(M·+, <1), 191 (2), 162 (10), 161 (71), 135 (48), 119 (17), 108 (12), 98 (19), 73 (10), and 48 (100).

The same product was also obtained by stirring the above reaction mixture at room temperature for 24 h.

N-(**Pyrazolo[3,4-*d*]pyrimidin-4-ylcarbamoyl**)-*o*-**chloroaniline (24). Method C.** A stirred mixture of 1.35 g (10 mmol) of 4-aminopyrazolo[3,4-*d*]pyrimidine (1), 3.08 g (20 mmol) of *o*-chlorophenyl isocyanate, and 20 ml of anhydrous Me_2SO was stirred in a glass bomb at room temperature for 24 h. The white solid was filtered and washed with hot EtOH. Additional product was obtained from the filtrate: total yield 1.89 g (65.4%); mp >300° dec; ir max 1700 (ureido C=O), 1590 and 1540 cm^{-1} (C=C, C=N); NMR (in Me_2SO-d_6) δ 7.65–6.97 (m, 4, phenyl ring), 8.60 (s, 1, 3 or 6-H), 8.68 (s, 1, 3 or 6-H), and 11.12 ppm (s, 1, NH); mass spectrum *m/e* (rel %) 245 (M·+, <1), 162 (1), 161 (7), 156 (4), 155 (32), 154 (9), 153 (100), 135 (19), 127 (21), 125 (32), 90 (28), and 63 (14).

These reactions, when carried out with excess alkyl or aryl isocyanates for a prolonged period of time (48 h) at room temperature, resulted in the formation of a mixture of the N^4-carbamoyl and the $N^1(N^2)$,N^4-dicarbamoyl-4-aminopyrazolo-[3,4-*d*]pyrimidine **5**. In these cases the mixture was dissolved in 1 N NaOH and heated on a steam bath for 10 min. After cooling to room temperature and then adjusting to pH 4.0 with concentrated HCl, the precipitated 4-ureidopyrazolo[3,4-*d*]pyrimidines (4) were collected on a filter and washed with water.

N^4-**Allylaminopyrazolo[3,4-*d*]pyrimidine (29). Method D.** A mixture of 1.54 g (10 mmol) of 4-chloropyrazolo[3,4-*d*]pyrimidine and 1.99 g (23.5 mmol) of allyamine in 10 ml of H_2O was heated at 100° for 4 h. Upon cooling to room temperature the white crystalline product was obtained: yield 1.19 g (70%); mp 187–191°; ir max 1600 cm^{-1} (C=C, C=N); NMR (in Me_2SO-d_6) δ, 4.22 (m, 2, NCH_2), 5.24 (m, 2, CH_2), 5.93 (m, 1, CH), 8.24 (s, 1, 3 or 6-H), and 8.30 ppm (s, 1, 3 or 6-H); mass spectrum *m/e* (rel %) 175 (M^+ + 1, 26), 174 (M·+, 32), 160 (75), 147 (11), 135 (10), 119 (15), 108 (10), 93 (15), 66 (29), and 56 (100).

Growth Inhibition Assays. Cultured cells derived from the buffy coat of a normal individual (Nc 37) and a patient with myeloblastic leukemia (RPMI 6410) and mouse leukemia cells L1210 were used for growth inhibition studies (Table I).[2b] The compounds were dissolved in Me_2SO and allowed to remain at 37° overnight; this achieved sterilization. Sterile growth medium (RPMI No. 1640 + 10% fetal calf serum) was then added to bring the final concentration of Me_2SO to 0.5% and that of a compound to 10^{-4} M. At this Me_2SO concentration, growth of control cultures was unaffected. The assays were performed in test tubes without agitation. The tubes were inoculated with cells from stock cultures in logarithmic growth to a starting density previously determined to be near minimum required for initiation of logarithmic growth of control cultures (between 2–5 × 10^5 cells/ml). At 24-h intervals, 0.2 ml was removed and mixed with trypan blue to a final concentration of 0.05% and the total and viable cells were counted in a hemocytometer. Cells able to exclude the dye were scored as viable. The compounds were rated as shown in Table I to indicate the following viable cell densities relative to controls after 72 h of incubation: >++, 0–30%; ++, 30–60%; +, 60–80%, ± 80–90%; NA, (not active) 90–110%.

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References and Notes

(1) This study was supported by a grant from the U.S. Public Health Service, CA 14185. Presented in part at the 169th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1975.
(2) (a) G. B. Chheda and C. I. Hong, *J. Med. Chem.*, **14**, 748 (1971); (b) C. I. Hong, G. B. Chheda, S. P. Dutta, A. O'Grady Curtis, and G. L. Tritsch, *ibid.*, **16**, 139 (1973); (c) W. H. Dyson, C. M. Chen, S. N. Alam, R. H. Hall, C. I. Hong, and G. B. Chheda, *Science*, **170**, 328 (1970); (d) W. H. Dyson, R. H. Hall, C. I. Hong, S. P. Dutta, and G. B. Chheda, *Can. J. Biochem.*, **50**, 327 (1972).
(3) (a) F. Skoog, H. Q. Hamzi, A. M. Szweyowska, N. J. Leonard, K. L. Carraway, T. Fujji, J. P. Helgeson, and R. N. Leoppky, *Phytochemistry*, **6**, 1169 (1967); (b) M. H. Fleysher, A. Bloch, M. T. Hakala, and C. A. Nichol, *J. Med. Chem.*, **12**, 1056 (1969).
(4) R. K. Robins, *J. Am. Chem. Soc.*, **78**, 784 (1956).
(5) M. E. Hedi Jellali, N. V. Bac, and N. Dat-Xuong, *Tetrahedron*, **31**, 587 (1975).

Catechol *O*-Methyltransferase. 8. Structure–Activity Relationships for Inhibition by 8-Hydroxyquinolines

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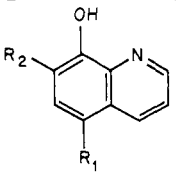
A series of 5- and 7-substituted 8-hydroxyquinolines was evaluated as inhibitors of catechol *O*-methyltransferase (COMT, E.C. 2.1.1.6). The electronic character of the substituents in the 5 position appeared to have only a small effect if any on the inhibitory activity of these compounds. A significant factor which contributes to the inhibitory activity of these compounds appears to be the nature of the 7-substituent. The structure–activity relationship for this series of inhibitors is discussed relative to the nature of the enzymatic binding site.

Catechol *O*-methyltransferase (COMT,[2] E.C. 2.1.1.6) plays an important role in the inactivation of catecholamines and the detoxification of various xenobiotic catechols. COMT is a soluble enzyme which requires magnesium to catalyze the transfer of a methyl group from *S*-adenosyl-L-methionine (SAM) to the catechol acceptor.[3,4] Of the various different classes of synthetic inhibitors of this transmethylation which have been identified some of the most potent in vitro inhibitors are derivatives of 8-hydroxyquinoline (8-HQ).[5] From kinetic studies carried out in our laboratory, we have shown that 8-HQ's inhibit COMT by binding reversibly to the catechol binding site on this enzyme.[5]

As part of our continuing studies of this enzymatic transmethylation, we have attempted to determine what structural modifications of 8-HQ would afford optimal inhibitory activity. In addition, we felt such comprehensive structure–activity relationship studies could provide useful information concerning the topography surrounding the catechol binding site of this enzyme. From earlier studies in our laboratory,[5] it was demonstrated that the 8-hydroxyl group and the quinoline nitrogen of 8-HQ were absolute

Table I. COMT Inhibitory Activity of Substituted 8-Hydroxyquinolines[a,b]



Compd no.	R_1	R_2	pI_{50} obsd	Compd no.	R_1	R_2	pI_{50} obsd
1	H	H[c]	4.67	13	CH_3	H[c]	4.35
2	Cl	H[c]	4.63	14	Et	H[c]	4.29
3	F	H[c]	4.22	15	*n*-Pr	H[c]	4.23
4	CHO	H[c]	4.23	16	NH_2	H[c]	4.53
5	$COCH_3$	H[c]	4.47	17	OH	H	3.97
6	NO_2	H[c]	4.70	18	Br	Br[d]	5.39
7	SO_3^-	H[c]	4.71	19	I	I[d]	5.25
8	CH_2CN	H[f]	4.88	20	Cl	I[e]	5.32
9	CH_2OH	H[c]	4.34	21	SO_3	I	5.15
10	CH_2OCH_3	H[c]	4.41	22	$COCH_3$	I[d]	5.56
11	$CH_2OC(=O)CH_3$	H[f]	4.72	23	NO_2	NO_2[d]	3.65
12	$CH_2CH_2OC(=O)CH_3$	H[f]	4.78				

[a] COMT was purified and assayed as previously described.[9,10] SAM concentration 1.0 mM; 0.05 μCi SAM-$^{14}CH_3$; DHB concentration 2.0 mM; Mg^{2+} concentration 1.2 mM; TES buffer concentration 40 mM, pH 7.60; incubation time, 10 min. [b] Unless otherwise noted the inhibitors were prepared as an aqueous stock solution of concentration 0.2-2.0 mM. [c] Stock solution prepared in 2 mM HCl. [d] Stock solution prepared in 25% Me_2SO-H_2O. [e] Stock solution prepared in 50% Me_2SO-H_2O. [f] Stock solution prepared in 10% Me_2SO-H_2O. Where inhibitor was added from a stock solution prepared in aqueous HCl or aqueous Me_2SO, the reactions in absence of inhibitors were run with addition of a similar amount of aqueous HCl or aqueous Me_2SO. (The aqueous HCl or Me_2SO utilized showed little effect on the rate of the standard reactions.)

requirements for maximum inhibitory activity. To further characterize the structural features of 8-HQ which gave maximum inhibitory activity, we have examined an extensive series of 8-HQ's as inhibitors of COMT. The inhibitory activities of the 8-HQ's were determined by measuring their pI_{50}'s, which represent the negative logs of the molar concentrations of the inhibitors needed to produce 50% inhibition of the enzyme activity.

In Table I are listed the observed pI_{50} values for various 5- and 7-substituted 8-HQ's. From earlier studies in our laboratory,[5] there were some preliminary indications that electron-withdrawing groups at the 5 position of 8-HQ's improved the ability of these compounds to inhibit COMT. However, the results shown in Table I for an extensive series of 5-substituted 8-HQ's (compounds 1–17) indicate that changes in the electronic and steric nature of the substituent in this position have little effect on inhibitory activities toward COMT (pI_{50}'s). Only very small changes in the pI_{50} values were observed, even when very drastic changes in the nature of 5-substituents on 8-HQ were made.[6]

Various 5,7-disubstituted 8-HQ's (18–23) were also tested for their abilities to inhibit this transmethylation. From the pI_{50}'s exhibited by these 8-HQ's (18–23) it can be concluded that incorporation of a halogen in the 7 position of an 8-HQ enhances the inhibitory activity toward COMT. For example, the inhibitory activity of compound **5** (pI_{50} = 4.47) was increased by a factor of 10 when an iodide group was incorporated into the 7 position (compound **22**, pI_{50} = 5.56). The strong dependence of the COMT inhibitory activity of 8-HQ's on the nature of the 7-substituent was somewhat surprising, since in earlier studies we had shown that inclusion of any substituent in the 2 position of 8-HQ (position adjacent to the quinoline nitrogen) resulted in complete loss of inhibitory activity.[5] This loss of inhibitory activity with 2-substituted 8-HQ's might be the result of a decrease in the availability for complexation of the electrons on the quinoline nitrogen. Alternately, since this appears to be a general effect regardless of the nature of the substituent in the 2 position, it may be the result of a strict steric effect in binding. Since the 2-substituted compounds were completely inactive toward COMT,[5] it was not possible to differentiate the possibilities. The enhanced inhibitory activity observed with incorporation of a halogen in the 7 position of 8-HQ might result because of the hydrophobic nature of this substituent and the possible existence of a hydrophobic cleft on the enzyme adjacent to the area which binds the 8-hydroxy group.[7]

By extensively studying the structure–activity relationships for a series of 8-HQ's, we are now in a better position to predict the structural features required to produce maximum enzymatic binding. In addition, we have also gained considerable insight into the characteristics of the enzymatic binding site for the catechol substrate.

Experimental Section

Materials. SAM-$^{14}CH_3$ (New England Nuclear, 55.0 mCi/mmol) was diluted to a concentration of 10 μCi/ml and stored at −20 °F. SAM iodide (Sigma) was prepared fresh as 0.01 M aqueous stock solutions. The following compounds were commercially available from the indicated sources: 1, **2**, **6**, 18, **19** (Aldrich); **5**, **20**, **22**, **23** (Alfred Bader); **21** (Baker); **7** (Sigma). Compounds 13–16[5] and **3**, 4, 8–12, and 17[8] were prepared as previously described.

Purification and Assay of COMT. COMT was purified from rat liver (male, Sprague–Dawley, 180–200 g) according to the methods previously described.[9,10] The enzyme was purified through the calcium phosphate gel step resulting in about a 50-fold purification from the crude supernatant. The enzyme activity was determined using SAM-$^{14}CH_3$ and DHB as substrates according to a previously described radiochemical procedure.[10]

Data Processing. Enzyme activities were calculated as nanomoles of product formed per milligram of protein per minute. Percent inhibitions for no less than five different concentrations of each inhibitor were determined. The percent inhibition was plotted vs. log of the inhibitor concentrations with a linear relationship resulting for each inhibitor. From these plots the pI_{50}'s were determined.

The regression analyses were performed with a Hansch-3 program using a Honeywell 635 computer; r is the correlation coefficient and s the standard deviation; the test F was calculated according to Mandel.[11] Correlation between COMT inhibitory activity and physicochemical properties of the substituted 8-HQ's

was sought as described by Hansch.[12]

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References and Notes

(1) Established Investigator of the American Heart Association.
(2) Abbreviations used are COMT, catechol *O*-methyltransferase; SAM, *S*-adenosyl-L-methionine; 8-HQ, 8-hydroxyquinoline; DHB, 3,4-dihydroxybenzoic acid.
(3) P. B. Molinoff and J. Axelrod, *Annu. Rev. Biochem.*, **40**, 465 (1971).
(4) L. Flohe, *Int. Pharmacopsychiatry*, **9**, 52 (1974).
(5) R. T. Borchardt, *J. Med. Chem.*, **16**, 382 (1973).
(6) Using linear regression analyses (see Experimental Section), attempts were made to derive equations which could correlate the inhibitory activities of the 8-HQ's and various substituent parameters. Attempts to correlate the changes in inhibitory activities (pI_{50}'s) observed for the 5-substituted 8-HQ's with electronic (σ_5), inductive (F_5), or resonance parameters (R_5) were unsucessful.
(7) We have observed a tenuous correlation (see Experimental Section) between the hydrophobic character (π_7) and the inhibitory activities of the various 5- and 7-substituted 8-HQ's [pI_{50} = 4.61 (±0.12) + 0.44 (±0.14) π_7; n = 22; r = 0.82; s = 0.27; F test ($F_{1,20}$ = 42.38; $F_{1,20;\alpha\ 0.005}$ = 9.94)]. The π_7 values for iodide and bromide groups were taken from values listed for 2-substituted phenols[13] and the π_7 value for the nitro group was calculated from the experimentally determined log P value. The terms π_7^2, E_{s7}, and σ_{o7} were also used in attempts to obtain reasonable correlations but without significant success. Because of the small number of analogs with substituents in the 7 position, any correlation observed between the inhibitory activities and substituent parameters must be interpreted with care.
(8) V. D. Warner, J. N. Sane, D. B. Mirth, S. S. Turesky, and B. Soloway, *J. Med. Chem.*, **19**, 167 (1976).
(9) R. T. Borchardt, *J. Med. Chem.*, **16**, 377 (1973).
(10) B. Nikodejevic, S. Senoh, J. W. Daly, and C. R. Creveling, *J. Pharmacol. Exp. Ther.*, **174**, 83 (1970).
(11) J. Mandel, "The Statistical Analysis of Experimental Data", Interscience, New York, N.Y., 1964.
(12) C. Hansch, *Acc. Chem. Res.*, **2**, 232 (1969).
(13) T. Fujita, J. Isawa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

Bridged Aminotetralins. 4. Resolution of Potent Analgesics of the Bridged Aminotetralin Type

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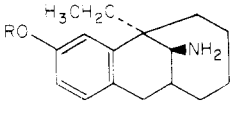
The resolution of three potent analgesics of the bridged aminotetralin type has been described, as well as the conversion of the enantiomers to the related phenols. Several of the compounds demonstrated analgesic activity up to 15 times that of morphine.

In previous reports[1,2] we described the synthesis and analgesic activity of a series of 1,3-bridged aminotetralins, including compounds with the potency level of morphine. Several of the more potent analgesics were selected for resolution into optical isomers. The compounds chosen were all β-epimers, in which the amine function is equatorially located relative to the tetralin ring and is trans to the alkyl function in the 5 position.[1] The resolution of these compounds (1–7) and the analgesic activities of the isomers are described herein.



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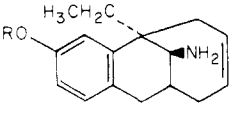
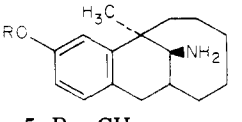
2, R = H



3, R = CH_3
4, R = H



5, R = CH_3
6, R = H
7, R = c-C_3H_5-C=O

Chemistry. The three methoxy derivatives **1**, **3**, and **5** were resolved by treatment with *d*- and *l*-tartaric acids and fractional crystallization of the tartrate salts from methanol to constant rotation. Both (+) and (−) rotamers were obtained. The resolved isomers **1** and **5** were demethylated to the corresponding phenols **2** and **6** in refluxing concentrated hydrobromic acid. The demethylation of **3** to **4** was carried out at low temperature with boron tribromide.

It was shown in a previous paper of this series[2] that acylation of the phenolic hydroxyl group gives derivatives of at least equivalent analgesic activity. Accordingly, the (−) rotamer of the phenol **6** was converted to its cyclopropylcarboxylate ester. This was done by the previously described route,[2] which involved blocking the primary amine with the benzyloxycarbonyl-protecting group, forming the ester of the phenolic OH, and then removing the blocking group by hydrogenation under acidic conditions.

Hydrogenation of the (+) rotamer of **3** over platinum oxide gave the (−) rotamer of **1**. Thus, despite the difference in signs of rotation, (−)-**1** and (+)-**3** are stereochemically related. In all likelihood, (−)-**5** is also of the same relative configuration as (−)-**1**.

Pharmacology. The resolved compounds were tested for analgesic activity in the D'Amour–Smith rat tail flick test.[3] In Table I are shown the analgesic ED_{50}'s of these compounds when administered by ip, im, and po routes. The table shows that phenols are more active than the corresponding methoxy derivatives, while in the case of the one example reported (**7**), esterification of the phenolic function resulted in diminished activity.

It can also be seen that the most active compounds are (−)-**2**, (+)-**4**, and (−)-**6**, with potencies up to 15 times that of morphine. It was shown above that these compounds, despite the sign of rotation of (+)-**4**, are of the same relative stereochemistry. In the case of compound **6**, virtually all of the analgesic activity is exhibited by the (−) rotamer.