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Chemical Structure-Activity Correlation in the O-Methylation of Substituted Catechols by Catechol O-Methyltransferase

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SUMMARY

The Hansch method of correlating biological activity with chemical structure, using regression analysis, was applied to the catechol *O*-methyltransferase-catalyzed *O*-methylation of neutral side chain-substituted catechols. A correlation of the ratio (*meta*:*para*) of *O*-methylated products with the steric effect of the substituent, as well as the transport factor, was found. The electronic character of the substituent did not effect the product distribution. The experimentally determined maximum velocities for the substrate reaction could be correlated with the steric and electronic parameters of the substrates. The Michaelis-Menten coefficient (K_m) was poorly correlated, apparently because of the inherent error in the extrapolation from which it was obtained.

Three major pathways have been proposed for the inactivation of catecholamines (1). The first is uptake by adrenergic nerves, followed partly by redistribution and storage. The second is deamination, performed by an intraneuronal enzyme, monamine oxidase (2). The third pathway is by an *O*-methylation reaction catalyzed by the extraneuronal enzyme catechol *O*-methyltransferase. This enzyme catalyzes the transfer of methyl groups from *S*-adenosylmethionine to the phenolic hydroxyl group of the catecholamines and a variety of other catechols. *m*-Methylation has been assumed to be the major pathway (3) *in vivo*. However, recent studies have demonstrated that *p*-methylation can be the predominant path for the reaction of certain catechols *in vitro* with catechol *O*-methyltransferase (4, 5). The significance of the *p*-methylation pathway *in vivo* is illustrated by the occurrence of homoisovanillic acid (3-hydroxy-4-methoxyphenylacetic acid) as a normal urinary constituent in man (6–8).

Our data are based on extensive studies by Creveling *et al.* (9–11) concerning the mechanism of transmethylation, using a catechol *O*-methyltransferase enzyme mixture *in vitro* with a wide variety of catecholamines and substituted neutral side chain catechols. In order to analyze the structure-activity relationship of substituted catechols in the presence of this enzyme mixture, the method of Leo, Hansch, and Elkins (12), using a double precision matrix inversion regression analysis, was applied to the neutral compounds listed in Table 1.

The structure-activity analysis promulgated by Hansch can correlate a biological activity expression (in our case the activity of the catechol *O*-methyltransferase system) with a partition coefficient, log *P* (the transport parameter, analytically determined in a 1-octanol–water system), the Hammett σ constant (electronic parameter), and the Taft E_s constant (steric parameter), among

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other possible parameters, via either a linear equation (shown as Eq. 1) or a related parabolic equation. The regression analysis solves the equation for the various k values.

$$\log \frac{1}{C} = k_1 + k_2\pi + k_3\sigma + k_4E_s \quad (1)$$

The compounds we chose to correlate were all neutral side chain-substituted catechols (11). We deliberately excluded amines and acids since we felt there would be inherently greater difficulty with these compounds using the Hansch method of analysis with the usual steric, electronic, and transport factors. For example, there is no a-priori way of knowing whether the amine or the amine salt, the carboxylic acid or the carboxylate anion, exists during interaction with the enzyme, and thus no way of knowing which should be correlated. Also, the necessary steric parameters (E_s) are unavailable for many of these substituents.

The partition coefficients, log P, have not been experimentally determined for the substituted catechols listed in Table 1. A calculated coefficient, π, estimating $\log_{10} P$, was assigned to the substituent R in *1* from the literature on the analogous *para*-substituted phenols (13), or it was calculated indirectly as noted below. In general, a slightly better correlation was obtained when the substituent R in *1* was considered to be oriented *para* rather than *meta* to the phenolic hydroxyl group. The unsubstituted catechol (1,2-dihydroxybenzene) was considered constant throughout the series and was given a π value of zero. Examples of the indirect calculation follow.

1. Substituent 1 ($COOC_2H_5$):

$$-COOC_2H_5 = p\text{-}OH-C_6H_5-COOCH_3 + -CH_3 - C_6H_5OH$$

$$(\pi) = +(1.96) + (0.5) - (1.46) = 1.00$$

2. Substituent 27 (CH_2CH_2OH):

$$-CH_2CH_2OH = m\text{-}OH-C_6H_5-C_2H_5 + -OH - C_6H_5OH$$

$$(\pi) = +(2.40) + (-1.81) - (1.46) = -0.87$$

– difference (−0.02) between *meta* and *para* —OH—C_6H_5—C_2H_5 = −0.89

3. Substituent 15 ($NHCOC_6H_5$):

$$-NHCOC_6H_5 = C_6H_5NHCOC_6H_5 - C_6H_6$$

$$(\pi) = (2.62) - (2.15) = 0.47$$

– difference (0.22) between the phenol and benzene series with conjugated carbonyl substituents; e.g., (p-OH—C_6H_5—$COCH_3$) − (C_6H_5—$COCH_3$) = 0.22 (difference in log P value) = 0.25 (π of $NHCOC_6H_5$)

Substituents 8, 11, 13, 14, 18, 20, 21, and 22 were similarly calculated. The π values determined for the various substituents are listed in Table 1.

Values of σ were obtained from the literature (14, 15), except for substituents 7, 21, and 22, for which the σ of a CH_2CH_3 group was used, and substituent 15, which was presumed to be comparable to an $NHCOCH_3$ group in σ value. Both a *meta*-σ and a *para*-σ term (*meta* or *para* to the phenolic hydroxyl group) were tried in the regression analyses, as were combinations (additive, subtractive, and multiplicative) of σ-*meta* and σ-*para*, since there was no a-priori way of determining which was the more important. The *para*-σ and *meta*-σ parameters, when used individually in the regression analysis, gave about the same correlation in the case of V_{max} (the maximum velocity of the reaction). They were found to be superior to their combination terms. Since, however, more *para*-σ values were directly obtainable from the literature, we chose these for the regression analysis; the values are listed in Table 1.

The limited availability of E_s constants[2] (16) forced the introduction of certain approximations. A carbonyl moiety, when present in the substituent R in *1*, was con-

[2] C. Hansch, personal communication.

TABLE 1

Substituent parameters used in structure-activity correlations; observed vs. predicted values from correlations

R = (in Fig. 1)	π	E_s	σ_p	Log 1/*M* (Eq. 2)		Log 1/*V* (Eq. 4)		*m*/*p* ratio (Eq. 2)	
				Observed	Predicted	Observed	Predicted	Observed	Predicted
1. —COO—CH_2—CH_3	1.00	−0.39	0.450	2.330	2.157	1.450[a]	2.390	0.85	1.27
2. —$C(CH_3)_3$	1.85	−1.54	−0.197	—[a, b, c]	3.105	1.600	1.894	—[a, b, c]	0.13
3. —CH_3	0.48	0.00	−0.170	2.160	2.089	1.940	1.961	0.85	1.01
4. —Br	1.13	0.08	0.232	2.520	2.230	2.090	2.299	0.57	1.11
5. —CHO	−0.38	0.00	0.220	1.820	1.934	2.190	2.314	2.10	1.61
6. —H	0.0	1.24	0.000	—[a]		1.680	1.648	—[a]	
7. —CH_2—CH_2OH	−0.89	−0.77	−0.151	1.850	1.934	1.990	2.067	2.20	1.79
8. —CH_2—CH_2—NH—CO—CH_3	−0.54	−0.40	−0.151	2.060	1.880	2.450	2.052	1.70	2.57
9. —NO_2	0.50	0.28	0.778	1.880	2.204	1.920	2.019	2.03	0.97
10. —Cl	0.93	0.27	0.227	2.390	2.276	2.150	2.244	0.67	0.77
11. —CO—CH_2—CH_3	0.39	−0.36	0.502	2.140	2.043	2.240	2.370	1.20	1.51
12. —F	0.31	0.78	0.062	2.560	2.560	2.110	1.951	0.35	0.35
13. —CH_2—CH_3	0.92	−0.07	−0.151	2.160	2.153	2.010	2.002	0.95	0.97
14. —CO—C_6H_5	1.54	−0.38	0.459	1.840	2.253	2.350	2.386	3.10	1.20
15. —NH—CO—C_6H_5	0.25	−0.36	0.000	1.950	2.020	2.280	2.228	2.60	2.19
16. —CO—CH_3	−0.11	−0.07	0.502	2.100	1.967	2.340	2.324	1.20	1.64
17. —CF_3	1.40	−1.16	0.540	2.690	2.617	2.760	2.341	0.36	0.43
18. —NH—CO—CH_3	−1.21	−0.38	0.000	1.580	1.754	—[a, c]		4.42	2.95
19. —CH_2OH	−1.26	−0.20	0.080	1.850	1.743	—[a, c]		2.00	2.53
20. —CO—CH_2—CH_2—CH_3	0.89	−0.39	0.502	2.080	2.137	—[a, c]		1.50	1.31
21. —CH_2—CO—NH_2	−1.92	−0.36	−0.170	2.140[a]	1.626	—[a, c]		1.20[a]	3.96
22. —CH_2—$COOCH_3$	−0.58	−0.39	−0.170	2.260[a]	1.868	—[a, c]		1.00[a]	2.4
23. —CN	0.14	0.73	0.660	2.470	2.479	—[a, c]	2.226[d]	0.46	0.45

[a] Not used for correlation.

[b] Selected for prediction: see the text.

[c] Data unavailble.

[d] Predictable, E_s and σ parameters available from the literature.

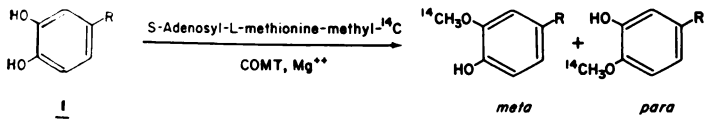

FIG. 1. *Methylation of substituted catechols*
COMT is catechol *O*-methyltransferase.

sidered sterically equivalent to a methylene; an amide or an ester was considered equivalent to an ethyl moiety, and the $(CH_2)_x$—OH group had the E_s of a $(CH_2)_x$—OCH_3 substituted for it. A dummy parameter was introduced as a correction term for these approximations. It did not help the correlation, and it was dropped from the final series of equations

Correlations were attempted with the enzymatic activity pertinent to the catechol *O*-methyltransferase enzyme mixture. They included (a) the Michaelis-Menten constant (K_m), (b) the maximum velocity (V_{max}) of the transmethylation reaction, (c) the product $K_m \cdot V_{max}$, possibly characteristic of substrate-enzyme interactions, and (d) the *meta*:*para* ratio (*m*/*p*) of the *O*-methylated products of the reaction shown in Fig. 1. The data were obtained from Creveling *et al.* (11).

The m/p and V_{max} values were given as ratios; K_m and our $K_m \cdot V_{max}$ product are expressed as millimoles per liter (11). In order to obtain better correlations for V_{max}, K_m, and $K_m \cdot V_{max}$, we had to divide these units by the molecular weight of the substrate. For consistency, we continued this for the m/p regression, although, without the molecular weight division, almost as good an equation could be obtained ($S = 0.21$, $R = 0.80$).

The kinetic data were obtained from a double-reciprocal ($1/v$ vs. $1/S$) plot (11). The least-squares straight line was extrapolated to infinite dilution to give V_{max}; the intercept with the abscissa provided K_m. The m/p ratio had usually been determined by separation of the two products on thin-layer plates, removal of 4-mm strips, and radiometric assay of extracts of these scrapings in a liquid scintillation counter. With compounds 3 and 13 (R = methyl and ethyl), separation and estimation of product ratios was obtained by gas chromatography. The concentration of enzyme, temperature, pH, buffer, and other reaction conditions were identical for the series of compounds in Table 1. With some of the m/p ratio determinations, the thin-layer separation was incomplete. From the original experimental data, the m/p ratio error was estimated as approximately 4.7% (maximum, in the case of compound 9, R = NO_2). The ratios of three other compounds (substitutents 4, 10, and 18) were recalculated from the original data, and these somewhat altered values were used in the regression and are listed in Table 1. For certain compounds (with substituents 21 and 22) data were available from only one experiment and appeared uncertain, and so these compounds were not included in the regression. An experimental error of approximately 20% could be estimated for the V_{max} and K_m data in the series of three or more determinations.

The following equations represent the best correlation which could be obtained from the regression analyses of the data in Table 1. In the equations, N = number of data points used in the regression analysis, S = standard deviation from regression, R = the correlation coefficient, and numbers in parentheses are 95% confidence limits.

$$\log \frac{1}{M} = 2.00(\pm 0.12) + 0.18\pi(\pm 0.12) + 0.26E_s(\pm 0.22) + 0.49E_s^2 \cdot(\pm 0.31), \quad N = 19, \cdot S = 0.19, R = 0.82 \tag{2}$$

where $M = m/p$ ratio divided by molecular weight of substrate.

$$\log \frac{1}{M} = 1.87(\pm 0.38) + 0.38\pi(\pm 0.42) + 0.32E_s(\pm 0.44) + 0.49E_s^2 \cdot(\pm 0.48), \quad N = 8, \cdot S = 0.19, R = 0.89 \tag{3}$$

where $M = m/p$ ratio divided by molecular weight substrate.

$$\log \frac{1}{V} = 2.17(\pm 0.18) - 0.22E_s(\pm 0.21) + 0.97\sigma(\pm 0.88) - 0.16E_s^2 \cdot(\pm 0.2) - 1.37\sigma^2(\pm 1.64), \cdot N = 16, S = 0.22, R = 0.74 \tag{4}$$

where $V = V_{max}$ divided by molecular weight of substrate.

$$\log \frac{1}{K} = 2.75(\pm 0.30) - 0.25E_s(\pm 0.4) + 0.83\sigma(\pm 0.86), \quad N = 16, \cdot S = 0.48, R = 0.74 \tag{5}$$

where $K = K_m$ divided by molecular weight of substrate.

$$\log \frac{1}{KV} = 2.62(\pm 0.39) - 0.30E_s \cdot(\pm 0.51) + 1.19\sigma(\pm 1.1), \cdot N = 16, S = 0.61, R = 0.58 \tag{6}$$

where $KV = K_m \cdot V_{max}$ divided by molecular weight of substrate.

Equation 2 was the best of the statistically valid equations. A correlation was observed using the m/p ratio, linear in π and parabolic in E_s. An ideal E_s value was found to be -0.26 (-0.66 to -0.05). The ideal π value

could not be ascertained from these data. However, from a related equation which was parabolic in π, an ideal π value was given as 1.50 ($\pm\infty$). The steric effect of the substituent appears to be a dominant feature in determining the m/p ratio of the products of the reaction. The transport of the substrate to the enzyme surface is also somewhat determined by the substituent. Apparently the electronic character of the substituent in these catechols does not affect the product distribution.

Equation 2 is surprisingly good when one considers the many rather gross approximations used for E_s and π and the uncertainty inherent in the biological data. It predicts the predominant isomer, formed in the reaction, correctly in all but four instances. Two of these are quite close, in fact, to the experimentally determined m/p ratio (substituents 1 and 3). Only compounds 4 and 9 are badly in error (the Br and NO_2 groups). An explanation for the discrepancy between the observed and predicted ratios of the nitro compound could lie in the fact that the nitro compound, at the pH at which the experiment was conducted, does not exist as the catechol. It presumably is the extreme case in these neutral catechols, in that it exists, almost entirely, as the *p*-monocatecholate anion.[3] Our calculations were based on a catechol moiety; thus an error might well arise here.

On the basis of Eq. 2, and using the ideal E_s value of -0.26 and a mean molecular weight of 161 (both constant), we calculated an ideal $\pi = 1.34$ (which would give an ideal m/p ratio of 1). It can be calculated that idealized π values greater than 1.34 lead to a predominance of *p*-*O*-methylated product; π values less than 1.34 lead to *meta* dominance. If we maintain a constant $\pi = 1.34$ and a molecular weight of 161, then calculated E_s values smaller or larger than -0.26 lead to increasing *para* isomer predominance in the product.

If only those compounds for which E_s values are known are used for the regression (compounds 3, 4, 9, 10, 12, 13, 17, and 23), Eq. 3, strikingly similar to Eq. 2, can be obtained. Equation 3 tends to indicate that our estimated E_s values (for the remaining compounds) did not change the character of the correlation; the σ parameter was still not involved. This equation has too few data points for the number of parameters involved and should not be used for predictions

Neither V_{max}, K_m, nor $K_m \cdot V_{max}$ could be correlated with statistical validity unless, in the case of V_{max}, the compound with substituent 1 was dropped from the calculation. Although we can think of no valid reason for not considering the data of compound 1, without it a fair correlation could be obtained, and this is shown in Eq. 4. The maximum velocity of the reaction is evidently determined by the electronic and steric parameters of the substituent. The effect of the substituent on transport (π) does not enter this equation. The ideal E_s value, from Eq. 4, is -0.72, and the ideal σ value is 0.35. Both have confidence limits of $\pm\infty$.

The increasing standard error observed in Eq. 4–6 is understandable. Equation 4 uses V_{max}, which is obtained through extrapolation of an experimental curve. Continued extrapolation gave K_m (and an increase in the error via the further extrapolation) used in Eq. 5. Equation 6, using a $K_m \cdot V_{max}$ product, will obviously magnify the inherent error in both extrapolations. Thus the extrapolated numbers become progressively inaccurate in going from V_{max} to K_m to $K_m \cdot V_{max}$, and this is reflected in the increasing standard deviation in this set of equations. The inaccuracy is such, and the standard deviation so large, that Eq. 5 and 6 are unreliable, even when compound 1 is not included in the correlation.

The importance of the conformation of the substituent (relatable to the steric parameter E_s) was recognized and given emphasis by Creveling *et al.* (11) for compounds related to dopamine and norepinephrine. No relationship between the inductive effect of the substituent (relatable to the σ parameter) and the m/p ratio at constant pH could be found in the regression analyses. Equation 2 tends to confirm the importance of a steric factor in the transmethylation reaction, and it additionally suggests that the substituent influences the transport of the catechol to

[3] J. Daly, personal communication.

the enzyme surface. Furthermore, Eq. 2 enables us to predict the m/p ratio of substrates not yet experimentally determined. For example, the catechol with the substituent R = *tert*-butyl in *1* (substituent 2) can be predicted to exhibit log $1/M$ = 3.11, equivalent to an m/p ratio of 0.13. The m/p ratio of this compound is difficult to determine experimentally (11); an experimentally satisfactory system for separating the *O*-methylated isomers has not yet been found. Future experimental work may confirm this point.

The determination of the minimum energy conformers of these substrates, and others in this series, by molecular orbital calculations might provide further evidence for the importance of the steric effect of the substituent on *meta*:*para* product ratios. This work will be reported in a future publication.

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