Acknowledgment. We thank Dr. G. U. Baig for synthesis of the sample of temozolomide, the Science and Engineering Research Council for support under the Molecular Recognition Initiative, and the Cancer Research Campaign for its sustained support of the antitumor imidazotetrazinone project.

Registry No. 1, 4342-03-4; 2, 28177-14-2; 3, 3413-72-7; 4, 85622-95-3; 5, 85622-93-1; 6, 142800-55-3; 7, 142800-56-4; 8, 142800-57-5; 9, 90521-24-7; 10, 90521-23-6; 11, 31384-86-8.

Supplementary Material Available: Tables listing bond distances, bond angles, and hydrogen bond geometry (3 pages). Ordering information is given on any current masthead page.

Energy Aspects of Oil/Water Partition Leading to the Novel Hydrophobic Parameters for the Analysis of Quantitative Structure–Activity Relationships

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Partition properties, that is partition coefficients and enthalpies ($\Delta H_p°$) and entropies ($\Delta S_p°$) of partition, have been measured for 50 benzoic acids in the 1-octanol/water system, and their role in QSAR (quantitative structure–activity relationship) analysis examined. The novel hydrophobic parameters have been introduced as a result of the separation of the Gibbs free energy term into the corresponding enthalpy and entropy terms. Application of these novel parameters to some available biological activity data supported the usefulness of these parameters in QSAR analysis. Relative contributions of the enthalpy and entropy terms are also discussed.

Introduction

The optimization between biological activity and structure in a series of drugs by variation of the substitution pattern is an important aim in quantitative structure–activity relationship (QSAR) analysis. A rational approach to this was first proposed by Hansch in 1962.[1] In the Hansch approach, hydrophobic, electronic, and steric substituent constants[2,3] are the three major factors used for a regression-analysis to determine QSAR. Electronic and steric substituent parameters can be estimated by such calculation methods.[4] On the other hand, hydrophobic parameters (log P) are difficult to estimate exactly for different kinds of compounds, despite the considerable efforts hitherto devoted to doing so. As of yet, most of the partition coefficients (P) have been obtained in the 1-octanol/water system, and the logarithms of P have been confirmed to be related to the logarithms of bioactive concentrations of drugs.[2-4] Now, these results are discussed widely for the development of new medicines with superb consequences, stimulating studies about hydrophobic factors from various points of view.

We have been studying partition properties especially from an energy standpoint, using various partition systems such as micelle/water, liposome/water, and so on.[5-7] The log P term reflects the Gibbs free energy of transfer ($\Delta G_p°$) for partition and includes the enthalpy term ($\Delta H_p°$) as well as the entropy term ($\Delta S_p°$):

$$\log P = -\Delta G_p°/(2.303RT) = -\Delta H_p°/(2.303RT) + \Delta S_p°/(2.303R) \quad (1)$$

where R is the gas constant and T is the absolute temperature. Hitherto, $\Delta H_p°$ and $\Delta S_p°$ have been reported for several solutes in several partition systems.[8-11] How-

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(7) (a) Fujiwara, H.; Da, Y.-Zh.; Ito, K.; Takagi, T.; Sasaki, Y. The Energy Aspect of Oil/Water Partition and Its Application to the Analysis of Quantitative Structure–Activity Relationships. Aliphatic Alcohols in the Liposome/Water Partition System. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 3707–3712. (b) Fujiwara, H.; Da, Y.-Zh.; Ito, K. The Energy Aspects of Oil/Water Partition. Proposition of Novel Hydrophobic Parameters for the Treatment of Quantitative Structure–Activity Relationships. *Chem. Lett.* **1992**, 215–218.

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ever, attention has not been given to the separation of the log P term into these two terms, and no attempts have been made to perform any analysis of the relation between these terms and biological activities.

In the present study we introduce some novel hydrophobic parameters that are deduced from a consideration of the energy aspect of partition and will be useful in QSAR analysis.

Definition of Novel Hydrophobic Parameters P_H, P_S and π_H, π_S

In an oil/water partition system, log P possesses enthalpic and entropic properties as shown in eq 1. When these enthalpic and entropic contributions are depicted as P_H and P_S, respectively (eq 2), P_H can be interpreted

$$\log P = P_H + P_S,\ P_H = -\Delta H_p^\circ/(2.303RT),\quad P_S = \Delta S_p^\circ/(2.303R) \quad (2)$$

to be a new hydrophobic parameter that reflects the heat evolved when a solute is transferred from water to a nonaqueous phase. Similarly, P_S can be interpreted to reflect the change of randomness or mobility induced in solution when a solute is transferred from water to a nonaqueous phase. When a series of compounds is used and the conventional hydrophobic substituent constant π applies, this parameter also can be separated into the enthalpy and entropy parts, symbolized as π_H and π_S (eqs 3–5).

$$\pi = \log P(\mathrm{R = X}) - \log P(\mathrm{R = H}) \quad (3)$$

$$\pi = \pi_H + \pi_S \quad (4)$$

$$\pi_H = P_H(\mathrm{R = X}) - P_H(\mathrm{R = H}),\quad \pi_S = P_S(\mathrm{R = X}) - P_S(\mathrm{R = H}) \quad (5)$$

Experimental Section

Benzoic acids from commercial sources were purified by recrystallization or distillation. The partition system was composed of 1-octanol and aqueous phase buffered at pH 2.00 by 50 mM KCl–HCl. The distilled 1-octanol and buffered aqueous solutions were presaturated by each other before use. Benzoic acid (10–100 mM) was first dissolved in 1-octanol, and then 2 mL of this solution was mixed with 10 mL of buffered solution and sealed in an ampule. The ampule was shaken in a constant-temperature bath for about 24 h, and the concentration of benzoic acid in the aqueous phase was measured by a spectrophotometer (Hitachi U-2000) to calculate the partition coefficient. The experiment was repeated after changing the bath temperature to ca. 25, 37, 50, 60, and 70 °C. At elevated temperatures any aqueous phase that separated from an ampule was half-diluted with buffered water before UV spectral measurement to avoid opalescence from a decreased solubility of 1-octanol at ambient temperature. The partition coefficient was calculated according to the following equation:

$$P = (C_{oil} - C_{water}V_{water}/V_{oil})/C_{water} \quad (6)$$

where C and V are the concentration and volume, respectively.

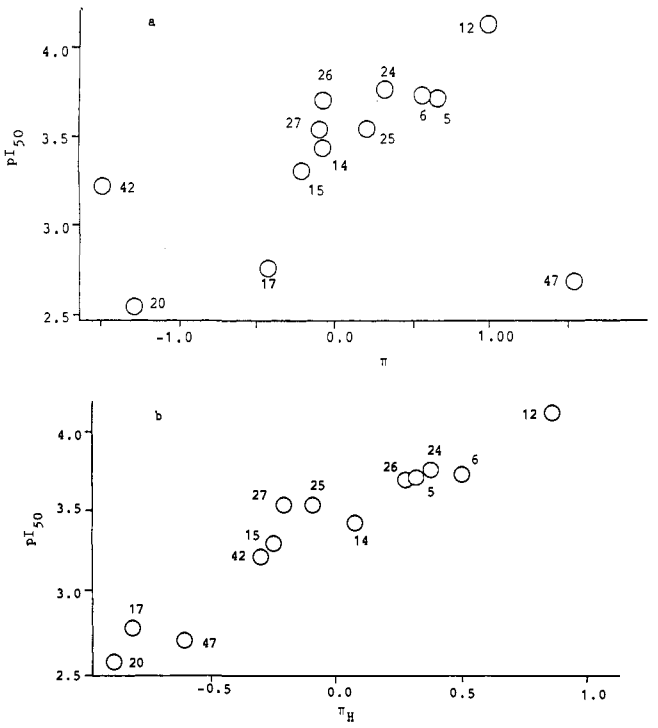

Figure 1. Correlation of pI_{50} and the hydrophobic substituent constants π (a) and π_H (b). The numbering corresponds to that in Table I.

The enthalpy and entropy of partition were then derived from a van't Hoff plot. In this plot the correlation coefficient usually amounted to about 0.98. This experiment were repeated several times to obtain averages and standard derivations of the partition properties. The experimental values are listed in Table I together with the available biological activities: IC_{50} (the concentration of compounds required to inhibit thiopurine methyltransferase's activity by 50%),[12] C' (the concentration of compounds required to induce 10% flowering in *Lemna-paucicostata-151*),[13] LD_{50} (the drug dose required to kill 50% of the mosquito larva in 24 h),[14] and M_{kid} and M_{liv} (the drug concentrations of glycine conjugates in kidneys and livers of rats, respectively).[15]

The log P values listed in Table I agree with the majority of those reported in the literature.[3,16] But some data deviate from the reported ones: included with these compounds are the NH_2 and multiply substituted congeners. The experimental condition of pH 2.00, which is adopted in the present study so that the partition properties of benzoic acids can be measured in an unionized (CO_2H) form, may produce such deviations.

Results and Discussion

From the thermodynamic parameters ΔH_p° and ΔS_p°, π_H and π_S are calculated according to the equations defined above (Table I). A correlation matrix between π, π_H, π_S,

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Table I. Partition Coefficients and Thermodynamic Parameters of Benzoic Acid Derivatives (X-Ph-COOH)

No.	X	log P^a	$\Delta H_p°$ (kJ/mol)	$\Delta S_p°$ (J/mol·K)	π	π_H	π_S	biological activities				
								log LD_{50}^b	pI_{50}^c	log $(1/C')^d$	log M_{kid}^e	log M_{liv}^e
1	H	2.04	−11.29	1.03	0.000	0.000	0.000	−1.64		1.40	1.44	1.28
2	2-F	1.52	−9.88	−3.85	−0.521	−0.247	−0.247			1.82		
3	4-F	2.05	−12.63	−3.02	0.013	0.235	−0.222	−1.85		1.27		
4	2-Cl	2.02	−11.26	0.76	−0.020	−0.005	−0.015	−1.62		2.15		
5	3-Cl	2.72	−13.20	7.67	0.675	0.328	0.347	−2.00	3.71	2.16	1.79	1.57
6	4-Cl	2.62	−14.18	2.51	0.577	0.506	0.071	−2.06	3.73	1.54	1.57	1.59
7	2-Br	2.14	−12.92	−2.39	0.095	0.285	−0.189			1.90		
8	3-Br	2.86	−17.57	−4.00	0.816	1.101	−0.285			1.71		
9	4-Br	2.81	−15.50	1.84	0.770	0.738	0.032	−2.03		1.19		
10	2-I	2.45	−13.41	2.37	0.412	0.372	0.040			1.19		
11	3-I	3.13	−17.06	2.91	1.093	1.011	0.082			1.29		
12	4-I	3.05	−16.22	3.86	1.008	0.864	0.144	−2.31	4.12	0.99		
13	2-NO_2	1.20	−3.05	12.69	−0.841	−1.450	0.609			1.54		
14	3-NO_2	1.69	−10.82	−7.24	−0.350	0.082	−0.432		3.43	0.22	1.23	0.73
15	4-NO_2	1.84	−9.92	2.00	−0.198	−0.240	0.042	−1.52	3.30		1.02	0.79
16	3-OH	1.55	−19.69	−36.34	−0.490	1.462	−1.952			−0.99		
17	4-OH	1.41	−6.70	4.38	−0.630	−0.805	0.175	−1.29	2.76	−1.69		
18	2-NH_2	0.51	1.26	14.54	−1.533	−2.239	0.706					
19	3-NH_2	−0.79	16.05	38.54	−2.829	−4.791	1.962			−1.00	1.45	0.76
20	4-NH_2	0.76	−6.33	−6.61	−1.279	−0.880	−0.399	−1.54	2.54	−1.67	0.79	0.59
21	3-CN	1.65	−9.53	−0.23	−0.389	−0.309	−0.080				0.95	0.69
22	4-CN	1.63	−9.89	−1.22	−0.414	−0.245	−0.169				1.57	0.62
23	2-CH_3	2.27	−13.32	−0.99	0.230	0.356	−0.126					
24	3-CH_3	2.38	−13.48	0.44	0.336	0.384	−0.048		3.76	0.41	1.83	1.71
25	4-CH_3	2.26	−10.78	6.86	0.217	−0.089	0.306	−1.66	3.54	−0.23	1.49	1.56
26	3-OCH_3	1.98	−13.02	−5.63	−0.064	0.284	−0.348		3.70	0.29	1.35	1.34
27	4-OCH_3	1.95	−10.13	3.21	−0.087	−0.203	0.116	−1.60	3.54	−0.66	1.00	1.09
28	4-OC_2H_5	2.45	−12.33	5.74	0.411	0.165	0.246				0.89	0.92
29	3-$NHCOCH_3$	1.32	−6.56	2.78	−0.717	−0.808	0.091				−0.10	−0.29
30	4-$NHCOCH_3$	1.27	−16.49	−31.23	−0.776	0.909	−1.685				−0.64	−0.08
31	3-$N(CH_3)_2$	0.22	6.94	28.06	−1.818	−3.230	1.412				0.45	0.20
32	4-$N(CH_3)_2$	1.28	1.19	28.23	−0.766	−2.187	1.421				0.08	0.17
33	2,5-Cl_2	2.82	−13.53	8.44	0.776	0.393	0.383	−1.77		1.63		
34	2,6-Cl_2	1.80	−4.08	22.43	−0.240	−1.263	1.024	−1.22				
35	3,4-Cl_2	3.25	−16.01	9.62	1.209	0.827	0.382	−2.28		2.47		
36	2,3,5-I_3	3.45	−19.20	1.45	1.406	1.386	0.020	−2.28				
37	3-CF_3	2.99	−13.50	12.43	0.951	0.356	0.595					
38	4-CF_3	3.18	−13.04	17.22	1.138	0.292	0.846					
39	4-CHO	1.76	−17.04	−22.84	−0.279	0.968	−1.247					
40	4-N_3	2.54	−11.68	9.54	0.495	0.051	0.444					
41	4-CH_2NH_2	1.03	−2.89	10.63	−1.016	−1.517	0.501					
42	4-SO_2NH_2	0.55	−9.69	−21.81	−1.488	−0.295	−1.193		3.2			
43	4-CH_2CH_3	2.89	−13.31	10.68	0.846	0.342	0.504					
44	4-$(CH_2)_2CH_3$	3.42	−11.12	27.92	1.375	−0.029	1.404					
45	4-$CH(CH_3)_2$	3.28	−11.53	23.97	1.238	0.040	1.198					
46	4-Bu	3.63	−7.96	42.69	1.589	−0.587	2.176					
47	4-t-Bu	3.59	−7.81	42.32	1.555	−0.601	2.156		2.68			
48	4-$O(CH_2)_2CH_3$	3.09	−12.49	17.24	1.047	0.201	0.846					
49	2,3-$(CH)_4$	2.98	−13.18	12.51	0.937	0.331	0.606	−1.46				
50	3,4-$(CH)_4$	2.78	−10.05	19.72	0.737	−0.239	0.976	−1.92				

[a] The value for 25 °C is listed. The error limits are less than 3%. [b] See ref 14. [c] See ref 12. [d] See ref 13. [e] See ref 15.

Table II. Correlation Coefficients between the Novel Hydrophobic Parameters and Other Physical Parameters[a]

	π	π_H	π_S	σ	L	B4	ΔVw	pK_a
π	1.00	0.69	0.22	0.20	0.44	0.29	0.35	0.09
π_H	0.69	1.00	−0.56	0.39	0.16	−0.03	0.13	0.29
π_S	0.22	−0.56	1.00	−0.30	0.21	0.26	0.23	−0.22
σ	0.20	0.39	−0.30	1.00	0.01	−0.22	0.29	−0.66
L	0.44	0.16	0.21	0.01	1.00	0.77	0.82	−0.08
B4	0.29	−0.03	0.26	−0.22	0.77	1.00	0.86	0.07
ΔVw	0.35	0.11	0.23	0.29	0.82	0.86	1.00	0.22
pK_a	0.09	0.29	−0.22	−0.66	−0.08	0.07	0.22	1.00

[a] L and B4 are the sterimol parameters. (Verloop, A.; Hoogenstraaten, W.; Tripker, J. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, *1976*; Vol. 7, Chapter 4.)

and other physical parameters is shown in Table II. π_H and π_S are not interrelated (r = −0.57) with each other, supporting independence between the newly defined parameters. Furthermore, correlation between π and π_H or π_S is low (r = 0.69 and 0.20, respectively). This fact shows that the original π constants are not determined exclusively by the enthalpy nor the entropy term, but that the two terms contribute cooperatively to π. This trend is obvious in the experimental values of $\Delta H_p°$ and $\Delta S_p°$: although $\Delta H_p°$ is much larger than $\Delta S_p°$ multiplied by T and dominates the $\Delta G_p°$ value for more than half of the congeners listed in Table I, contributions of the entropy, i.e., $T\Delta S_p°$, become comparable to or larger than $\Delta H_p°$ for the remaining congeners. In the following text, biological data are treated by several physical parameters including π_H and π_S, and the results are discussed in full detail.

Table III. QSAR of Benzoic Acids Using π, π_H, π_S and Other Physical Parameters

equations[a]	n[b]	r[c]	r[d]	r'[e]	F[f]	SD[g]
$pI_{50} = 0.214(0.347)\pi + 3.373(0.280)$	13	0.379	0.256	0.000	1.84	0.458
$pI_{50} = 0.609(1.316)\pi_3' - 0.095(0.603)MR_{3,4} + 3.405(0.610)$	13	0.338	0.000	0.000	0.644	0.489
$pI_{50} = 0.867(0.172)\pi_H + 3.431(0.087)$	13	0.958	0.954	0.951	122.63	0.142
$pI_{50} = 0.853(0.175)\pi_H - 0.062(0.123)\pi_S + 3.437(0.088)$	13	0.963	0.955	0.949	20.52	0.230
$-\log LD_{50} = 0.407(0.145)\pi + 1.674(0.106)$	17	0.839	0.827	0.816	35.56	0.186
$-\log LD_{50} = 0.462(0.106)\pi_H + 0.078(0.200)\pi_S + 1.737(0.082)$	17	0.930	0.920	0.911	45.02	0.130
$-\log LD_{50} = 0.451(0.101)\pi_H + 1.755(0.067)$	17	0.927	0.922	0.917	91.19	0.128
$-\log C' = 2.765(1.247)\sigma - 0.670(0.856)\Delta Vw(m,p) - 0.274(1.050)\Delta Vw(o) + 1.022(0.989)$	24	0.763	0.720	0.679	9.27	0.858
$-\log C' = 0.737(0.346)\pi + 2.001(0.967)\sigma - 1.004(0.637)\Delta Vw(m,p) - 0.361(0.757)\Delta Vw(o) + 1.451(0.740)$	24	0.892	0.867	0.844	18.44	0.616
$-\log C' = 0.880(0.282)\pi_H + 1.489(0.506)\pi_S + 1.985(0.753)\sigma - 1.246(0.514)\Delta Vw(m,p) - 0.629(0.608)\Delta Vw(o) + 1.740(0.599)$	24	0.940	0.923	0.906	27.29	0.477
$-\log M_{kid} = -0.251(0.220)\pi + 3.424(1.639)Vw(m) + 4.262(1.495)Vm(p) - 2.297(0.482)$	18	0.874	0.844	0.817	15.05	0.359
$-\log M_{kid} = -0.041(0.160)\pi_H + 3.713(1.937)Vw(m) + 4.323(1.767)Vw(p) - 2.263(0.568)$	18	0.818	0.773	0.731	9.44	0.425
$-\log M_{kid} = -0.117(0.272)\pi_S + 3.885(1.865)Vw(m) + 4.293(1.736)Vw(p) - 2.237(0.559)$	18	0.825	0.783	0.743	9.98	0.417
$-\log M_{kid} = -0.371(0.227)\pi_H - 0.668(0.394)\pi_S + 3.259(1.447)Vw(m) + 4.029(1.308)Vw(p) - 2.218(0.418)$	18	0.915	0.887	0.861	16.68	0.309
$-\log M_{liv} = -0.375(0.207)\pi + 2.614(1.540)Vw(m) + 2.872(1.406)Vw(p) - 1.857(0.453)$	18	0.857	0.823	0.792	12.94	0.338
$-\log M_{liv} = -0.129(0.168)\pi_H + 2.871(2.031)Vw(m) + 2.948(1.852)Vw(p) - 1.818(0.596)$	18	0.734	0.664	0.593	5.46	0.445
$-\log M_{liv} = 0.007(0.318)\pi_S + 3.196(2.178)Vw(m) + 2.978(2.027)Vw(p) - 1.796(0.653)$	18	0.670	0.575	0.475	3.80	0.487
$-\log M_{liv} = -0.472(0.240)\pi_H - 0.694(0.406)\pi_S + 2.399(1.520)Vw(m) + 2.643(1.383)Vw(p) - 1.772(0.431)$	18	0.907	0.876	0.848	13.89	0.302

[a] In parentheses are listed the 95% confidence intervals. [b] Number of data. [c] Multiple correlation coefficient. [d] Multiple correlation coefficient adjusted for the degree of freedom. [e] Multiple correlation coefficient doubly adjusted for the degree of freedom. [f] Variance ratio. [g] Standard deviation.

QSAR Analyses of Benzoic Acids. The QSAR analyses of benzoic acids are shown in Table III. In this table, correlation coefficients are also shown after adjusting for the degree of freedom.[17] For the pI_{50} data, the correlation coefficient (r) is as low as 0.379 (Figure 1a), when π is used as an independent parameter. However, this is remarkably improved to 0.963 (Figure 1b) when π is separated into π_H and π_S. Also, a higher coefficient is obtained ($r = 0.958$) when the data are treated with π_H only, indicating an important role for the enthalpy term. When compared with the freedom-doubly-adjusted correlation coefficients (r') or F values, π_S becomes useless and π_H turns out to be an essential parameter. This result is interpreted to show that an important process in the inhibition activity, where drugs penetrate into a hydrophobic cleft[18] in an enzyme, occurs, reflecting the enthalpy change in the model 1-octanol/water partition. This is probable since hydrogen-bonding interactions between drug molecules and water become interrupted when drugs are transferred from an aqueous phase to a hydrophobic environment. The pI_{50} data have been correlated with π_3' (relative hydrophobicity of the more hydrophobic of the two meta substituents) and $MR_{3,4}$ (molar refractivity of the more hydrophobic of the two meta substituents and of the para substituent on the phenyl ring). However, these parameters do not give a sufficient result for the compounds treated here ($r = 0.338$, Table III). Inclusion of trisubstituted congeners has given a reported high correlation coefficient ($r = 0.891$),[12] but π_3' and $MR_{3,4}$ are not the effective parameters for the simple derivatives of benzoic acid. It is noteworthy that the pI_{50} data treated here cannot be correlated with π even if the Hammett constant σ is incorporated.

The LD_{50} data are better correlated with π_H ($r = 0.927$) than with π ($r = 0.839$), the π_S term contributing negligibly. This trend is quite similar to the case of pI_{50} mentioned above. The Hammett constant σ is reported to be useful for the LD_{50} data,[19] but in the present case it gives only a low correlation coefficient ($n = 17$, $r = 0.394$). In this correlation the congeners having Cl atoms in the ortho position (2-Cl, 2,5-Cl_2, and 2,6-Cl_2) and having 4-NO_2 and 4-NH_2 groups deviate appreciably. Some steric effect might be operative, but it fails to explain the reason for the deviation of the 4-NO_2 and 4-NH_2 groups.

The $-\log C'$ data have been interpreted by means of σ, $\Delta Vw(m,p)$, and $\Delta Vw(o)$,[13] but some unresolvable factors

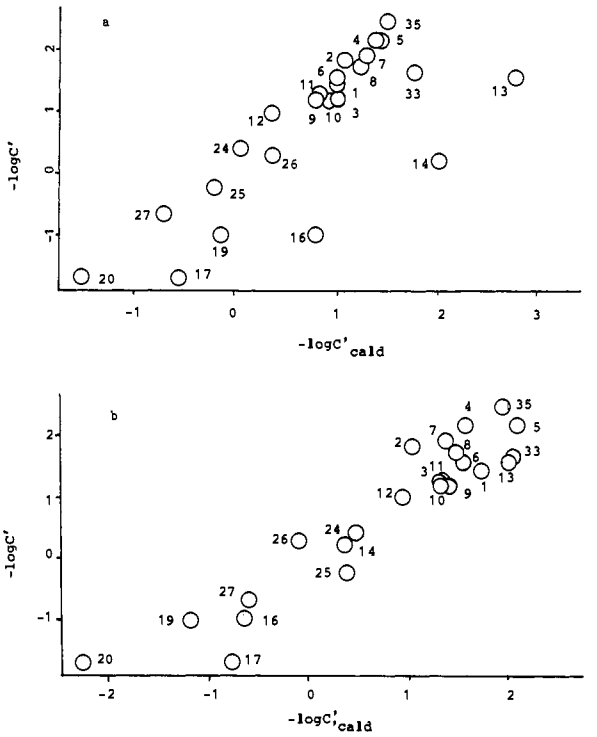

Figure 2. Correlation of $-\log C'$ and the calculated values. (a) Calculated using σ, $\Delta Vw(m,p)$, and $\Delta Vw(o)$. (b) Calculated using π_H and π_S as well as σ, $\Delta Vw(m,p)$, and $\Delta Vw(o)$. The numbering corresponds to that in Table I.

(17) Yanai, H.; Takane, Y. In *Shinpan Tahenryo Kaisekiho* (Japanese); Asakura: Tokyo, 1990, p 59.
(18) Woodson, L. C.; Ames, M. M.; Selassie, C. D.; Hansch, C.; Weinshilboum, R. M. Thiopurine Methyltransferase. Aromatic Thiol Substrates and Inhibition by Benzoic Acid Derivatives. *Mol. Pharmacol.* 1983, *24*, 471–478.
(19) Seydel, J. K. In *Drug Design*; Ariens, E. J., Ed.; Academic: New York, 1971; Vol. 1, Chapter 3.

Table IV. The Novel Hydrophobic Constants P_H and P_S of Fatty Alcohols

alcohols	log P	P_H	P_S
ethanol	−0.25	−2.37	2.12
1-propanol	0.24	−2.10	2.34
1-butanol	0.82	−1.79	2.61
1-pentanol	1.41	−1.47	2.88
1-hexanol	2.11	−1.15	3.26
1-heptanol	2.72	−0.47	3.19
2-propanol	0.11	−2.54	2.65
2-methyl-1-propanol	0.74	−1.84	2.58
2,2-dimethyl-1-propanol	1.29	−1.75	3.04
2-butanol	0.62	−2.49	3.11
2-chloroethanol	−0.08	−1.14	1.06
2,2-dichloroethanol	0.71	−0.51	1.22
2,2,2-trichloroethanol	1.59	−0.16	1.75
2-bromoethanol	0.29	−0.86	1.15
2-fluoroethanol	−0.68	−1.66	0.98

seemed to intervene, and the OH and NO_2 groups needed be excluded from the treatment. In fact, similar treatment of the data for the commercially available benzoic acids studied here, including congeners with OH and NO_2 groups, gives a correlation coefficient (r) as low as 0.763 (Figure 2a). However, when π_H and π_S are included as independent variables, the value of r increases to 0.940 (Figure 2b). Therefore, hydrophobicity is thought to contribute to this activity. The contributions from the enthalpy and entropy terms are comparable with each other, being slightly weighted to the latter term as judged from the coefficients in the regression analyses (Table III). In this calculation, the value of r is reduced to 0.892 when π_H and π_S are replaced by the conventional parameter π. This trend is unchanged when compared with the freedom-adjusted or freedom-doubly-adjusted multiple correlation coefficients.

The log M_{kid} and log M_{liv} data were interpreted by means of Vw and log P after separating the meta and para derivatives and collecting the log P values from different sources.[15] For the log M_{kid} data a treatment with the three parameters π, Vw(m), and Vw(p) affords $r = 0.874$ (Table III) for $n = 18$ data points.[20] This correlation coefficient is not improved even if π is replaced with π_H or π_S. However, when both π_H and π_S are included, r improved to 0.915. This situation does not change even if σ is incorporated as judged from the values of r'' or F (Table III). Quite similar trends are observed for the log M_{liv} data (Table III). In this case, separation of π into π_H and π_S does not improve the result as clearly as observed above. This is explained by the roughly equal contribution from the π_H and π_S terms in the regression analyses. That is, if the regression coefficients for these two terms are equal, then π_H and π_S can be summed up to π with the same results.

QSAR Analyses of Fatty Alcohols. The hydrophobic enthalpy and entropy constants P_H and P_S can be applied to QSAR analyses of any drugs for which thermodynamic parameters of partition are measured. As an example of such cases, fatty alcohols for which enthalpies and entropies of partition have been reported in 1-octanol/water[6] are treated here. The P_H and P_S constants are summarized in Table IV. The QSAR analyses using these constants (Table V) are essentially the same as those[6] using directly the enthalpies and entropies of partition. However, use of the P_H and P_S constants is convenient for a discussion of the relative contribution of the enthalpy and entropy terms as stated below. The entropy term P_S contributes nothing to the data for in vivo toxicity (LD_{50}, the single intraperitoneal dose required to kill 50% of the mice in 7 days). It contributes only partly (about one-third) to the activities of ID_{50} (the concentration required to produce 50% inhibition of growth) and T_i (the slope of the dose–response curve in tissue culture). Finally it contributes about one-half of the enthalpy term for the activity of H_{50} (the concentration required to produce 50% hemolysis in saline). It is reported[21] from the constancy of the T_i/P to LD_{50} ratio that the in vivo toxicity LD_{50} can be accounted for by the intrinsic toxicity which may be represented by T_i and the equilibrium concentration of the toxicant which may be represented by $1/P$. But this explanation assumes that the model 1-octanol/water system is an adequate model of the partition system in vivo. To test the validity of this assumption, the ratio LD_{50}/T_i is analyzed by the regression using log P, P_H, and P_S (Table V). The results show that log (LD_{50}/T_i) is simulated not by log P but by P_H or ($P_H + 0.2P_S$). Therefore, the enthalpy term plus about $^1/_5$ of the entropy term in the model 1-octanol/water system is a useful estimate for the factor of equilibrium concentration, i.e., the transportion factor, in the biophase system. It is of great interest that such a mixed parameter can explain activities of all the alcohols, including halogenated as well as nonhalogenated ones.

Relative Contribution of the Enthalpy and Entropy Terms. As is discussed above, separation of log P, which corresponds to the Gibbs free energy change of partition, into its constituent terms of enthalpy and entropy has proven to widen the applicability of QSAR analysis: in some cases the enthalpy term is very important and in other cases both terms are important. Since the enthalpy and entropy terms P_H and P_S as well as π_H and π_S are normalized with respect to each other so as to contribute equally to the Gibbs free energy change of partition in the 1-octanol/water system, the ratio of the coefficient of the entropy term (P_S or π_S) to the coefficient of the enthalpy term (P_H or π_H) expresses the relative contribution of these two terms. When this ratio is unity, it means that partition in the biophase system takes place for the drugs just as it does in the model 1-octanol/water system. On the other hand, when the entropy/enthalpy ratio is much smaller than unity, it means that the Gibbs free energy change in the biophase system is related to the *enthalpy* change (not the entropy change) in the 1-octanol/water system.

The entropy/enthalpy ratio can be discussed more closely when linear relations are assumed for the enthalpy and entropy terms between the 1-octanol/water and biophase systems:

$$\Delta H_p^\circ(\text{bio}) = k_1 \Delta H_p^\circ(\text{oct}) + k_2 \quad (7)$$

$$\Delta S_p^\circ(\text{bio}) = l_1 \Delta S_p^\circ(\text{oct}) + l_2 \quad (8)$$

(20) The separated treatment for the meta- and para-substituted congeners affords the following results, where $n = 9$ (meta) and $n = 10$ (para), including unsubstituted benzoic acid in both cases:

$$\log M_{liv}(\text{meta}) = 0.120(0.337)\pi - 3.212(2.514)\text{Vw(m)} + 2.125(0.770)$$

$$r = 0.818,\ F = 6.09,\ \text{SD} = 0.418$$

$$\log M_{liv}(\text{para}) = 0.621(0.412)\pi - 3.236(1.634)\text{Vw(p)} + 2.016(0.539)$$

$$r = 0.923,\ F = 20.26,\ \text{SD} = 0.301$$

(21) Dillingham, E. O.; Mast, R. W.; Bass, G. E.; Autian, J. Toxicity of Methyl- and Halogen-Substituted Alcohols in Tissue Culture Relative to Structure-Activity Models and Acute Toxicity in Mice. *J. Pharm. Sci.* 1973, *62*, 22–30.

Table V. Regression of Alcohol Toxicities by Means of the Novel Hydrophobic Parameters

QSAR equations	n	r^a	F	SD
$-\log ID_{50} = 0.678(0.615) \log P + 1.475(0.466)$	11	0.639	6.22	0.570
$-\log ID_{50} = 1.006(0.289)P_H + 0.335(0.291)P_S + 2.680(0.472)$	11	0.952	38.63	0.241
$-\log LD_{50} = 0.328(0.594) \log P + 2.380(0.455)$	10	0.410	1.62	0.453
$-\log LD_{50} = 0.490(0.193)P_H - 0.053(0.213)P_S + 3.436(0.317)$	10	0.965	47.57	0.139
$-\log H_{50} = 1.006(0.720) \log P + 0.118(0.567)$	8	0.813	11.69	0.512
$-\log H_{50} = 1.211(0.361)P_H + 0.579(0.412)P_S + 1.336(0.696)$	8	0.970	40.03	0.234
$\log T_i = 0.673(0.677) \log P + 3.193(0.513)$	11	0.600	5.06	0.629
$\log T_i = 1.015(0.399)P_H + 0.316(0.402)P_S + 4.449(0.651)$	11	0.917	21.08	0.333
$\log (LD_{50}/T_i) = -1.222(1.361) \log P - 5.401(1.041)$	10	0.591	4.29	1.036
$\log (LD_{50}/T_i) = -1.584(0.591)P_H - 0.376(0.622)P_S - 7.750(0.925)$	10	0.955	36.63	0.406
$\log (LD_{50}/T_i) = -1.331(0.387)P_H - 8.141(0.686)$	10	0.942	63.02	0.431

[a] Multiple correlation coefficient.

Then, the actual partition coefficient for the biophase can be expressed as follows:

$$\begin{aligned}\log P(\text{bio}) &= -\Delta H_p^\circ(\text{bio})/(2.303RT) + \Delta S_p^\circ(\text{bio})/(2.303R) \\ &= -[k_1\Delta H_p^\circ(\text{oct}) + k_2]/(2.303RT) + [l_1\Delta S_p^\circ(\text{oct}) + l_2]/(2.303R) \\ &= k_1P_H(\text{oct}) + l_1P_S(\text{oct}) + \text{constant} \\ &= k_1[P_H(\text{oct}) + \alpha P_S(\text{oct})] + \text{constant}\end{aligned} \quad (9)$$

where α is equal to l_1/k_1. Therefore, when biological activity (BA) is expressed as a linear function of log P(bio), as expected from a model calculation:[22]

$$\log BA = a \log P(\text{bio}) + b \quad (10)$$

and the following relation is reached:

$$\begin{aligned}\log P(BA) &= ak_1P_H(\text{oct}) + al_1P_S(\text{oct}) + \text{constant} \\ &= ak_1[P_H(\text{oct}) + \alpha P_S(\text{oct})] + \text{constant}\end{aligned} \quad (11)$$

Therefore, $P_H + \alpha P_S$ in the 1-octanol/water system is equivalent to log P(bio) in eq 10. Here, α is determined from a regression equation of biological activity and equals the ratio of coefficients for P_H and P_S. Equation 7 shows that k_1 expresses a susceptibility of the enthalpy of transfer of the biophase relative to that of the 1-octanol/water system, and eq 8 shows that l_1 expresses a relative susceptibility of the entropy term of the biophase with reference to that of the 1-octanol/water system. Therefore, α (or l_1/k_1) is interpreted to reflect the entropy/enthalpy ratio of the relative susceptibilities.

The α values derived from regression equations in Table III are summarized in Table VI together with the correlation coefficients reached using the free energy terms π or log P and using the separated enthalpies π_H or P_H and entropies π_S or P_S. The pI_{50} and $-\log LD_{50}$ data for benzoic acids and the $-\log LD_{50}$ data for fatty alcohols indicate that a replacement of the free energy term by the enthalpy term strikingly enhances the correlation coefficient and the enthalpy term plays the decisive role in the transportion of drugs.[23] For the $-\log H_{50}$ data of alcohols, α amounts to 0.5 and a replacement of π by π_H and π_S is not a prerequisite for a high correlation coefficient. This is also the case for $-\log C'$, $-\log M_{kid}$, and $-\log M_{liv}$ of benzoic acids, for which α amounts to 1.5–1.8. These trends are typical when $\alpha = 1$, for which π and log P are equivalent to $\pi_H + \pi_S$ and $P_H + P_S$, respectively. For the $-\log ID_{50}$ and log T_i data, for which $\alpha = 0.3$, the separation of log P into P_H and P_S still enhances the correlation coefficients greatly.

Table VI. α and r Values for the Benzoic Acids and Alcohols

	benzoic acids		
	α	$r(\pi)^a$	$r(\pi_H,\pi_S)^b$
pI_{50}	0.0	0.379	0.958
$-\log LD_{50}$	0.2	0.839	0.927
$-\log C'$	1.7	0.892	0.940
$-\log M_{kid}$	1.8	0.874	0.915
$-\log M_{liv}$	1.5	0.857	0.907
	alcohols		
	α	$r(\log P)^c$	$r(P_H,P_S)^d$
$-\log ID_{50}$	0.3	0.639	0.952
$-\log LD_{50}$	−0.1	0.410	0.965
$-\log H_{50}$	0.5	0.813	0.970
$\log T_i$	0.3	0.600	0.917
$\log (LD_{50}/T_i)$	0.2	0.591	0.955

[a] Multiple correlation coefficient when analyzed using π constants. [b] Multiple correlation coefficient when analyzed using π_H and π_S. [c] Multiple correlation coefficient when analyzed using log P. [d] Multiple correlation coefficient when analyzed using P_H and P_S.

Conclusions

Separation of the conventional hydrophobic constant log P and π into its enthalpy and entropy parts was useful for the improved estimation of the biological activities of benzoic acid derivatives and alcohols. Such an improvement can be brought about when relative contributions of the enthalpic and entropic portions of the partition properties differ between the biophase and the model system.

(22) Hansch, C.; Clayton, J. M. *Lipophilic Character and Biological Activity of Drugs II*: The Parabolic Case. *J. Pharm. Sci.* 1973, *62*, 1–21.

(23) For the $-\log LD_{50}$ data of benzoic acids, r is exceptionally high, even if π is used as a parameter. This is because π and π_H are interrelated ($r = 0.833$) for the compounds treated.