Thermochemical Aspects of Partition. A Study on The Novel Hydrophobic Parameters of Multiply-Substituted Benzoic Acids

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The thermochemical aspects of partition have been measured for multiply-substituted benzoic acids with the intention of disclosing the mode of change of the proposed novel hydrophobic parameters on multiple substitution in the phenyl ring. The substituent hydrophobic parameter (π) has been separated into two terms of enthalpic and entropic nature (π_H and π_S). It is necessary to take into account the effect of solvation around the hydrogen-bond-participating groups, such as OH, OCH_3, and NO_2, besides the benzoic CO_2H in interpreting the substituent dependency of π, π_H and π_S. Some Hammett sigma parameters, such as σ_o(→OH) and σ_o(→OCH_3), which mean the *ortho* substituent effect to the functional OH and OCH_3 groups, respectively, act in a compensating manner to π_H and π_S, resulting in a vanishing effect on π. These trends are in good conformity with the enthalpy–entropy compensation rule in chemical thermodynamics.

We recently studied the partition phenomenon of benzoic acid derivatives, fatty alcohols, and others in an oil/water system from the standpoint of the free energy, and applied the results to analyses of quantitative structure-activity relationships (QSAR).$^{1-3)}$ Hitherto, in QSAR analyses of drugs, it has been usual to use the partition coefficient ($\log P$) or fragment constant (f), which was derived from data of $\log P$, as a measure of drug hydrophobicity. Sometimes, electronic and steric factors are also included to accomplish a QSAR analysis. However, there are still many examples that can not be fully explained in such a conventional manner. In order to improve our estimation of the hydrophobicity, we considered the partition phenomenon from thermochemical points of view, and have introduced novel hydrophobic parameters (P_H and P_S, or π_H and π_S), which are defined as follows based on the enthalpy (ΔH_P°) and entropy (ΔS_P°) of partition:

$$\log P = P_H + P_S \tag{1a}$$

$$P_H = -\Delta H_P^\circ/2.303RT;\quad P_S = \Delta S_P^\circ/2.303R \tag{1b}$$

$$\pi = \pi_H + \pi_S \tag{1c}$$

$$\pi_H = P_H(X) - P_H(H);\quad \pi_S = P_S(X) - P_S(H) \tag{1d}$$

Here, R is the gas constant and T is the absolute temperature. As given in Eq. 1d, the π_H and π_S are defined as the difference in the P_H and P_S, respectively, when a substituent (X) is introduced instead of H. These novel parameters have provided splendid QSAR analyses for monosubstituted benzoic acid derivatives, fatty alcohols, and others, which are superior to the results reached by using the conventional parameters, $\log P$ or π. As these consequences, we have confirmed the necessity and feasibility of the above-mentioned novel hydrophobic parameters (P_H and P_S, or π_H and π_S).$^{1-3)}$

In the present study, to further clarify the characteristics of novel hydrophobic parameters, we measured the π_H and π_S constants for multiply-substituted benzoic acid derivatives and investigated the mode of change of the π_H and π_S depending on multiple substitutions in the phenyl ring.

Experimental

Multiply-substituted benzoic acid derivatives from commercial sources were purified by recrystallization in methanol–water mixed solvents. The partition system comprised a 1-octanol and aqueous phase buffered at pH 1.98 by 50 mM KCl–HCl (1 M=1 mol dm^{-3}). The distilled 1-octanol and the buffered aqueous solution were presaturated with each other before use. Each derivative was dissolved to 0.1—20 mM in 1-octanol. Two ml of this solution was mixed with a 10 ml buffered aqueous phase and sealed in an ampoule. The ampoule was shaken in a constant-temperature bath for about 24 h, and the concentration of the derivative was measured in the aqueous phase with a Hitachi U2000 spectrophotometer. In the case that the solute is dissolved in an organic phase before partitioning, the partition coefficient (K_P) is obtained according to Eq. 5, which is derivable from Eqs. 2, 3, and 4.

$$[S]_{org} = M_{org}/V_{org} \tag{2}$$

$$[S]_{aq} = M_{aq}/V_{aq} \tag{3}$$

$$[S_0] = (M_{org} + M_{aq})/V_{org} \tag{4}$$

Table 1. Partition Properties log P, $\Delta H^\circ_\mathrm{P}$, $\Delta S^\circ_\mathrm{P}$, and π, π_H, π_S of Multiply-Substituted Benzoic Acid Derivatives in 1-Octanol/Water System

No.	Substituents	log P[a)]	$\Delta H^\circ_\mathrm{P}$ kJ mol^{-1}	$\Delta S^\circ_\mathrm{P}$ J K^{-1} mol^{-1}	π	π_H	π_S
1	H	2.04(0.00)	−11.29	1.03	0.00	0.00	0.00
2	2-Cl, 5-Cl	2.82(0.01)	−13.53	8.44	0.78	0.39	0.38
3	2-Cl, 6-Cl	1.80(0.01)	−4.08	22.43	−0.24	−1.26	1.02
4	3-Cl, 4-Cl	3.25(0.01)	−16.01	9.62	1.21	0.83	0.38
5	2-Cl, 4-Cl	2.76(0.00)	−12.79	10.13	0.72	0.26	0.46
6	3-Cl, 5-Cl	3.29(0.01)	−13.08	19.29	1.25	0.32	0.93
7	2-F, 3-F	1.88(0.01)	−8.74	6.82	−0.16	−0.45	0.29
8	2-I, 3-I, 5-I	3.45(0.03)	−19.20	1.45	1.41	1.39	0.02
9	2-Cl, 4-NO_2	1.86(0.00)	−3.58	23.66	−0.18	−1.35	1.17
10	2-Cl, 5-NO_2	1.88(0.00)	−6.09	15.55	−0.16	−0.91	0.75
11	4-Cl, 3-NO_2	2.53(0.01)	−13.02	4.68	0.49	0.30	0.18
12	2-CH_3, 3-NO_2	2.16(0.01)	−9.63	8.95	0.12	−0.29	0.41
13	3-CH_3, 4-NO_2	2.42(0.01)	−12.67	3.79	0.38	0.24	0.14
14	4-CH_3, 3-NO_2	2.35(0.01)	−13.67	−0.83	0.31	0.42	−0.11
15	5-CH_3, 2-NO_2	1.63(0.00)	−2.32	23.40	−0.41	−1.57	1.16
16	3-NO_2, 5-NO_2, 4-OCH_3	1.73(0.00)	−9.71	0.33	−0.31	−0.28	−0.04
17	2-OH, 3-CH_3	3.04(0.01)	−21.22	−13.04	1.00	1.74	−0.74
18	2-OH, 4-CH_3	2.80(0.02)	−17.14	−3.93	0.76	1.03	−0.27
19	2-OH, 4-OH	1.75(0.00)	−21.62	−38.95	−0.29	1.81	−2.10
20	4-OH, 3-OCH_3	1.40(0.00)	−14.44	−21.67	−0.64	0.55	−1.19
21	5-Br, 2-OH	3.30(0.02)	−17.80	3.43	1.26	1.14	0.12
22	4-Br, 3-OH, 5-OH	1.75(0.00)	−25.81	−53.06	−0.29	2.55	−2.83
23	4-OH, 3-OCH_3, 5-OCH_3	1.13(0.00)	−13.65	−24.12	−0.91	0.41	−1.32
24	3-NO_2, 5-NO_2, 2-OH	0.80(0.00)	−13.44	−29.80	−1.24	0.38	−1.61
25	3-OCH_3, 4-OCH_3	1.48(0.00)	−6.39	6.81	−0.56	−0.86	0.30
26	3-OCH_3, 4-OCH_3	2.20(0.00)	−12.48	0.31	0.19	0.21	−0.05
27	3-OCH_3, 4-OCH_3, 5-OCH_3	1.66(0.00)	−2.35	23.88	−0.38	−1.57	1.18

a) The value at 25 °C is listed with a standard deviation in parenthesis.

$$K_\mathrm{p} = \frac{[\mathrm{S}]_\mathrm{org}}{[\mathrm{S}]_\mathrm{aq}} = \frac{[\mathrm{S}_0]}{[\mathrm{S}]_\mathrm{aq}} - \frac{V_\mathrm{aq}}{V_\mathrm{org}} \tag{5}$$

In the above Eqs., [S] and M are the concentration and mole number of the solute after partitioning, respectively; V denotes the volume of the phase mixed, the suffixes org and aq mean the organic phase and the aqueous phase, respectively, and $[\mathrm{S}_0]$ stands for the initial solute concentration (molarity) in 1-octanol before partitioning. The enthalpy and entropy of the partition were determined from a van't Hoff plot after variable-temperature measurements of log P at nearly 25, 32, 40, 50, and 60 °C. At elevated temperatures each aqueous phase separated from the ampoule was half-diluted with the same volume of buffered water before measuring UV spectra, thus avoiding opalescence of the separated solution sometimes induced from a decreased solubility of 1-octanol at ambient temperature. These experiments were repeated several times to obtain averages and standard deviations of the partition properties.

Results and Discussion

The log P and other thermochemical properties of multiply-substituted benzoic acid derivatives are listed in Table 1. The novel hydrophobicity parameters (π_H and π_S) are determined according to Eqs. 1a, 1b, 1c, and 1d from the experimental thermodynamic parameters ($\Delta H^\circ_\mathrm{P}$ and $\Delta S^\circ_\mathrm{P}$); they are also included in Table 1. A correlation matrix is shown in Table 2 for π, π_H, and π_S. The correlation between π and π_H or π and π_S is low (r=0.390 and r=0.299, respectively), showing that the original π constant is not dependent solely on the enthalpy or the entropy term, but that the latter two terms contribute cooperatively to π. It can be seen from the negative value of r (−0.762) for the correlation between π_H and π_S that the enthalpy and entropy terms contribute to π in a compensative manner. However, this enthalpy–entropy correlation is not so high as to make the partner term fully dependent on the other one. This fact is evidenced more directly in the experimental values of $\Delta H^\circ_\mathrm{P}$ and $\Delta S^\circ_\mathrm{P}$; $T\Delta S^\circ_\mathrm{P}$ contributes to the free energy term ($-2.303RT$ log P) decisively in some derivatives and is comparable with $\Delta H^\circ_\mathrm{P}$ in other some cases, although it is almost negligible in other derivatives (Table 1).

Table 2. Correlation Coefficients between π, π_H, and π_S

	π	π_H	π_S
π	1.000	0.390	0.299
π_H	0.390	1.000	−0.762
π_S	0.299	−0.762	1.000

Following the definition of the hydrophobic substituent constant (π), the π value of a multiply-substituted derivative can be expressed, as a first approx-

Table 3. Surveyed and Calculated Values of Hydrophobic Properties[a)]

No.	Substituents	π	π'	π_H	π'_H	π_S	π'_S
1	H	0.00	0.00	0.00	0.00	0.00	0.00
2	2-Cl, 5-Cl	0.78	0.66	0.39	0.32	0.38	0.33
3	2-Cl, 6-Cl	−0.24	−0.04	−1.26	−0.01	1.02	−0.03
4	3-Cl, 4-Cl	1.21	1.25	0.83	0.83	0.38	0.42
5	2-Cl, 4-Cl	0.72	0.56	0.26	0.50	0.46	0.06
6	3-Cl, 5-Cl	1.25	1.35	0.32	0.66	0.93	0.69
7	2-F, 3-F	−0.16	−0.46	−0.45	−0.27	0.29	−0.20
8	2-I, 3-I, 5-I	1.41	2.60	1.39	2.39	0.02	0.20
9	2-Cl, 4-NO_2	−0.18	−0.22	−1.35	−0.25	1.17	0.03
10	2-Cl, 5-NO_2	−0.16	−0.37	−0.91	0.08	0.75	−0.45
11	4-Cl, 3-NO_2	0.49	0.23	0.30	0.59	0.18	−0.36
12	2-CH_3, 3-NO_2	0.12	−0.12	−0.29	0.44	0.41	−0.56
13	3-CH_3, 4-NO_2	0.38	0.14	0.24	0.14	0.14	−0.01
14	4-CH_3, 3-NO_2	0.31	−0.13	0.42	−0.01	−0.11	−0.13
15	5-CH_3, 2-NO_2	−0.41	−0.51	−1.57	−1.07	1.16	0.56
16	3-NO_2, 5-NO_2, 4-OCH_3	−0.31	−0.70	−0.28	0.16	−0.04	−0.86
17	2-OH, 3-CH_3	1.00	0.51	1.74	1.39	−0.74	−0.88
18	2-OH, 4-CH_3	0.76	0.39	1.03	0.92	−0.27	−0.53
19	2-OH, 4-OH	−0.29	−0.46	1.81	0.20	−2.10	−0.66
20	4-OH, 3-OCH_3	−0.64	−0.69	0.55	−0.52	−1.19	−0.17
21	5-Br, 2-OH	1.26	0.99	1.14	2.11	0.12	−1.12
22	4-Br, 3-OH, 5-OH	−0.29	−0.21	2.55	3.66	−2.83	−3.87
23	4-OH, 3-OCH_3, 5-OCH_3	−0.91	−0.76	0.41	−0.24	−1.32	−0.52
24	3-NO_2, 5-NO_2, 2-OH	−1.24	−0.53	0.38	1.17	−1.61	−1.70
25	3-OCH_3, 4-OCH_3	−0.56	−0.15	−0.86	0.08	0.30	−0.23
26	3-OCH_3, 4-OCH_3	0.19	−0.13	0.21	0.57	−0.05	−0.70
27	3-OCH_3, 4-OCH_3, 5-OCH_3	−0.38	−0.22	−1.57	0.37	1.18	−0.58

a) The calculated values π', π'_H, and π'_S were obtained from mono-substituted derivatives followed to the data in Ref. 3.

imation, by the sum of the π of each substituent that is defined from monosubstituted derivative. Similarly, π_H and π_S of a multiply-substituted derivative may be expressed as a sum of those of monosubstituted derivatives. These first-approximation relations are designated as follows:

$$\pi' = \sum \pi(R_i), \tag{6}$$

$$\pi'_H = \sum \pi_H(R_i) \tag{7}$$

and

$$\pi'_S = \sum \pi_S(R_i), \tag{8}$$

where π', π'_H, and π'_S mean the π' values of multiply-substituted derivatives predicted from a simple sum rule, $\sum$ means the summation for all substituents, and π (R_i) is the π constant of the ith substituent assigned based on the data for monosubstituted derivatives. According to Eqs. 6, 7, and 8, π', π'_H, and π'_S are calculated by using the π constants of monosubstituted benzoic acids already reported by the authors.[3)] These values are summarized in Table 3. Linear regressions of π, π_H, and π_S against π', π'_H, and π'_S, respectively, are summarized in Table 4. Table 4 shows that the regression coefficients are smaller than unity, indicating a decreased substituent effect in multiply-substituted derivatives, and that the correlation coefficients are not satisfactorily high. In the case of π, a quadratic equation afforded the correlation coefficient of 0.931, i.e., $\pi=-0.264(0.132)(\pi')^2 +1.181(0.230)\pi' +0.1699(0.121)$; such a relation is interpreted as simply suggesting a saturation profile of the substituent effect on multiple substitution. That is, an electronic effect of one substituent becomes decreased upon multiple substitution, since the substituents already attached to a phenyl ring have changed the electronic distribution on the ring so that there remains insufficient room for a newly introduced substituent to change the distribution effectively.

To discuss π, π_H, and π_S further in detail, these parameters are tested with respect to substituent electronic and steric effects. When the Hammett substituent constants (σ)[4)] and the incremental van der Waals volume (ΔV_w)[5)] are introduced into the regression analyses, the correlations listed in Table 4 (Eqs. 9, 10, and 11) are slightly improved, as shown in the same Table 4 (Eqs. 12, 13, and 14), where $\sigma_o(\rightarrow CO_2H)$ is the Hammett substituent constant acting on the CO_2H group from the *ortho* position and $\sigma_{m,p}(\rightarrow CO_2H)$ is a similar constant for a substituent located at the *meta* or *para* position. It can be seen from these equations that the Hammett constant plays only a very limited role. The substituents treated in the present study include hydrogen-bond-participating groups, such as NO_2, OCH_3, and OH, besides the ben-

Table 4. Formulated Equation of Surveyed Hydrophobic Properties π, π_H, and π_S against the Simple Sum Predictions

Eq. No.	Equations[a]	n[b]	R[c]	R'[d]	SD[e]	F[f]
9	$\pi=0.82(0.18)\ \pi'+0.06(0.14)$	27	0.88	0.87	0.12	84.7
10	$\pi_H=0.77(0.31)\ \pi'_H-0.20(0.34)$	27	0.71	0.70	0.56	25.7
11	$\pi_S=0.83(0.33)\ \pi'_S+0.30(0.32)$	27	0.72	0.71	0.51	26.7
12	$\pi=0.92(0.08)\ \pi'-1.23(0.36)\ \Delta V_W+0.02(0.12)\ \sum\sigma_{m,p}(\rightarrow CO_2H)$ $-0.06(0.23)\ \sum\sigma_o(\rightarrow CO_2H)+0.47(0.14)$	27	0.93	0.91	0.29	32.7
13	$\pi_H=0.74(0.15)\ \pi'_H-1.23(0.78)\ \Delta V_W-0.36(0.27)\ \sum\sigma_{m,p}(\rightarrow CO_2H)$ $-1.13(0.55)\ \sum\sigma_o(\rightarrow CO_2H)+0.39(0.28)$	27	0.83	0.80	0.62	12.5
14	$\pi_S=0.72(0.17)\ \pi'_S+0.25(0.79)\ \Delta V_W+0.39(0.29)\ \sum\sigma_{m,p}(\rightarrow CO_2H)$ $+1.10(0.57)\ \sum\sigma_o(\rightarrow CO_2H)+0.01(0.30)$	27	0.79	0.74	0.68	9.1

a) The values in parentheses express standard errors. b) Number of data. c) Multiple correlation coefficient. d) Multiple correlation coefficient adjusted for the degree of freedom. e) Standard deviation. f) Variance ratio.

Table 5. Formulated Equation of Surveyed Hydrophobic Properties π, π_H, and π_S Considering "Forward and Backward" Effects

Eq. No.	Equations[a]	n[b]	R[c]	R'[d]	SD[e]	F[f]
15	$\pi=2.00(0.52)\ \Delta V_W+0.38(0.26)\ \sum\sigma_{m,p}(\rightarrow CO_2H)-1.28(0.28)\ \sum\sigma_o(\rightarrow CO_2H)$ $-0.96(0.25)\ \sum\sigma_{m,p}(\rightarrow OH)+0.31(0.32)\ \sum\sigma_o(\rightarrow OH)-0.95(0.18)\ \sum\sigma_{m,p}(\rightarrow NO_2)$ $-1.12(0.34)\ \sum\sigma_{m,p}(\rightarrow OCH_3)-0.04(0.51)\ \sum\sigma_o(\rightarrow OCH_3)+0.25(0.10)\ \sum Es_o$ $+0.17(0.15)$	27	0.95	0.92	0.28	15.9
16	$\pi_H=2.00(0.52)\ \Delta V_W+0.38(0.26)\ \sum\sigma_{m,p}(\rightarrow CO_2H)-1.28(0.28)\ \sum\sigma_o(\rightarrow CO_2H)$ $-0.96(0.25)\ \sum\sigma_{m,p}(\rightarrow OH)+0.31(0.32)\ \sum\sigma_o(\rightarrow OH)-0.95(0.18)\ \sum\sigma_{m,p}(\rightarrow NO_2)$ $-1.12(0.34)\ \sum\sigma_{m,p}(\rightarrow OCH_3)-0.04(0.51)\ \sum\sigma_o(\rightarrow OCH_3)+0.25(0.10)\ \sum Es_o$ $-0.06(0.23)$	27	0.94	0.90	0.45	13.7
17	$\pi_S=2.00(0.52)\ \Delta V_W+0.38(0.26)\ \sum\sigma_{m,p}(\rightarrow CO_2H)-1.28(0.28)\ \sum\sigma_o(\rightarrow CO_2H)$ $-0.96(0.25)\ \sum\sigma_{m,p}(\rightarrow OH)+0.31(0.32)\ \sum\sigma_o(\rightarrow OH)-0.95(0.18)\ \sum\sigma_{m,p}(\rightarrow NO_2)$ $-1.12(0.34)\ \sum\sigma_{m,p}(\rightarrow OCH_3)-0.04(0.51)\ \sum\sigma_o(\rightarrow OCH_3)+0.25(0.10)\ \sum Es_o$ $+0.23(0.20)$	27	0.95	0.92	0.39	18.0

a) The values in parentheses are standard errors. b) Number of data. c) Multiple correlation coefficient. d) Multiple correlation coefficient adjusted for the degree of freedom. e) Standard deviation. f) Variance ratio.

zoic CO_2H group. The profile of the hydrogen-bonding solvation around these groups may change, reflecting the electronic and steric effects from other groups on the same phenyl ring. For example, for 3-methyl-4-nitrobenzoic acid, NO_2 group affects solvation around the CO_2H group (forward effect); also, the CO_2H group affects the NO_2 group (backward effect). In this case, however, the CO_2H and NO_2 groups do not affect the CH_3 group significantly, since CH_3 is not a substituent that participates considerably in solvation (by hydrogen bonding). Such "forward and backward" effects have already been proposed[6)] to successfully interpret the $\log P$ data of multiply-substituted aromatic compounds. For a full application of such a treatment, the collection of much more data is necessary with different congeners, including several congeners other than the benzoic acid treated here. However, a rather simple extension of such a "forward and backward" idea may be possible by including the Hammett substitutent constants for all of the hydrogen-bonding groups, besides CO_2H. For example, the inclusion of $\sigma(\rightarrow OH)$ in a regression analysis, where $\rightarrow OH$ means that the electronic effect is acting on the OH group in the phenol derivatives, may allow for changes in the hydrogen-bonding profile around the phenolic OH group. In actual regression analyses, σ constants are included at first toward all of the hydrogen-bonding substituents (CO_2H, OH, NO_2, and OCH_3) on the phenyl ring. Then, any unnecessary σ constants are removed one by one following a step-by-step regression analysis. The final analyses are summarized in Table 5. In these results the σ constants $\sigma_o(\rightarrow OH)$ and $\sigma_o(\rightarrow OCH_3)$ contribute to π_H and π_S in a compensating manner, resulting in an insignificant contribution to the π term. The same is true, more or less, for the σ constants ($\sigma_{m,p}(\rightarrow CO_2H)$, $\sigma_o(\rightarrow CO_2H)$, $\sigma_{m,p}(\rightarrow OH)$, and $\sigma_o(\rightarrow OH)$) and for ΔV_w. As exceptional cases, $\sigma_{m,p}(\rightarrow NO_2)$ contributes to π_H and π_S in the same manner, and $\sigma_{m,p}(\rightarrow OCH_3)$ and $\sum Es_o$ contribute only to π_H (and hence to π), but not to π_S. These trends may be interpreted as showing the importance of solvation around functional groups by hydrogen-bonding; usually, when such solvation is strengthened enthalpically, solvent molecules are tightly bound to the functional groups, and hence, solvation is weakened in an entropic sense.

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