The Correlation of Localization Rates of Benzeneboronic Acids in Brain and Tumor Tissue with Substituent Constants

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SUMMARY

Substituent constants and regression analysis have been used to correlate the localization of substituted benzeneboronic acids in brain and tumor tissue of mice. It is shown that localization in the brain can be rationalized in terms of one parameter, π, obtained from octanol:water partition coefficients. Localization in the tumor depends on an additional electronic parameter, σ. Localization in the boronic acid series is compared with biologic activity in a series of barbituric acids.

INTRODUCTION

We have recently shown (1, 2) that the variation in equivalent biologic response of the members of a set of congeneric drugs in which steric effects can be neglected may often be rationalized in terms of substituent constants using Eq. 1.

$$\log \frac{1}{C_X} = -k\pi^2 + k'\pi + \rho\sigma + k'' \quad (1)$$

Here C_X is the molar concentration of derivative X necessary to cause an equivalent biologic response such as LD_{50} or ED_{50}. The free energy-related substituent constant, π, is obtained by measuring the partition coefficient of a series of derivatives between octanol and water (3, 4) and is defined as:

$$\pi = \log P_X - \log P_H \quad (2)$$

where P_X is the partition coefficient of derivative X, and P_H that of the parent molecule. By this definition, the more lipophilic the substituent is, the greater its π value. Thus π can be taken as a measure of the hydrophobic bonding power of a substituent such as CH_3 or NO_2. σ is the well known Hammett function (5), a measure of the way a substituent modifies the electron density on the benzene ring; and ρ is the reaction constant.

Equation 1, when used with regression analysis as described in detail previously (1), becomes a powerful tool for uncovering new leads in the design and modification of drugs. The purpose of this report is to analyze an illustrative example from the field of cancer research and to provide further support for the use of substituent constants in drug design. Soloway *et al.* (6) injected boronic acids into mice bearing subcutaneously transplanted gliomas and measured the concentration of boron in brain and tumor tissue. The object of the work was to find substituents which, when attached to benzeneboronic acid,

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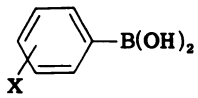
TABLE 1

Observed and calculated rates of penetration and localization ratios for benzeneboronic acids in brain and tumor tissue



Function X	$\Sigma\sigma$[a]	$\Sigma\pi$[b]	Tumor		Brain		Tumor:brain	
			Obs.[c] log C_t	Calcd.[d] log C_t	Obs.[c] log C_b	Calcd.[e] log C_b	Obs. log $\frac{C_t}{C_b}$	Calcd.[f] log $\frac{C_t}{C_b}$
1. 3-CF_3	0.42	1.10	0.85	1.014	1.62	1.692	−0.78	−0.680
2. 4-Br	0.23	1.01	1.20	1.115	1.85	1.726	−0.65	−0.612
3. 4-OEt	−0.25	0.61	1.42	1.394	1.66	1.770	−0.25	−0.377
4. 4-Cl	0.23	0.80	1.36	1.165	1.72	1.770	−0.35	−0.607
5. 3-CH_3	−0.07	0.52	1.18	1.334	1.67	1.756	−0.50	−0.423
6. 4-CH_3	−0.17	0.48	1.26	1.380	1.72	1.733	−0.47	−0.368
7. 4-OCH_3	−0.27	0.11	1.46	1.449	1.64	1.582	−0.18	−0.134
8. 4-F	0.06	0.22	1.20	1.310	1.79	1.646	−0.59	−0.337
9. H	0.00	0.00	1.53	1.341	1.71	1.504	−0.18	−0.164
10. 3-NO_2	0.71	−0.02	1.08	1.053	1.62	1.489	−0.54	−0.436
11. 3-OH	0.00	−0.35	1.37	1.325	1.22	1.170	0.16	0.154
12. 4-OH	−0.36	−0.27	1.46	1.477	1.24	1.258	0.22	0.218
13. 3-$NHCONH_2$	0.00[g]	−1.01[h]	1.28	1.208	0.40	0.180	0.88	1.027
14. 4-$B(OH)_2$	0.45	−0.55[i]	1.04	1.119	0.35	0.920	0.69	0.199
15. 2-CH_3	−0.17	0.68[h]	1.36	1.350	1.72	1.776	−0.50	−0.426
16. 2-NO_2	0.78	−0.35[h]	1.40	1.009	1.61	1.171	−0.22	−0.162
17. 3-COOH	0.36	−1.05[j]	1.15	1.052	0.40	0.106	0.75	0.945
18. 4-COOH	0.27	−1.05[j]	1.16	1.088	0.51	0.106	0.65	0.982
19. 3-NH_2	−0.16	−1.15[k]	1.36	1.234	1.31	−0.089	0.06	1.323
20. 2-NO_2, 4-$B(OH)_2$	1.23	−0.78[i]	1.00	0.764	0.30	0.580	0.70	0.184
21. 3-NO_2, 4-COOH	0.98	−1.07[j]	1.28	0.795	0.88	0.062	0.41	0.727
22. 2-NO_2, 4-COOH	1.32	−0.70[j]	1.32	0.743	0.44	0.705	0.88	0.038
23. 3-NH_2, 4-CH_3	−0.33	−0.72[k]	1.48	1.407	1.52	0.680	−0.04	0.733
24. 4-CH_2-$CHCOO^-$ | NH_3^+	—[l]	−3.50[m]	0.93	−0.657	0	−9.142	0.93	7.131
25. 2,4,6-$TriCH_3$	−0.51	1.79	1.36	1.131	1.60	1.144	−0.24	−0.013

[a] Values of σ come from the compilation of Jaffé (5).

[b] Except as noted, the values of π were obtained with benzoic acids (3).

[c] Relative rates of localization are in terms of μg B/g tissue in 15 min. In a few instances data were not available for the 15-min experiment and data for the 30-min experiment were corrected, assuming linear uptake with time.

[d] Calculated from Eq. 9.

[e] Calculated from Eq. 5.

[f] Calculated from Eq. 11.

[g] σ is not reported for this function; we assume it is close to zero, as for the acetamido group.

[h] This value of π was obtained with the phenoxyacetic acid derivative. Experience has shown these to be close to benzoic acid values (3).

[i] The value of π for $B(OH)_2$ was obtained from benzeneboronic acid. C was divided by 2 for this molecule having two atoms of boron. We are indebted to M. F. Hawthorne for a sample of this material.

[j] The π' value for COOH was used; see text for explanation.

[k] This value was obtained from aniline.

[l] No value for σ is available; although we have used zero for calculation purposes, the value of σ is probably not insignificant.

[m] π was determined by subtracting log P for ethylbenzene from log P for $\phi CH_2CH_2CH_2CH(NH_2)COOH$.

would lead to high concentrations of boron in the tumor. Radiation of the boron with neutrons would then cause the reaction sequence:

$$B^{10} + \text{neutron} \rightarrow B^{11} \rightarrow Li^{7} + He^{4} + 2.5 \text{ mev} \quad (3)$$

Tissues containing sufficient boron would be destroyed by the high energy alpha radiation and, if a high tumor boron:brain boron ratio could be established, this would form a basis for the treatment of brain tumors. Their data (Table 1) provide us with a direct test of the validity of Eq. 1, and of the relative importance of its several terms. Different terms were assumed to be negligible, and in each case the coefficients in the corresponding simplified equation were evaluated by the method of least squares, to see which form of the equation described the data best. Computations were performed on a Clary DE-60 computer. The results are summarized in Table 2. For penetration of the brain tissue, Eq. 5 provides the best rationalization, and adding a σ term (Eq. 6) does not improve the correlation. Equation 5 with the squared term "explains" 20% more variance in the concentrations than does the linear relationship expressed in Eq. 4, and these two variances differ significantly ($P < 0.01$). This is quite clear support for our hypothesis (1) that the movement of organic compounds into tissue is parabolically rather than linearly dependent on π or log P.

Equation 9 affords the best rationalization of the relative rates of localization in the tumor tissue. Here the correlation is not as good as that obtained for penetration of the brain. This may be due in part to the variations in tumor, from transplant to transplant, resulting in greater inhomogeneity in the tissue (A. H. Soloway, personal communication). The most interesting fact brought to light here is the dependence of the concentration in this tissue on σ. The variance accounted for by Eq. 9 is significantly greater ($P < 0.01$) than that accounted for by Eq. 7, which lacks the term in σ. The negative sign associated with the σ term means that electron-releasing groups promote the localization of boronic acids in tumor tissue.

The fact that localization in tumor is (in contrast to brain itself) dependent on σ, reveals an important difference at

TABLE 2

Regression analysis of localization of benzeneboronic acids in brain and tumor tissue

Data for the first 14 compounds of Table 1[a] were fitted by regression analysis to several forms of Eq. 1 in which various terms were omitted.

Equation[b]	r^2	r[c]	s[c]	Equation number
Brain				
$\log C_b = 0.642\pi + 1.324$	0.640	0.800	0.304	(4)
$\log C_b = -0.540\pi^2 + 0.765\pi + 1.505$	0.837	0.915	0.214	(5)
$\log C_b = -0.514\pi^2 + 0.766\pi - 0.127\sigma + 1.504$	0.843	0.918	0.220	(6)
Tumor				
$\log C_t = -0.213\pi^2 + 1.345$	0.283	0.532	0.173	(7)
$\log C_t = -0.199\pi^2 - 0.030\pi + 1.345$	0.292	0.540	0.179	(8)
$\log C_t = -0.130\pi^2 - 0.405\sigma + 1.341$	0.653	0.808	0.125	(9)
$\log C_t = -0.116\pi^2 - 0.029\pi - 0.405\sigma + 1.342$	0.661	0.813	0.130	(10)
Tumor: brain				
$\log \frac{C_t}{C_b} = 0.410\pi^2 - 0.765\pi - 0.405\sigma - 0.164$	0.862	0.928	0.212	(11)

[a] Since π and σ values are known only for these.

[b] C is concentration of boron, μg/g tissue at 15 min.

[c] r is the multiple correlation coefficient; s is the standard deviation.

the molecular level between these two tissues. Two possible mechanisms come to mind. It is known from the work of Snyder and Wyman (7) that electron-releasing groups greatly increase the ease of hydrolysis of the carbon-boron bond in the benzeneboronic acids. Thus, one could imagine that sufficiently lipophilic boronic acids might penetrate into a compartment in a tumor cell where cleavage of the molecule would produce boric acid that would then be too hydrophilic to escape easily through the lipophilic barrier it had crossed in reaching the site. Alternatively, tumor tissue might contain a special kind of electron-deficient component with which electrons in the boron p orbital could overlap.[2]

Equation 11 shows how a "therapeutic index," an important guide for further work, can be constructed in terms of substituent constants. It is apparent from the positive sign associated with the squared term in Eq. 11 that either large negative or positive values of π result in compounds with high localization ratios in favor of tumor tissue. The significance of this relationship is depicted in Fig. 1. However, we require more than a simple high ratio for therapeutic purposes. The most lipophilic compounds, with large positive values of π, were toxic. Moreover, since Eqs. 5 and 9 both have negative π^2 terms, the concentration of boronic acid localized within the test interval falls off with large values of π. Hence, substituents must be chosen with values of π such that the minimum concentration of boron necessary for destruction of the tumor upon radiation will reach the tumor. Thus, while large negative values for π would obviously be suitable for investigation, a limit is soon reached, set by Eq. 5, where the concentration localized in the tumor will not be high enough for therapeutic purposes. Ideal functions or combinations of functions to investigate would be those with $\Sigma\pi$ values[3] in the range —1.0 to —2.0. For example, 4-OH, 3-$NHCONH_2$ would offer the advantage of electron release by OH and a high negative $\Sigma\pi$. The importance of the negative σ term in Eq. 11 suggests that great advantage could be obtained with a stable borate anion, provided its log P were in the correct range. A complete discussion of the use of boron compounds in cancer therapy has recently been published by Soloway (8).

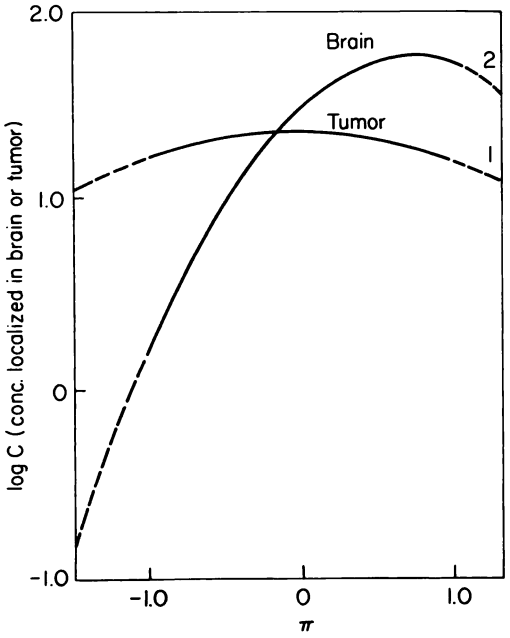


FIG. 1. *Log concentration as a function of π for brain and tumor tissue*

Curve *1* results from a plot of Eq. 9 with $\sigma = 0$. Curve *2* comes from Eq. 5. The solid lines represent the experimentally realized portions; the broken lines represent extrapolations.

[2] An effect of σ upon ionization need not be considered inasmuch as none of the compounds is significantly ionized at physiologic pH values.

The compounds beyond 14 in Table 1 are those for which uncertainty exists concerning their π and σ constants. We have found that π, like σ, is often unpredictable for substituents in ortho positions. Ionizable groups such as carboxyl present a more difficult problem. We have found that π obtained in our octanol:water system gives poor correlations for such functions. The reason for this is that under the conditions of biological testing, these groups

[3] I.e., the sum of π values for all substituent groups on a molecule.

are more or less dissociated, depending on the degree of dilution and the pH of the surroundings.[4] The first difficulty of this type was met (9) with the very polar sulfone group —SO_2CH_3, whose π value is —1.26. Since this value gave poor results in the equations for several different biological systems, we suggested the use of —0.47 (π'), a value which gave good results. Using a similar approach for the carboxyl group, we suggest $\pi' = -1.05$ instead of the experimentally found value of about —0.2. This gives moderately good results for the boronic acids (Table 1) and for a number of other systems (1, 9). Poor correlations are also found for other charged groups such as 4-$CH(NH_2)COOH$ and NH_2. In time it may be possible to work out π' values for these functions.[5]

An ideal partition coefficient, log P_0, for penetration of the brain by boronic acids, can be found by setting $d\log C/d\pi$ equal to zero in Eq. 5. This yields $\pi_0 = 0.7$, where π_0 is the value of $\Sigma\pi$ for substituents giving the drug ideal hydrophobic/hydrophilic character. Since for benzeneboronic acid, $\log P = 1.58$, and since π and $\log P$ are additive constants (3, 4), we have $\log P_0 = 0.7 + 1.58 = 2.3$.

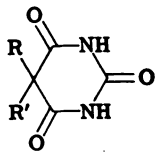
[4] In addition to the partitioning process which a molecule undergoes in moving through cellular material, many sorption–desorption steps onto more or less charged surfaces must also occur. As a first approximation, we considered this to be a special case of partitioning. While this may be satisfactory for uncharged groups even with moderate dipole moments, evidence begins to accumulate that for charged groups such approximations will not be suitable.

[5] Although insufficient data are in hand to give one much confidence in this method for evaluating π', the results so far obtained are helpful and much better than one might have anticipated. For ionizable functions where the degree of ionization varies with dilution and the environment, no single constant can be expected to apply. Perhaps biologic systems can be divided into a few main types for each of which a π' constant for the common ionizable functions could be evaluated. Even constants which gave only semiquantitative results could be quite useful to those designing new drugs.

It is of interest to compare this value with that for a series of barbiturates. While a great amount of work has been published on barbiturates, we have chosen the data of Shonle and Moment (10) (Table 3)

TABLE 3

Observed and calculated equivalent biological response of Barbiturates on Rabbits[a]



R	R'	$\Sigma\pi$[b]	Obs.[c] $\log\frac{1}{C}$	Calcd. $\log\frac{1}{C}$
Et	Et	2.00	3.09	3.01
Pr	Pr	3.00	3.55	3.68
Pr	i-Pr	2.82	3.63	3.66
n-Bu	*n*-Bu	4.00	2.84	3.04
i-Pr	Et	2.32	3.30	3.37
i-Bu	Et	2.82	3.63	3.66
n-Bu	Et	3.00	3.72	3.68
i-Am	Et	3.32	3.75	3.62
i-Am	Pr	3.82	3.48	3.25

[a] From the work of Shonle and Moment (10). Biological response was inability of rabbit to rise when shaken.

[b] The value for CH_3 or CH_2 was taken as 0.50; that for i-Pr as 1.32. Isobutyl was calculated by adding 0.50 to 1.32.

[c] The concentration in grams per kilogram of body weight given by Shonle and Moment was converted to moles/kg, and the log of the reciprocal of this used to represent equivalent biological response.

for analysis because the relative simplicity of the derivatives reported minimizes steric and electronic effects.[6] A least-squares fit yields Eq. 12:

$$\log\frac{1}{C} = -0.657\pi^2 + 3.956\pi - 2.275; \qquad (12)$$

n	r	s
9	0.903	0.154

Computation, as above, yields $\pi_0 = 3.0$;

[6] A more complete analysis of the structure–activity relationship of these compounds will be published later.

log$P$ for barbituric acid is about —0.6,[7] so logP_0 for a barbiturate effect on brain function is 2.4. This value, based on a biologic effect in brain, is remarkably close to that for the boronic acids, based on direct measurements of drug concentration. The coincidence is at least suggestive and may offer a point of departure for development of drugs for the penetration of the central nervous system.

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[7] It should be noted that P for barbituric acid is not as constant as the values for the other molecules we have investigated. It varied from 0.3 to 0.5, depending on the dilution at which the acid was partitioned (10^{-5} to 10^{-6} M).

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