Synthesis and Biological Activity of 2- and 4-Substituted 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinolines†

Edward E. Smissman,[1a] Jack R. Reid, David A. Walsh,

Department of Medicinal Chemistry, School of Pharmacy

and Ronald T. Borchardt*,[1b]

Department of Biochemistry, McCollum Laboratories, University of Kansas, Lawrence, Kansas 66044. *Received May 9, 1975*

Various 2- and 4-substituted 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines were synthesized and evaluated as substrates and inhibitors of catechol *O*-methyltransferase (COMT). In addition, these compounds were tested for their ability to release norepinephrine-^3H from mouse hearts in vivo. Methyl substituents in the 2 and/or 4 positions of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline had little effect on the interaction of these molecules with COMT. In general, the substrate kinetic (K_m, V_{max}) and inhibitory kinetic (K_{is}) properties toward COMT were similar for each of these compounds. In contrast, norepinephrine depleting activity showed more strict structural requirements. Methyl substituents in the 2 or 4 positions of the parent compound, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, eliminated the norepinephrine depleting activity. The interesting exception was 6,7-dihydroxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide, which was found to be more active than the parent molecule as a depleter of norepinephrine from mouse hearts.

Appropriately substituted tetrahydroisoquinolines (THIQ's)[2] are known to produce a variety of pharmacological and biochemical actions on the adrenergic nervous system. The compound 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1) has been shown to be both a direct and indirect acting sympathomimetic agent.[3] The pharmacological effects of THIQ's include lypolytic,[4-8] bronchial relaxant,[8-10] and hypotensive activity.[6] Of recent interest has been the proposal[11] that alcohol ingestion may lead to the endogenous formation of THIQ's such as 6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline or 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1). These compounds could be produced in vivo by condensation reactions between dopamine and acetaldehyde or between dopamine and formaldehyde. Both 6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline and 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1) have been shown to satisfy many of the criteria established for false adrenergic transmitters. It has been demonstrated that they are taken up and stored by nerve endings, and they are released by nerve stimulation.[12-14]

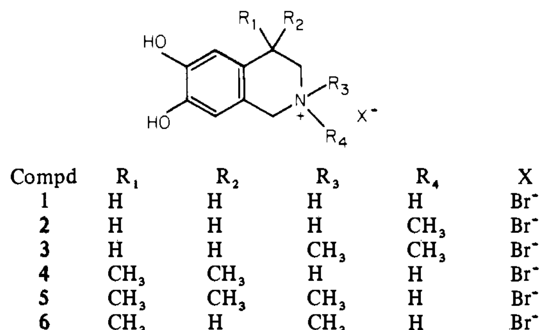
THIQ's have also been reported to be metabolized by O-methylation catalyzed by catechol *O*-methyltransferase[15] (COMT, E.C. 2.1.1.6). Because THIQ's are known to be good substrates for COMT,[15,16] they represent a new class of alternate substrate type inhibitors of this transmethylation.[17] In an effort to identify those THIQ's which will inhibit COMT but not act as false neurotransmitters, we have prepared a series of 2- and/or 4-substituted 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines (Chart I, 1–6). These THIQ's (1–6) have been evaluated as substrates and inhibitors of COMT and tested for their ability to stimulate norepinephrine release from mouse hearts. The present paper reports the results of this study.

Experimental Section

Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Microanalyses were conducted on a Hewlett-Packard Model 185B C, H, N analyzer, The University of Kansas, Lawrence, Kan. Unless otherwise stated, the ir, NMR, and uv data were consistent with the assigned structures. Ir data were recorded on a Perkin-Elmer Model 727 spectrophotometer and NMR data on a Varian Associates Model T-60 spectrophotometer (Me_4Si).

† This paper is dedicated to Professor Edward E. Smissman—esteemed teacher, scientist, and first and foremost friend.

Chart I. Substituted 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinolines Synthesized and Evaluated in Norepinephrine Depletion Studies and COMT Inhibition Studies



Compd	R_1	R_2	R_3	R_4	X
1	H	H	H	H	Br^-
2	H	H	H	CH_3	Br^-
3	H	H	CH_3	CH_3	Br^-
4	CH_3	CH_3	H	H	Br^-
5	CH_3	CH_3	CH_3	H	Br^-
6	CH_3	H	CH_3	H	Br^-

Scintillation counting was done on a Beckman LS-150 scintillation counter.

Materials. *S*-Adenosyl-L-methionine-*methyl*-^{14}C (SAM-$^{14}CH_3$) (New England Nuclear, 55.0 mCi/mmol) and DL-epinephrine-*7*-^{14}C (Epi-^{14}C) (New England Nuclear, 51 mCi/mmol) were diluted to concentrations of 10 μCi/ml and stored at −20°F. A solution of DL-norepinephrine-*7*-^3H (Norepi-^3H) (New England Nuclear, 7.5 Ci/mmol) was freshly prepared daily by dilution with isotonic NaCl to a concentration of 50 μCi/ml. SAM iodide (Sigma) was stored as a 0.01 *M* aqueous stock solution.

6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (7). The 6,7-Dimethoxy-3,4-dihydroisoquinoline[18] (19.2 g, 0.1 mol) was dissolved in 200 ml of EtOH and the ethanolic solution acidified with concentrated HCl. Platinum oxide (0.3 g) was added and the reaction mixture hydrogenated at 25° under 2 atm of pressure. Crystallization of the crude product from ethanol afforded 19.1 g (83%) of 7: mp 254–256° (lit.[19,20] mp 251–252°).

Method A. General Procedure for Hydrolysis of 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolines to 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinolines. 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrobromide (1). A mixture of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (7, 3.4 g, 15 mmol) and 30 ml of 48% HBr was heated at reflux under nitrogen atmosphere for 3 hr. The reaction mixture was concentrated and the residue washed with ethanol and collected by filtration. Recrystallization ($EtOH-H_2O-Et_2O$) gave 3.5 g (95%) of 1: mp 267–268° (lit.[20,21] mp 268°). The various 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines (1–6) reported in Table I were prepared from the corresponding 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines (7–9, **13b**, **14a,b**) using this general hydrolysis procedure.

Method B. General Procedure for Methylation of 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolines to 6,7-Dimeth-

Table I. 2- and 4-Substituted 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinolines

Compd	Mp, °C	Recrystn solvent	Method of prepn	Yield, %	Formula	Analyses
3	263-266[a]	EtOH	A	61	$C_{11}H_{16}BrNO_2$	C, H, N
4	211-215	*i*-PrOH-H_2O	A	72	$C_{11}H_{16}BrNO_2$	C, H, N
5	285-287	*i*-PrOH-Et_2O	A	84	$C_{12}H_{18}BrNO_2$	C, H, N
6	120-122	*i*-PrOH-Et_2O	A	88	$C_{11}H_{16}BrNO_2 \cdot {}^1/_6H_2O$	C, H
8	219-221[b]	*i*-PrOH	B	90	$C_{12}H_{18}ClNO_2$	C, H, N
14a	149-151	*i*-PrOH	B	88	$C_{13}H_{20}ClNO_2$	C, H, N
14b	249-251	*i*-PrOH	B	58	$C_{14}H_{22}ClNO_2$	C, H, N

[a] Lit.[22] mp 285°. [b] Lit.[21] mp 215°.

oxy-2-methyl-1,2,3,4-tetrahydroisoquinolines. **6,7-Dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (8).** The 6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (7, 13.9 g, 61 mmol) was partitioned between CH_2Cl_2 (75 ml) and 10% NaOH (75 ml). The CH_2Cl_2 layer was dried (K_2CO_3) and filtered, and the solvent was removed under reduced pressure to yield the solid free base. The free base was dissolved in 98% formic acid (35 ml) and 37% formaldehyde (8.20 g). The pale yellow mixture was heated at 100° for 18 hr while stirring. The reaction mixture was concentrated in vacuo at 100°; then 150 ml of 3 *N* HCl was added. The acidic reaction mixture was extracted twice with 50-ml portions of Et_2O and then made basic with 4 *N* NaOH. The neutralized reaction mixture was extracted with ether and the ether dried over K_2CO_3. The ether was removed in vacuo and the free base converted to the hydrochloride salt to yield 11.7 g (93%): mp 219–221° (lit.[21] mp 215°).

6,7-Dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium Iodide (9). The compound 6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (8, 19.0 g, 75 mmol) was partitioned between 100 ml of CH_2Cl_2 and 100 ml of 10% aqueous NaOH. The layers were separated and the aqueous phase was extracted once with 100 ml of CH_2Cl_2. The CH_2Cl_2 layers were combined, dried over K_2CO_3, and filtered, and the solvent was removed in vacuo to yield 16.6 g (97%) of a white solid. This solid was dissolved in 300 ml of anhydrous Et_2O and a solution of 15.6 g of MeI in 50 ml of anhydrous Et_2O was added with rapid stirring. The resulting white solid was collected by filtration and washed with two 50-ml portions of anhydrous Et_2O. After drying in vacuo (P_2O_5), 27.95 g (99%) of the title compound was isolated: mp 248–249° (lit.[22] 267–268°, prepared by an alternate procedure). Anal. ($C_{13}H_{20}INO_2$) C, H, N.

5,6-Dimethoxy-3,3-dimethylindanone 1-Oxime (11b). The intermediate 5,6-dimethoxy-3,3-dimethylindanone (**10b**) was prepared by a modification of a previously published procedure.[23] To a mixture of veratrole (42.3 g, 0.31 mol) and 200 g of polyphosphoric acid was added 3,3-dimethylacrylic acid (31.5 g, 0.31 mol). The reaction mixture was heated on a steam bath with agitation to initiate the exothermic reaction. After the initial reaction had subsided, heating was continued for 45 min on a steam bath, after which 600 g of crushed ice was added. The reaction mixture was maintained for 24 hr and then extracted with two 300-ml portions of C_6H_6. The C_6H_6 layers were combined and washed with aqueous 1 *N* NaOH, H_2O, aqueous 1 *N* NaCl, and H_2O. The C_6H_6 layer was dried (Na_2SO_4) and filtered, and the C_6H_6 was removed under reduced pressure. Vacuum distillation afforded 37.54 g (55%) of a colorless, viscous oil. Spectral data were consistent with the proposed structure of 5,6-dimethoxy-3,3-dimethylindanone (**10b**).

A mixture of the ketone **10b** (11.01 g, 50 mmol), $NH_2OH \cdot HCl$ (5.56 g, 80 mmol), NaOAc (12.25 g, 150 mmol), 100 ml of EtOH, and 30 ml of H_2O was refluxed for 1 hr, after which 100 ml of H_2O was added. The reaction mixture was allowed to cool slowly overnight to ambient temperature and then placed in a refrigerator. The resulting crystals were collected by filtration to yield 11.76 g (83%) of the desired oxime **11b**: mp 140–142°. Anal. ($C_{13}H_{17}NO_3$) C, H, N.

***dl*-5,6-Dimethoxy-3-methylindanone 1-Oxime (11a).** Using a procedure identical with that described above for the preparation of **11b**, veratrole (42.3 g, 0.31 mol) and *cis*-crotonic acid were converted to the corresponding oxime **11a**. After work-up, 21.1 g (77%) of **11a** was isolated: mp 124–126°. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

6,7-Dimethoxy-4,4-dimethyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (13b). To a solution of P_2O_5 (20 g) dissolved in 200 ml of methanesulfonic acid[24] was added 5,6-dimethoxy-3,3-dimethylindanone 1-oxime (**11b**, 6.0 g, 25 mmol) in 0.5-g increments, waiting until each successive portion had dissolved. The reaction mixture was heated on an oil bath at 100° for 75 min and then cooled to ambient temperature. The reaction mixture was poured cautiously into 450 ml of saturated $NaHCO_3$ solution with stirring. Solid $NaHCO_3$ was added until gaseous evolution had ceased. The aqueous solution was extracted three times with 200-ml portions of $CHCl_3$. The $CHCl_3$ extract was dried ($MgSO_4$) and filtered, and the $CHCl_3$ was removed under reduced pressure to yield a yellow residue. The residue was treated with a mixture of 75 ml of C_6H_6 and 400 ml of Skelly B to yield 4.80 g (80%) of a tan amorphous powder: mp 170–174°. The spectral data were consistent with the structure of the proposed isocarbostyril derivative **12b**.

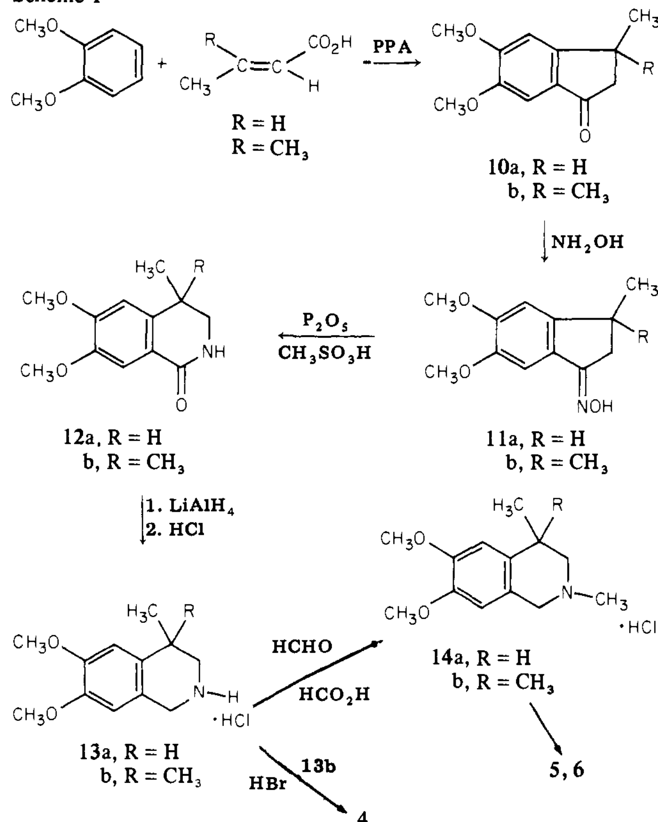
The crude isocarbostyril derivative **12b** (4.80 g, 20 mmol) was dissolved in 250 ml of dry THF to which was added $LiAlH_4$ (5.00 g) in portions. The reaction mixture was refluxed overnight (18 hr) and the excess $LiAlH_4$ decomposed by addition of aqueous 4 *N* NaOH solution (40 ml) and H_2O (750 ml). The inorganic salts were removed by filtration and washed with 250 ml of Et_2O to remove any occluded product. The aqueous phase was extracted two times with 250-ml portions of Et_2O, the combined Et_2O solutions were dried (Na_2SO_4) and filtered, and the volume was reduced in vacuo to 100 ml. HCl gas was bubbled into the Et_2O solution and the white solid which formed was collected by filtration. Recrystallization (*i*-PrOH–Et_2O) afforded 2.70 g (51%) of the desired **13b**: mp 209–212°. Anal. ($C_{13}H_{20}ClNO_2$) C, H, N.

***dl*-6,7-Dimethoxy-4-methyl-1,2,3,4-tetrahydroisoquinoline Hydrochloride (13a).** Using a procedure identical with that described above for the preparation of **12b**, *dl*-5,6-dimethoxy-3-methylindanone 1-oxime (**11a**) was converted to the isocarbostyril derivative **12a** in 72% yield: mp 160–161°. Anal. ($C_{12}H_{15}NO_3$) C, H, N. The isocarbostyril derivative **12a** was reduced to the desired **13a** in 33% yield using a procedure identical with that described above for the preparation of **13b**. Recrystallization (*i*-PrOH) resulted in pure product: mp 222–225°. Anal. ($C_{12}H_{18}ClNO_2$) C, H, N.

Enzyme Purification and Assay. COMT was purified from rat liver (male, Sprague–Dawley, 180–200 g). Purification was carried through the calcium phosphate stage resulting in approximately a 50-fold purification of COMT activity.[25-27] In determining the kinetic parameters (V_{max} and K_m) for epinephrine and the various THIQ's **1–6** as substrates for COMT, SAM-$^{14}CH_3$ was used as a methyl donor and a standard radiochemical assay utilized.[25,26] For determining the inhibitory constants (K_{is}) for the THIQ's **1–6** toward a COMT-catalyzed transmethylation, SAM and Epi-^{14}C were used as substrates. Using this type of radiochemical assay only methylation of epinephrine was detected. The radiolabeled metanephrine and paranephrine which were generated could be extracted [toluene–isoamyl alcohol (3:2)] from the incubation mixtures and measured.[25,26] Processing of the kinetic data was achieved by plotting reciprocal velocities against reciprocals of the substrate concentrations. Substrate kinetic parameters (V_{max} and K_m) and inhibitory constants (K_{is}) were calculated using Cleland's equations[28] as described in earlier publications from this laboratory.[26,29,30]

Assay of Norepinephrine Release. The general procedure used to detect norepinephrine release was that previously described by Daly et al.[31] A 0.1-ml solution (isotonic NaCl containing

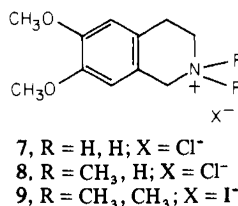
Scheme I



50 mg of heparin/l.) of 1.0 nmol (5 μCi) of Norepi-^{3}H was injected into the tail vein of mice [approximately 20 g, ARS HA(ICR)f outbred Swiss albino mice]. After 1 hr the THIQ's 1-6 (10 mg/kg) were administered subcutaneously. After 3 hr the mice were sacrificed and the hearts (4-5 mice per assay) were immediately removed and homogenized in 10 vol of 0.4 N $HClO_4$. After centrifugation, the tritium content of 0.2 ml of the supernatant solution was measured. Control animals were given Norepi-^{3}H, but instead of administering a drug, an injection of an equal volume of saline was used.

Results and Discussion

Chemistry. The 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (7) was prepared by reduction of 6,7-dimethoxy-3,4-dihydroisoquinoline.[18] Hydrolysis of 7 using 48% HBr afforded the desired 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1).[20,21] Methylation of 7 using the Eschweiler-Clark procedure afforded 8, which was hydrolyzed to 6,7-dihydroxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2). Exhaustive methylation of 8 using methyl iodide afforded 6,7-dimethoxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide (9), which was hydrolyzed



7, R = H, H; X = Cl$^-$
8, R = CH_3, H; X = Cl$^-$
9, R = CH_3, CH_3; X = I$^-$

to 3. The 4-methylated THIQ's 4-6 were synthesized from the intermediate indanones **10a** and **10b**, which were prepared by reaction of veratrole with 3,3-dimethylacrylic acid or crotonic acid. The indanones **10a** and **10b** were converted to the corresponding oximes **11a** and **11b**. Treatment of **11a** and **11b** with P_2O_5 in methanesulfonic acid[24] under Beckmann rearrangement conditions afforded the corresponding isocarbostyril derivatives **12a** and **12b**.

Table II. Kinetic Data for Tetrahydroisoquinolines 1-6 as Substrates and Inhibitors of Catechol O-Methyltransferase[a]

Compd no.	Substrate constants[b] K_m ± SEM, μM	V_{max} ± SEM[c]	Inhibition constants[d] K_{is} ± SEM, μM[e]
1-Epinephrine	398 ± 8.89	19.8 ± 2.56	
1	540 ± 66	15.5 ± 1.18	196 ± 29
2	670 ± 13	18.5 ± 0.2	152 ± 17
3[f]			191 ± 27
4	310 ± 37	17.4 ± 0.8	259 ± 29
5	410 ± 33	12.1 ± 0.55	187 ± 22
6	754 ± 64	21.2 ± 3.12	101 ± 35

[a] COMT was purified as described in the Experimental Section. [b] Assay mixtures contained SAM concentration = 1.0 mM; SAM-$^{14}CH_3$ = 0.05 μCi; substrate concentration = 50-500 μM. Incubations were carried out for 10 min, after which the reaction was stopped with 0.25 ml of borate buffer, pH 10.0. The assay mixtures were extracted with 10 ml of toluene-isoamyl alcohol (3:2).[25,26] [c] nmol of product/mg of product/min. [d] Assay mixtures contained SAM concentration = 1.0 mM; Epi-^{14}C = 0.05 μCi; Epi concentration = 54-404 μM. Assay conditions and work-up identical with those described in footnote b. [e] In all cases competitive kinetics were observed and inhibition constants calculated according to the procedure of Cleland.[28] [f] The high aqueous solubility of 3 prevented extraction of methylated products and thereby prevented the accurate determination of K_m and V_{max}.

Earlier works by Marsili[32,33] had shown that Beckmann or Schmidt rearrangements on related indanones resulted in the formation of the isocarbostyril derivatives. Reduction of **12a** and **12b** using $LiAlH_4$ afforded the desired THIQ's **13a** and **13b**. Hydrolysis of **13b** yielded **4**. Methylation of **13a** and **13b**, followed by hydrolysis, produced the desired THIQ's **5** and **6** (Scheme I).

Biological. The various THIQ's reported in this study (1-6, Chart I) were prepared in an effort to determine if the COMT inhibitory effects of THIQ's could be separated from their indirect sympathomimetic effects. To evaluate this possibility the THIQ's 1-6 were tested as substrates and inhibitors of purified COMT. In addition, they were evaluated for their ability to release Norepi-^{3}H from mouse hearts, which would be an index of their indirect sympathomimetic activity.[31,35,36]

In Table II is shown the substrate kinetic constants (K_m, V_{max}) for the methylation of 1-epinephrine and the THIQ's 1, 2, and 4-6 by COMT. The results of this study show that COMT has a similar affinity (K_m) and can methylate these THIQ's at a rate (V_{max}) comparable to that of epinephrine. Substitution of methyl groups in the 2 and/or 4 positions of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (1) appears to have little effect on the enzyme's affinity for these substrates or the rate at which the enzyme can methylate them. These results would be consistent with the earlier observations by Creveling et al.[16] that the THIQ's 1 and 2 were substrates for COMT. In addition, Collins et al.[15] have shown that the 1-substituted THIQ's, salsolinol and 1,2,3,4-tetrahydropapaveroline, are also substrates for this transmethylation reaction.

The THIQ's 1-6 were also evaluated as inhibitors of the COMT-catalyzed transfer of a methyl group from SAM to Epi-^{14}C and the results are shown in Table II. Using reciprocal velocity vs. reciprocal substrate plots, the kinetic patterns for COMT inhibition by THIQ's 1-6 were determined. In all cases linear competitive patterns of inhibition were observed when epinephrine was the variable substrate. The observation that the magnitude of the inhibition constants for THIQ's 1-6 is similar would suggest that methyl substituents on the 2 and/or 4 positions of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline have little effect on the ability of these compounds to interact

Table III. Release of Norepinephrine-^3H from Mouse Heart in Vivo by Tetrahydroisoquinolines **1-6**[a]

Compd no.	Norepinephrine-^3H[b,c] in heart, % of control
1	63 ± 4.2
2	104 ± 7.8
3	43 ± 3.8
4	103 ± 9.2
5	115 ± 10.4
6	107 ± 7.3

[a] THIQ'S were administered subcutaneously 1 hr after intravenous administration of Norepi-^3H and radioactivity was measured in hearts 2 hr later (see Experimental Section). [b] THIQ'S administered at a dose of 10 mg/kg. Controls were given an equivalent volume of saline. [c] Values are the average of duplicate determinations using five mice per determination.

with the catechol binding site on COMT. This conclusion would be further supported by the substrate kinetic data discussed above. The lack of strict structural requirements for the binding of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines to COMT is also apparent from the earlier observation by Borchardt[17] that the THIQ derived from the condensation of norepinephrine and pyridoxal 5'-phosphate is a potent inhibitor of COMT. In addition, Ho et al.[34] have shown that various 1-substituted 5,6-dihydroxy-1,2,3,4-tetrahydroisoquinolines derived from the condensation of dopamine or norepinephrine with aromatic aldehydes were good substrates and inhibitors of COMT. These data would suggest that appropriately substituted 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolines could be potentially useful alternate substrate type inhibitors of this enzymatic reaction.

In an effort to evaluate the structural specificity for the norepinephrine depleting activity of THIQ's, compounds **1-6** were tested for their abilities to release Norepi-^{3}H from mouse hearts in vivo. The depleting activities were measured using a previously published procedure[31,35,36] and the results are shown in Table III. These data are expressed as the percent of controls of Norepi-^{3}H remaining in mouse hearts after administration of 10 mg/kg of the THIQ's **1-6**. In using this experimental procedure we have assumed that the compounds being tested reach the cardiac nerve endings in the same concentrations and the same time, so that the structure-activity relationships we have observed are those for depleting abilities of the compounds, not differences in transport, metabolism, etc. Since the changes in the basic THIQ structure are minor and we have already observed little differences in rates of metabolism by COMT, we feel this is a valid assumption. Consistent with earlier observations,[12-14] our results indicate that 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (**1**) can displace norepinephrine in cardiac nerve endings. However, methyl substituents in the 2 and/or 4 positions of the parent molecule resulted in compounds (**2, 4-6**) which were unable to release Norepi-^{3}H from cardiac sites. These findings would appear to be consistent with the earlier observations of Daly et al.[31] that introduction of a methyl substituent into the benzylic position of *p*-tyramine also significantly decreases its releasing activity. In contrast, however, 6,7-dihydroxy-2,2-dimethyl-1,2,3,4-tetrahydroisoquinolinium iodide was found in our studies to be more active as a depleter of norepinephrine than the parent compound, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline. These results appear to contradict the earlier observations of Daly et al.[31] that quaternization of the terminal nitrogen of dopamine produced a sharp reduction in norepinephrine depleting activity.

In conclusion, our data suggest fairly strict structural requirements for the norepinephrine releasing activity of THIQ. This appears to be in sharp contrast to the less stringent structural requirements observed for the COMT inhibitory activity. The results of this study show that by minor structural modifications of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, the COMT inhibitory effects can be separated from the indirect sympathomimetic effects.

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References and Notes

(1) (a) Deceased July 14, 1974; (b) Established Investigator of the American Heart Association.
(2) Abbreviations used are SAM, *S*-adenosyl-L-methionine; SAM-$^{14}CH_3$, *S*-adenosyl-L-methionine-*methyl*-^{14}C; Epi-^{14}C, DL-epinephrine-*7*-^{14}C; Norepi-^{3}H, DL-norepinephrine-*7*-^3H; COMT, catechol *O*-methyltransferase; THIQ, tetrahydroisoquinoline; K_{is}, inhibition constant for the slope.
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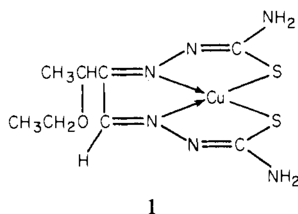
Comparative Analysis of the Cytotoxicity of Substituted [Phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) Chelates†

Eugene A. Coats,* Stanley R. Milstein, Gary Holbein, John Mc Donald, Ronald Reed, and Harold G. Petering

College of Pharmacy and Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45221. *Received May 5, 1975*

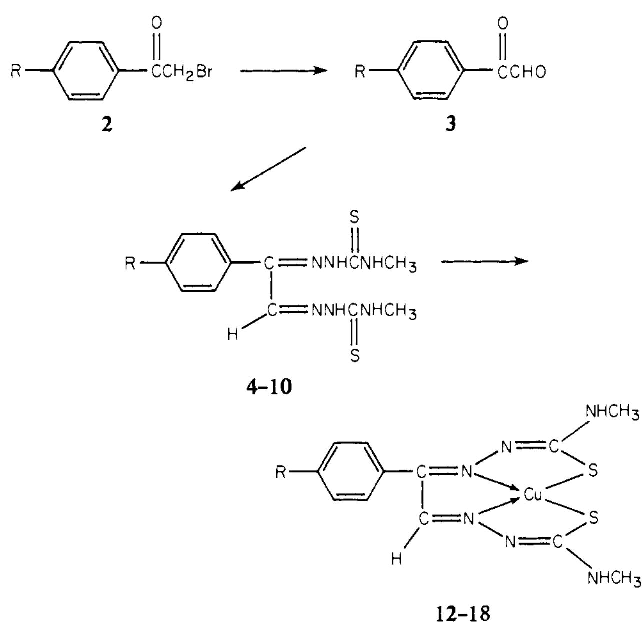
Seven para-substituted [phenylglyoxal bis(4-methyl-3-thiosemicarbazone)]copper(II) chelates (**12–18**) have been designed, synthesized, and tested for their ability to inhibit the respiration of rat liver slices as a normal cell model and Ehrlich ascites cells as a tumor cell model. Relationships between chemical structure and respiratory inhibition are described on a quantitative basis using substituent constants (π, E_s, and σ_p) by computerized multiparameter regression analyses. The correlations indicate that changes in E_s have the largest effect on liver slice toxicity of chelates while π and σ_p account for most of the variation in toxicity to ascites cells. A comparative analysis strongly suggests that electron-donating substituents with greater water solubility should increase cytotoxicity to ascites cells at the expense of cytotoxicity to the rat liver cells. The predictions of the equations were checked by synthesizing and testing an additional derivative. The results strengthen the initial predictions.

The development of metal chelates and chelating agents as potential antitumor agents has led to the selection of a class of copper chelates of bis(thiosemicarbazones) for initial quantitative SAR studies. Many reports have appeared dealing with KTS [2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone), kethoxal bis(thiosemicarbazone)] and its copper(II) chelate **1** as antitumor agents.[1–9]



Considerable evidence has been presented in support of the view that **1** serves as a vehicle for transporting copper into the cell and that the cytotoxicity is actually due to the accumulation of copper within the cell and its effect on enzymes involved in DNA synthesis. More recent investigations[10] have indicated that an additional mechanism of cytotoxicity may reside in the ability of the copper chelate of KTS to uncouple oxidative phosphorylation in isolated rat liver mitochondria. These chelates are also capable of inhibiting the respiration of Ehrlich ascites cell suspensions and of rat liver slices.[10] In light of these findings a new series of bis(thiosemicarbazone)copper(II) chelates (**12–18**) was designed in an attempt to allow further delineation of the mechanism of action and to allow investigation of possible quantitative relationships between chelate structure and biological activity. The molecules examined in this stage of our investigation contain completely conjugated phenyl rings for the primary purpose of determining the electronic effect of substituents on biological activity. If the chelate is in fact a metal ion transport system, it seemed reasonable that the substituted phenyl rings would affect chelate stability and hence the biological effectiveness of the bis(thiosemicarbazone) chelate. The substituents were selected, therefore, to obtain the largest feasible variance in electron-donating and -withdrawing ability as measured by the Hammett σ constant.[11] In addition, the variance in steric[12] and hydrophobic[13] properties was considered with care being taken to select only those substituents for which accurate physicochemical parameters were readily available.

Scheme I



Chemistry. The chelates were prepared according to that shown in Scheme I, starting with the appropriately substituted phenacyl bromide (**2**).

Following procedures established by Kornblum, the known phenylglyoxals **3** were prepared by treating **2** with dimethyl sulfoxide at room temperature.[14] Conversion of **3** to the bis(4-methyl-3-thiosemicarbazones) **4–10** proceeded smoothly in the presence of excess 4-methyl-3-thiosemicarbazide and an acid catalyst.[15] Treatment of **4–10** with stoichiometric amounts of cupric acetate in hot

† This paper is dedicated to the memory of Professor Edward E. Smissman whose scientific insight, personal integrity, and constant warmth and concern continue to serve as an example to one young medicinal chemist (Eugene A. Coats).