH–H_2O–HOAc (4:1:1) as the developer gave single spots with R_f 0.61. Exposure of the spots to NH_3 fumes for 15 sec followed by a spray of 1% ethanolic trichlorobenzoquinonimine gave raspberry-colored spots. $Fe[Fe(CN)_6]$ after NH_3 afforded deep blue spots. The presence of latent thiol group was also shown by means of the Ellman reagent. Spots obtained on electrophoresis or tlc gave vivid yellow colors but only after a 15-sec treatment with ammonia fumes: mass spectrum molecular ion at *m/e* 211; also peaks at *m/e* 181, 153, and 149. The compound was extremely hygroscopic. The ir spectrum (KBr) showed absorption at 3400 (OH), 2600 and 2700 (substituted ammonium), and 1755 cm^{-1} (carbonyl). Due to difficulties in recrystallizing this material, a satisfactory analytical sample was not obtained.

Attempted Preparation of DL-3-Mercaptooctopamine (DL-4-Hydroxy-3-mercaptophenylethanolamine) (33). In a typical experiment, crude **31** (129 mg, 0.52 mmole), dithiothreitol (110 mg), and thiourea (20 mg, 0.25 mmole) were dissolved in 1 ml of MeOH, and the mixture was allowed to stand. After 1 hr, electrophoresis of the reaction mixture in 5 *M* HOAc showed the presence of essentially one cationic component which afforded a deep yellow color with Ellman reagent, an immediate red-orange color with 2,6,*N*-trichlorobenzoquinoneimine, and a vivid blue color with $Fe[Fe(CN)_6]$. Similar results were obtained in aqueous solution with dithiothreitol and $NaHCO_3$; Li_2S and H_2S; H_2O, 2-mercaptoethanol, and $NaHCO_3$; and dithiothreitol and Na_2CO_3. Thus far, attempts to isolate **33** as the free base or as the picrate have afforded only oils. Hydrazinolysis of **32** also afforded solutions which appeared to contain **33**.

Assay of Enzymatic Activity. COMT was purified as described[22] to yield a preparation containing 3.8 mg of protein per ml with a specific activity of 34 nmoles/mg per min with 3,4-dihydroxybenzoic acid as substrate. Enzyme activity was assayed as described previously[22] except that the order of addition of components was as follows, water, *S*-adenosylmethionine, $MgCl_2$, substrate, and sodium phosphate buffer (pH 8.0). The buffer was added 15 sec before the addition of enzyme. The reaction was stopped after 5 min.

Preincubation of Enzyme. The enzyme preparation (0.5 ml) in 0.02 *M* sodium phosphate buffer, pH 7.0, was incubated with varying amounts of 3-mercaptotyramine at 37°. At time intervals from 0 to 30 min, aliquots were removed and assayed for enzymatic activity. The 3-mercaptotyramine was prepared fresh as a 20 m*M* solution in water. Results were compared to controls of enzyme treated in identical manner without the addition of 3-mercaptotyramine. Reactivation studies were carried out by the addition of dithiothreitol to a final concentration of 2 m*M* to the enzyme before preincubation, or to the assay mixture before addition of enzyme. Dithiothreitol was prepared as a 100 m*M* solution in water.

Dialysis of Inhibited Enzyme. The enzyme preparation (1.0 ml) was preincubated at 37° for 7 min with 3-mercaptotyramine (2 m*M*), cooled in an ice bath, and transferred to a collodion bag (Schleicher and Schuell, No. 100) immersed in 3 l. of 0.02 *M* sodium phosphate buffer (pH 7.0). Both solutions were stirred slowly and the temperature maintained at 1–2°. Aliquots of inhibited enzyme were removed at times up to 3 hr and assayed. Results were compared to enzyme treated in the same manner without addition of 3-mercaptotyramine.

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