Structure–Activity Relationship in Benzamides Inhibiting Alcohol Dehydrogenase

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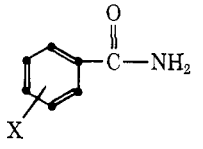
Abstract: Regression analysis using substituent constants has been applied to the structure-activity relationship in the complex formation between benzamides and alcohol dehydrogenase and diphosphopyridine nucleotide (DPNH). Electron-releasing functions and large apolar groups in the para position promote complex formation. Substituents in the meta position have no hydrophobic effect. This constitutes another example of the importance of the directional nature of hydrophobic bonding in enzyme–substrate interactions. The quantitative structure–activity relationship found for the aromatic benzamides agrees well with earlier findings for aliphatic amides.

We have been interested in the quantitative structure–activity relationship (QSAR) of various types of amides interacting with alcohol dehydrogenase.[1,2] Using substituent constants and regression analysis, it has been found[3,4] that it is possible to factor the substituent effect on rate or equilibrium processes into three primary factors: electronic, hydrophobic, and steric. These characteristics of substituents can be modeled using substituent constants so that their roles can be more clearly understood.

In a first treatment of substituent effects on $K_{ER,I}$, dissociation constants for the complex of alcohol dehydrogenase with benzamides (I) and DPNH, it was



I

$$K_{ER,I} = \frac{[ER][I]}{[ERI]}$$

E = enzyme concentration
I = inhibitor concentration
R = DPNH concentration

found[1] that complex formation of the amides depended on the electronic and hydrophobic characteristics of X. This analysis was made using estimated values of the hydrophobic parameter π for X. We have now experimentally determined values for X by measuring octanol/water partition coefficients for a series of benzamides. These values have been measured as usual[5] and are listed in Table I. The hydrophobic constant π_X represents the logarithm of the partition coefficient of a substituent and is defined as: $\pi_X = \log P_X - \log P_H$, where P_H refers to the partition coefficient of the parent compound (benzamide in the present discussion).

Using measured π constants, it is now possible to make a more refined structure–activity analysis of the $K_{ER,I}$ constants. Regression equations were generated from the data in Table II from all possible linear combinations of the following variables: $\Sigma\pi$, $\Sigma\pi^2$, π-3, π-4, σ, σ^+, E_s-3, E_s-4, P_E-3, P_E-4. This yielded 1023 equations ($2^n - 1$, where n is the number of variables). Of the above symbols, the notation π-3, E_s-4, etc.

Table I. Octanol/Water Partition Coefficients (P) for X-$C_6H_4CONH_2$

X	Log P	π_X
H	0.64	0.00
4-F	0.91	0.27
4-Cl	1.55	0.91
4-Br	1.76	1.12
4-CH_3	1.18	0.54
4-CF_3	1.71	1.07
4-$CH(CH_3)_2$	2.14	1.50
4-OH	0.33	−0.31
4-OCH_2CH_3	1.30	0.66
4-NO_2	0.82	0.18
4-$N(CH_3)_2$	1.14	0.50
4-NH_2	0.02	−0.62
3-F	0.91	0.27
3-Cl	1.51	0.87
3-Br	1.65	1.01
3-CH_3	1.18	0.54
3-NH_2	−0.33	−0.97
3-OH	0.39	−0.25
3-NO_2	0.77	0.13
3-$N(CH_3)_2$	0.95	0.31
4-OCH_3	0.86	0.22

Table II. Structure–Activity Parameters for Complex Formation between X-$C_6H_4CONH_2$, Alcohol Dehydrogenase, and DPNH

X	—Log $K_{ER,I}$— Obsd[a]	Calcd[b]	$\lvert\Delta$ log $K_{ER,I}\rvert$	σ	E_s-4	π-4
H	−2.72	−2.656	0.06	0.0	1.24	0.0
3-NO_2	−3.30	−3.227	0.07	0.71	1.24	0.0
3-Cl	−2.90	−2.954	0.05	0.37	1.24	0.0
3-Br	−2.99	−2.970	0.02	0.39	1.24	0.0
3-F	−2.89	−2.930	0.04	0.34	1.24	0.0
3-OH	−2.90	−2.753	0.15	0.12	1.24	0.0
3-$N(Me)_2$	−2.30	−2.487	0.19	−0.21	1.24	0.0
3-Me	−1.50[c]	−2.600	1.10	−0.07	1.24	0.0
4-NO_2	−2.62	−2.618	0.00	0.78	−1.28	0.18
4-Cl	−1.93	−2.204	0.27	0.23	0.27	0.91
4-F	−2.62	−2.475	0.14	0.06	0.78	0.27
4-OH	−2.48	−2.372	0.11	−0.37	0.69	−0.31
4-Me	−1.78	−1.987	0.21	−0.17	0.0	0.54
4-$CH(ME)_2$	−1.70	−1.459	0.24	−0.15	−0.47	1.50
4-OMe	−2.20	−2.239	0.04	−0.27	0.69	0.22

[a] From ref 1. [b] Calculated using eq 7. [c] This data point not used in the derivation of eq 1–8.

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(2) C. Hansch, J. Schaeffer, and R. Kerley, *J. Biol. Chem.*, **247**, 4703 (1972).
(3) C. Hansch, *Accounts Chem. Res.*, 2, 232 (1969).
(4) C. Hansch in "Drug Design," Vol. 1, E. J. Ariëns, Ed., Academic Press, New York, N. Y., 1971, p 271.
(5) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).

indicates the ring position of the substituent. P_E is the sum of the atomic refractivities.[6] This parameter has been used as a rough measure of the total bulk of the substituent in contrast to the steric parameter E_s which has a partial directional nature. The parameters σ and σ^+ are well defined[7] and there is no reason for factoring these according to the position of the substituent.

$$\log \frac{1}{K_{ER,I}} = 0.39(\pm 0.54)\sum\pi - 2.68(\pm 0.34)$$

n	r	s	
14	0.417	0.457	(1)

$$\log \frac{1}{K_{ER,I}} = -0.35(\pm 0.32)E_s\text{-}4 - 2.29(\pm 0.32)$$

14	0.573	0.412	(2)

$$\log \frac{1}{K_{ER,I}} = -0.84(\pm 0.67)\sigma - 2.41(\pm 0.25)$$

14	0.619	0.395	(3)

$$\log \frac{1}{K_{ER,I}} = 0.76(\pm 0.44)\pi\text{-}4 - 2.70(\pm 0.22)$$

14	0.739	0.339	(4)

$$\log \frac{1}{K_{ER,I}} = 0.089(\pm 0.05)P_E\text{-}4 - 2.86(\pm 0.27)$$

14	0.749	0.333	(5)

$$\log \frac{1}{K_{ER,I}} = 0.69(\pm 0.30)\pi\text{-}4 - 0.71(\pm 0.39)\sigma - 2.59(\pm 0.16)$$

14	0.904	0.224	(6)

$$\log \frac{1}{K_{ER,I}} = 0.453(\pm 0.28)\pi\text{-}4 - 0.804(\pm 0.30)\sigma - 0.232(\pm 0.17)E_s\text{-}4 - 2.369(\pm 0.20)$$

14	0.953	0.168	(7)

$$\log \frac{1}{K_{ER,I}} = -0.42\sum\pi^2 + 0.96\sum\pi - 0.43\pi\text{-}3 - 0.93\sigma - 0.25E_s\text{-}4 - 2.37$$

14	0.981	0.118	(8)

$$\pi_0 = 1.13$$

Of the pertinent single-variable equations (eq 1–5), π-4 and P_E-4 give the highest and essentially the same correlation. This indicates the great importance of the apolar interaction of the para substituents. There is a very high correlation between these two variables for this particular set of substituents ($r^2 = 0.698$). The best two-variable equation is eq 6, indicating that after hydrophobic interaction of the para substituents, the next most important substituent effect is electron release by ring functions toward the amide group. The use of P_E-4 instead of π-4 in eq 6 does not yield as good a correlation ($r = 0.885$). The best three-variable equation is eq 7. It is unrealistic with so few data points to attempt to obtain a more detailed resolution of the structure–activity relationship by the use of more variables.

Of the 1023 equations considered, the one with the lowest standard deviation is eq 8. In eq 8, with five variable terms, there are less than three data points per variable term. Topliss and Costello[8] have pointed out the danger of finding meaningless chance correlations with three or four data points per variable. The π-3 term in eq 8 appears to be a kind of correction on $\Sigma\pi$ since subtraction of these two coefficients yields roughly the coefficient of π-4 in eq 7. While eq 8 cannot be accepted, it is interesting in that it predicts an optimum $\Sigma\pi$ value of 1.13. In our previous studies with aliphatic amides, log P_O for eq 2 (log $1/K_{ER,I}$) of ref 2 is 0.72. For the benzamides, log P_O can be calculated as follows

$$\pi_O = \log P_O - \log P_H = 1.13 = \log P_O - 0.64$$
$$\log P_O = 1.77$$

Unfortunately, good confidence limits cannot be placed on log P_O in either example. Dropping the π-3 term from eq 8 gives an equation of standard deviation 0.168 with π_O of 1.01, in good agreement with eq 8.

It thus appears that for the interaction of amides with alcohol dehydrogenase, optimum binding occurs with amides having log P values below the range 1.0–1.7. This suggests that more apolar molecules may cause some kind of a conformational change so that enzyme, DPNH, and inhibitor do not form as stable a complex.

The log P_O value for log $K_{E,I}$ for aliphatic amides would appear to be higher (1.71 from eq 4, ref 2) although again, good confidence limits cannot be placed on this figure.

The "best" of eq 1–8 is eq 7. The correlation coefficient is good and there are almost five data points per variable. The stepwise application of the F statistic indicates that each of the terms is valid. For eq 4, $F_{1,12} = 14.5$; $F_{1,12\,\alpha.005} = 11.8$. Comparing eq 6 with eq 4, $F_{1,11} = 16.3$; $F_{1,11\,\alpha.005} = 12.2$ and, comparing eq 7 with eq 6, $F_{1,10} = 9.7$; $F_{1,10\,\alpha.025} = 6.9$. For eq 7, $F_{3,10} = 32.7$; $F_{3,10\,\alpha.005} = 8.1$.

Not only is eq 7 highly significant as judged by the F statistic, it makes sense with respect to the linear combination of eq 2–4. The coefficients with the three variable terms in eq 7 are close in value to those associated with these variables in the single-variable equations. This testifies to the independent additive character of these three variables.

Possibly the most important test of a mathematical structure–activity relationship is, how does it square with other biochemical or physical–chemical information about this system?[9] The negative coefficient found with σ in eq 3, 6, and 7 agrees with the negative coefficients found for σ^* in aliphatic amides.[2]

In each series, an increase in electron density in the amide function results in an increase in complex formation. This has been interpreted to mean that one important binding point of amides is the oxygen atom of the carbonyl function.[2] Equation 9 correlates[2] binding of aliphatic amides with alcohol dehydrogenase. Although the intercept of eq 9 cannot be compared

(6) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 5th ed, Wiley, New York, N. Y., 1964, p 54.
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(8) J. C. Topliss and R. J. Costello, *J. Med. Chem.*, **15**, 1068 (1972).
(9) S. H. Unger and C. Hansch, *J. Med. Chem.*, **16**, 745 (1973).

$$\log \frac{1}{K_{ER,I}} = 0.81(\pm 0.34) \log P - 0.77(\pm 0.20)\sigma^* + 3.53(\pm 0.33) \quad n = 15 \quad r = 0.929 \quad s = 0.302 \qquad (9)$$

with eq 6 or 7, the slopes for the hydrophobic and electronic terms are in reasonable agreement.

A most interesting aspect of eq 7 is the negative coefficient of the E_s-4 term. Bearing in mind that the more negative the E_s value of a substituent, the larger it is, this coefficient means that the bulkier groups in the para position *increase* binding. While this could be interpreted as a kind of correction term for π-4 (note that the value of the coefficient with π-4 drops in going from eq 6 to eq 7), it could also mean that the substituents help in producing an induced fit of the type postulated by Koshland.[10]

It is most interesting that eq 1, using $\Sigma\pi$, does not give as good a correlation as eq 4, using π-4, even though the former parameter contains more information. Also, in eq 8 the π-3 term has a negative coefficient. In the 1023 equations there is no evidence that 3 substituents aid complex formation *via* a hydrophobic interaction. In most of the equations where a π-3 term occurs, its coefficient has a negative sign. In a number of the equations the coefficient is positive, but esentially zero (*i.e.*, <0.1). In most of the few examples where the coefficient is positive and greater than 0.1, the 95% confidence intervals are quite large and, in fact, overlap zero. At best, 3 substituents have no hydrophobic or steric effect; at worst, there may be an inhibitory effect by such substituents on complex formation.

The lack of importance of π-3 in the above analysis indicates the directional nature of the hydrophobic effect in enzyme–substrate interaction. A similar effect has been uncovered in the interaction of phenyl glucosides with emulsin[11] and in the interacton of phenethanolamines with *N*-methyl transferase.[12] No doubt many other such examples will be discovered.

In deriving eq 1–8, one data point (3-CH_3) was not used. This derivative was invariably poorly fit. The reason for this is not clear. One might speculate that since there is no hydrophobic interaction by 3 substituents, the region in which these functions find themselves is polar. It may be that polar 3 functions such as Cl, NO_2, OH, etc. fit into this region better because of their dipole moments and that CH_3, lacking a strong dipole moment, fits poorly.

In summary it can be said that the present analysis of $K_{ER,I}$ values for aromatic amides agrees well with the previous analysis of aliphatic amides.[2] The important difference is that the more rigid aromatic molecules show a directional hydrophobic bonding effect which it is not easy to see with the more flexible aliphatic compounds. The two studies show that it should be possible to obtain more precise information about the inhibitor complex *via* a study of well-designed derivatives using regression analysis.

Acknowledgment. This work was supported by Grants CA-11110 and CA-12462 from the National Institutes of Health and GB-28015 and GP-28061 from the National Science Foundation.

(10) D. E. Koshland, Jr., in "The Enzymes," Vol. 1, P. D. Boyer, H. A. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1960, p 305.

(11) C. Hansch, E. W. Deutsch, and R. N. Smith, *J. Amer. Chem. Soc.*, 87, 2738 (1965).

(12) C. Hansch and W. R. Glave, *J. Med. Chem.*, **15**, 112 (1972).

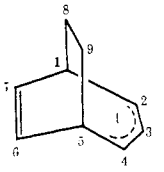
Communications to the Editor

The Bicyclo[3.2.2]nona-2,6-dienyl Carbanion. Preparation, Basicity, and Laticyclic Stabilization[1]

Sir:

The elegant theoretical analysis of bicycloaromatic stabilization in π-bridged ions by Goldstein and Hoffmann[2] prompted us to seek experimental verification of these concepts. In our previous work,[3] it was concluded that longicyclic stabilization was negligible. We now wish to report a test of laticyclic stabilization using the bicyclo[3.2.2]nona-2,6-dienyl carbanion I. The preparation, nmr spectrum, and relative basicity of this ion are reported.

The carbanion I was generated from the methoxy ether precursor IIIc by previously reported procedures.[3] The ether IIIc was prepared by standard procedures[3,4] from the ketone IIIb.[5] Quenching of the



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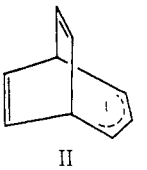

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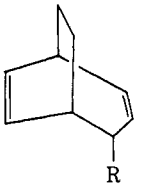
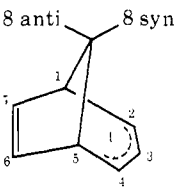


III a, R = H
b, R = O
c, R = OCH_3



IV

(1) Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

(2) M. J. Goldstein and R. Hoffmann, *J. Amer. Chem. Soc.*, **93**, 6193 (1971).

(3) J. B. Grutzner and S. Winstein, *J. Amer. Chem. Soc.*, **94**, 2200 (1972).

(4) All new compounds gave satisfactory elemental analyses and structures were verified by spectral techniques.

(5) T. Uyehara and Y. Kitahara, *Chem. Ind. (London)*, 354 (1971).