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Studies on Absorption and Excretion of Drugs. X.[1] Relation between Chemical Structure and Absorption Rate. (2). Substituent Constant for Absorption Rate Coefficient of Foreign Organic Compounds

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When Danielli's model, the transition state theory and the formal treatment on the basis of the extrathermodynamic relationships were applied to the intestinal absorption of foreign organic compounds, it was expected that there should be the following relation between the absorption rate coefficients for the compound (RS) having a substituent group (S) and for the mother compound (RH).

$$\log k_{RS}/k_{RH} = \text{constant}$$

The perfusion experiments through the small intestine of anesthetized rats for a number of benzene derivatives were carried out. The logarithmic plots of the residual ratio against the perfusion time gave straight lines and from the slopes the absorption rate coefficients were obtained.

The results indicate that the values of $\log k_{RS}/k_{RH}$ for a given substituent group (S) have a proper value which is called the substituent constant.

In our preceeding paper,[1] from the comparison of intestinal absorption rates of many benzoyl derivatives, it was found that the number and the position of the OH-groups were important factors relating absorption rates to chemical structure and that Danielli's activated diffusion model could be applied to the intestinal absorption of foreign organic compounds.

The purpose of the present study is to investigate the effect of substituent groups on the absorption rate coefficients of foreign organic compounds.

Let us consider the processes of intestinal absorption of drugs on the basis of Danielli's model.[3]

Generally speaking, drug molecules in solution will be vibrating about a mean position between adjacent energy barriers and only those having more than the minimum energy necessary to pass over an energy barrier will in fact be able to transfer. Also, when a drug molecule transfers from the solution in the intestinal lumen into the biophase, it must surmount the energy barrier in the interface. This process will be accompanied with the replacement of the interaction with water surrounding the solute molecule in the solution by the interaction with the components of the biophase. According to the transition state theory,[4–10] these processes can be treated as follows: We shall represent the drug mole-

1) Part IX: H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), **16**, 389 (1968).
2) Location: *a*) *Hongo, Tokyo*; *b*) *Fukushima-ku, Osaka.*
3) H. Davson and J.F. Danielli, "The Permeability of Natural Membranes," Cambridge, 1952, p. 324.
4) S. Glasstone, K.J. Laidler, and H. Eyring, "The Theory of Rate Processes," Chapter 1, 3, 4, 9, McGraw-Hill Co., 1941.
5) H. Eyring and E.M. Eyring, "Modern Chemical Kinetics," Chapter 2, 3, 4, Reinhold, 1963.
6) K.J. Laidler, "Reaction Kinetics," Vol. I, Chapter 2, Pergamon, 1963.
7) H. Eyring, J. Walter, and G.E. Kimball, "Quantum Chemistry," Chapter 16, John Wiley and Sons, 1944.
8) B.J. Zwolinski, H. Eyring, and C.E. Reese, *J. Phys. Colloid Chem.*, **53**, 1426 (1949).
9) R.B. Parlin and H. Eyring, in "Ion Transport Across Membrane," ed. H.T. Clark, Academic Press, 1954, p. 103.
10) F.H. Johnson, H. Eyring, and M.J. Polissar, "The Kinetic Basis of Molecular Biology," Chapter 14, John Wiley and Sons, 1954.

cule interacting with water molecules in the luminal solution by $D_{in\,W}$, and that interacting with the components in the biophase by $D_{in\,B}$. In this treatment, it is assumed that drug molecules must pass the transition state in the top of the barrier. When $D_{in\,X^{\neq}}$ denotes the drug molecule in the transition state, the process is shown in Fig. 1. When the drug concentration in the biophase is so small that the transfer of the drug from the biophase to the luminal solution can be negligible, the net amount of the drug transferring from the luminal solution into the biophase can be approximately shown in equation (1).

$$-\frac{d(V\times C_{D\,(in\,W)})}{dt}=\lambda A\left(\frac{kT}{h}\right)\exp\left[-\frac{\Delta G^{\neq}}{RT}\right]\times C_{D\,(in\,W)} \quad (1)$$

In this equation V is the volume of the luminal solution, $C_{D\,(in\,W)}$ is the molar concentration of the drug in the solution, λ is the distance between the transition state and the last barrier in the aqueous phase as shown in Fig. 1, A is the surface area of the mucosal membrane, $\Delta G^{\neq}$ is the molar free energy difference between $D_{in\,W}$ and $D_{in\,X^{\neq}}$, R is the gas constant and (kT/h) is a frequency factor involving the Boltzmann constant, k, the absolute temperature, T, and h, Planck's constant. The transmission coefficient is assumed to be unity.[8–10] When the volume of the solution, the temperature, the surface area, and the characteristics of the membrane can be regarded as constant, the equation (2) is obtained by integration of equation (1),

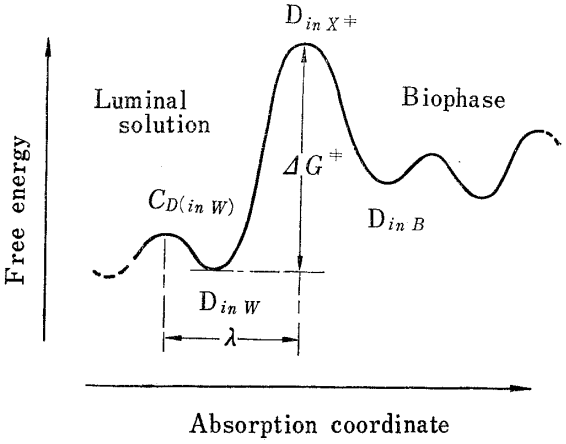


Fig. 1. Free Energy Profile Curve

$$\log\frac{D_{in\,W}}{D^{\circ}_{in\,W}}=-\frac{0.434}{V}kt=-k't \quad (2)$$

where $D_{in\,W}$ and $D^{\circ}_{in\,W}$ are the amount of the drug in the luminal solution in $t=t$ and $t=0$, respectively, and k is the constant defined in equation (3).

$$k=\lambda A\left(\frac{kT}{h}\right)\exp\left[-\frac{\Delta G^{\neq}}{RT}\right] \quad (3)$$

From equation (2), it is clear that k is the absorption rate coefficient shown in our preceeding paper.[1]

In the field of organic chemistry, the experimental relationships between chemical structure and reactivity are widely found and they are called extrathermodynamic relationships, since they are not directly derived from the axioms of thermodynamics alone. According to Leffler, *et al.*,[11] the molecule is divided into two zones, —the substituent group in question and a second zone. Each of these zones will be regarded as contributing an additive term to the free energy.[12] We shall represent the zones in a molecule by S for the substituent group and by R for the second zone. In the treatment on the basis of extrathermodynamic relationships, the standard partial molar free energy of a substance, $\bar{G}^{e}_{RS}$, is expressed as in equation (4).

11) J.E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reaction," Chapter 1—6, John Wiley and Sons, 1963.

12) Such treatments are seen also in the reports on thin-layer chromatography (A.J.P. Martin, *Ann. Rev. Biochem.*, **19**, 520 (1950); D. French and G.M. Wild: *J. Am. Chem. Soc.*, **75**, 2612 (1953); E.C. Bate-Smith and R.G. Westall, *Biochim. Biophys. Acta*, **4**, 427 (1950)).

$$\bar{G}^{\ominus}_{RS}=G_R+G_S \qquad (4)$$

The quantities G_R and G_S are independent additive terms (per mole).

For the drug molecules which are expressed in RS and RH, the rate coefficients for the processes can be described in the same way as equation (3).

$$k_{RS}=\lambda_{RS}A\left(\frac{kT}{h}\right)\exp\left[-\frac{\Delta G^{\neq}_{RS}}{RT}\right] \qquad (5)$$

$$k_{RH}=\lambda_{RH}A\left(\frac{kT}{h}\right)\exp\left[-\frac{\Delta G^{\neq}_{RH}}{RT}\right] \qquad (6)$$

By the application of the formal treatment,[11-13] the activation free energies are shown as equation (7) and (8).

$$\begin{aligned}\Delta G^{\neq}_{RS}&=\bar{G}^{\ominus}_{RS\,(in\,X^{\neq})}-\bar{G}^{\ominus}_{RS\,(in\,W)}\\&=(G_{R\,(in\,X^{\neq})}+G_{S\,(in\,X^{\neq})})-(G_{R\,(in\,W)}+G_{S\,(in\,W)})\\&=\Delta G^{\neq}_{R}+\Delta G^{\neq}_{S}\end{aligned} \qquad (7)$$

$$\begin{aligned}\Delta G^{\neq}_{RH}&=\bar{G}^{\ominus}_{RH\,(in\,X^{\neq})}-\bar{G}^{\ominus}_{RH\,(in\,W)}\\&=(G_{R\,(in\,X^{\neq})}+G_{H\,(in\,X^{\neq})})-(G_{R\,(in\,W)}+G_{H\,(in\,W)})\\&=\Delta G^{\neq}_{R}+\Delta G^{\neq}_{H}\end{aligned} \qquad (8)$$

where $\Delta G^{\neq}_{R}$, $\Delta G^{\neq}_{S}$ or $\Delta G^{\neq}_{H}$ is an additive term of the activation free energy for R, S or H, respectively. From equations (7) and (8), equation (9) is obtained.

$$\Delta G^{\neq}_{RS}-\Delta G^{\neq}_{RH}=(\Delta G^{\neq}_{R}+\Delta G^{\neq}_{S})-(\Delta G^{\neq}_{R}+\Delta G^{\neq}_{H})=\Delta G^{\neq}_{S}-\Delta G^{\neq}_{H} \qquad (9)$$

According to Danielli,[3] λ can be treated approximately as a constant for analogous compounds. Thus, under the condition that A can be regarded as constant, from equations (5), (6) and (9) as a first approximation the following equations are obtained.

$$\frac{k_{RS}}{k_{RH}}=\exp\left[-\frac{\Delta G^{\neq}_{RS}-\Delta G^{\neq}_{RH}}{RT}\right] \qquad (10)$$

$$\log\left(\frac{k_{RS}}{k_{RH}}\right)=-\frac{0.434}{RT}[\Delta G^{\neq}_{S}-\Delta G^{\neq}_{H}] \qquad (11)$$

When H is a standard substituent, $\Delta G^{\neq}_{H}$ is fixed, and the right hand side in equation (11) should have a proper value to a given substituent group, S. We shall express this value by K_S, as shown in equation (12).

$$-\frac{0.434}{RT}[\Delta G^{\neq}_{S}-\Delta G^{\neq}_{H}]=K_S \qquad (12)$$

Thus, equation (13) is obtained.

$$\log\left(\frac{k_{RS}}{k_{RH}}\right)=K_S \qquad (13)$$

From the above considerations, it seems that the substituent constant shown in equation (13) exists for each substituent group. This prediction should be confirmed by animal experiments.

13) A.I. Shatenshtein, in "Advances in Physical Organic Chemistry," ed. V. Gold, Vol. I, Academic Press, 1963, p. 193.

In this paper, compounds with a strong intramolecular interaction are excluded from the object of study, because as indicated in the previous paper, the absorption rate of the compound with intramolecular interaction differs considerably from that of the isomer without it. The effect of intramolecular interaction will be discussed in the following paper.[14]

Experimental

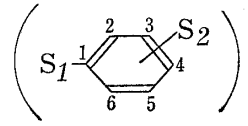
Sample Solution——The nineteen compounds tested here are listed in Table I. The sample solution contained 2 mM of the compound to be tested and 0.9% NaCl. All the sample solutions were adjusted to pH 6.0 by the addition of NaOH or HCl.

Experimental Procedure——The experimental technique employed was the same as that described in our previous paper.[1] The volume of the perfusion solution was 50 ml for *p*-hydroxybenzenesulfonamide and 200 ml for other compounds.

Analytical Methods

a) *p*-Hydroxybenzenesulfonamide: The sample solution (50 ml) and washings were transferred to a 250 ml-measuring flask and the volume was made up to 250 ml with distilled water. Ten milliliters of this solution were pipetted into a 20 ml-measuring flask and 7 ml of M/15 Na_2HPO_4, 4 drops of 1% 4-aminoantipyrine and 2 ml of 2% $K_3Fe(CN)_6$ were added. The volume was made up to 20 ml with M/15 Na_2HPO_4 and the optical density was read on a spectrophotometer (Hitachi Co., Ltd., Model EPU-2) at 500 mμ.

b) Other samples: Two hundred milliliters of the sample solution and washings were transferred into a 1 liter-measuring flask and the volume was made up to 1 liter with distilled water. The recovered sample solutions were diluted with 0.1 N NaOH for the compounds No. (25) to (32) and with 0.1 N HCl for the compounds No. (33) to (42). The optical density was measured at 267 mμ for (25), at 389 mμ for (26), at 398 mμ for (27), at 332 mμ for (28), at 330 mμ for (29), at 315 mμ for (30), at 355 mμ for (31), at 378 mμ for (32), at 293 mμ for (33), at 270 mμ for (34), at 275 mμ for (35), at 273 mμ for (36), at 277 mμ for (37), at 272 mμ for (38), at 276 mμ for (39), at 254 mμ for (40), at 250 mμ for (41), and at 266 mμ for (42).

TABLE I. Absorption Rate Coefficients 

No.	Compounds	Substituent groups S_1	Substituent groups S_2[a]	Absorption rate coefficients k
25	Nitrobenzene	$-NO_2$		1.29
26	*m*-Nitrophenol	$-NO_2$	-OH(3)	0.87
27	*p*-Nitrophenol	$-NO_2$	-OH(4)	0.90
28	*o*-Nitroanisole	$-NO_2$	$-OCH_3$(2)	1.05
29	*m*-Nitroanisole	$-NO_2$	$-OCH_3$(3)	1.06
30	*p*-Nitroanisole	$-NO_2$	$-OCH_3$(4)	1.05
31	*m*-Nitroaniline	$-NO_2$	$-NH_2$(3)	0.93[b] {0.81; 1.04}
32	*p*-Nitroaniline	$-NO_2$	$-NH_2$(4)	0.91[b] {0.94; 0.87}
33	*p*-Acetaminobenzaldehyde	-CHO	$-NHCOCH_3$(4)	0.86
34	Phenol	-OH		0.53
35	Pyrocatechol	-OH	-OH(2)	0.53
36	*m*-Ethoxyphenol	-OH	$-OC_2H_5$(3)	0.87
37	*p*-Cresol	-OH	$-CH_3$(4)	0.70
38	*o*-Ethylphenol	-OH	$-C_2H_5$(2)	0.79
39	*p*-Ethylphenol	-OH	$-C_2H_5$(4)	0.77
40	Ethyl *p*-hydroxybenzoate	$-COOC_2H_5$	-OH(4)	0.99
41	*p*-Hydroxybenzamide	$-CONH_2$	-OH(4)	0.34
42	*p*-Nitrobenzamide	$-CONH_2$	$-NO_2$(4)	0.71[b] {0.77; 0.64}
43	*p*-Hydroxybenzenesulfonamide	$-SO_2NH_2$	-OH(4)	0.08

a) The position is shown in the parentheses.
b) This is a mean of the values in the brace.

14) H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), **16**, 586 (1968).

Results and Discussion

The absorption rate coefficients obtained by the same method as in the previous paper are shown in Table I.

From the estimations of pK_a values for these compounds with a potentiograph (Metrohm A.G. Model-E336), it was confirmed that the contribution of the ionic form of each compound might be negligible at the virtual pH in the absorption site.[15] The results for 3 compounds (*p*-nitrobenzamide, *m*- and *p*-nitroanilines), indicate that the reproducibility was as good as shown in the previous paper. From the values in Table I, together with the data in the previous paper,[1] the changes in log k when H is replaced by a given substituent group, S, can be calculated. These values are shown in Table II.

Table II. Values of Substituent Constant

Substituent groups -S	RS (No.)[a]	log k_{RS}	RH (No.)[a]	log k_{RH}	log (k_{RS}/k_{RH})=K_S		
						(Means)	(Mean deviations)
$-CONH_2$	(41)	−0.47	(34)	−0.27	−0.20	−0.23	±0.03
	(42)	−0.15	(25)	+0.11	−0.26		
-OH [I]	(13)	−0.06	(11)	+0.09	−0.15	−0.16	±0.02
	(14)	−0.03	(11)	+0.09	−0.12		
	(19)	−0.04	(17)	+0.16	−0.20		
	(20)	+0.01	(17)	+0.16	−0.15		
	(26)	−0.06	(25)	+0.11	−0.17		
	(27)	−0.05	(25)	+0.11	−0.16		
$-NH_2$	(31)	−0.03	(25)	+0.11	−0.14	−0.15	±0.01
	(32)	−0.04	(25)	+0.11	−0.15		
$-OCH_3$	(23)	−0.02	(11)	+0.09	−0.11	−0.07	±0.03
	(24)	+0.09	(11)	+0.09	0.00		
	(28)	+0.02	(25)	+0.11	−0.09		
	(29)	+0.03	(25)	+0.11	−0.08		
	(30)	+0.02	(25)	+0.11	−0.09		
$-C_2H_5$	(38)	−0.10	(34)	−0.27	+0.17	+0.17	±0.01
	(39)	−0.11	(34)	−0.27	+0.16		
$-NO_2$	(26)	−0.06	(34)	−0.27	+0.21	+0.22	±0.01
	(27)	−0.05	(34)	−0.27	+0.22		
-CHO	(13)	−0.06	(34)	−0.27	+0.21	+0.23	±0.01
	(14)	−0.03	(34)	−0.27	+0.24		
	(16)	−0.04	(35)	−0.28	+0.24		
$-COOCH_3$	(19)	−0.04	(34)	−0.27	+0.23	+0.26	±0.03
	(20)	+0.01	(34)	−0.27	+0.28		
$-SO_2NH_2$	(43)	−1.09	(34)	−0.27	−0.82	−0.82	.....
$-NHCOCH_3$	(33)	−0.07	(11)	+0.09	−0.16	−0.16	.....
$-OC_2H_5$	(36)	−0.06	(34)	−0.27	+0.21	+0.21	.....

a) Compounds represented by No. (1) to No. (24) are shown in Part IX of this series of studies and No. (25) to No. (43) are shown in this paper.

The mean and the deviation of log (k_{RS}/k_{RH}) for each substituent group are listed in the last two columns. The difference between the obtained values for *m*- and *p*-isomers is not significant, so that the means and the mean deviations calculated without distinction between *m*- and *p*-positions are described in this table.

15) C.A.M. Hogben, D.J. Tocco, B.B. Brodie, and L.S. Schanker, *J. Pharmacol. Exptl. Therap.*, **125**, 275 (1959).

The results indicate that, considering the errors of the animal experiments, the values of $\log(k_{RS}/k_{RH})$ for each substituent group are almost equal, as expected from equation (13). Thus, K_S in equations (12) and (13) can be called the substituent constant in this study.

From the application of the formal treatment on the basis of the extrathermodynamic relationships to the intestinal absorption of foreign organic compounds, it appears that the substituent constant is, as shown in equation (12), proportional to the contribution of the substituent group, S, to the free energy change of the compound in the process from the hydrated state in an aqueous solution to the transition state. Since in this process, as discussed in the previous paper, the breakage of the hydrogen bond with surrounding water molecules may be an important factor, some relationship between the hydrogen bond forming power and the substituent constant may be expected.

From the studies on infrared light absorption,[16,17] the decreasing order of the H–bond forming powers of proton acceptors is estimated as follows: $-SO_2NH_2$, $-CONH_2$, $-CHO$, $-COOCH_3$, $-NO_2$. This essentially agrees with the order of the values of the substituent constant in Table II; *i.e.*, the more the H–bond forming power of a substituent group, the more the decreasing effect of the group on the intestinal absorption rate.

These findings support the application of Danielli's activated diffusion model to the intestinal absorption of foreign organic compounds and also possibly suggests the application of the formal treatment on the basis of extrathermodynamic relationships.

16) M. Tsuboi, *Bull. Chem. Soc. Japan*, **25**, 60 (1952).
17) S. Seki, H. Chihara, and K. Suzuki, "Suiso Ketsugō," in "Iwanami Kōza Gendai Kagaku," VI G, Iwanami, 1956, p. 24.