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Biological Activity of Drugs. XI.[1] Relation of Structure to the Bacteriostatic Activity of Sulfonamides. (2)

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Hydrophobicity constant π, which was introduced by Hansch, of sulfonamides was measured in the systems of *n*-octyl alcohol-water (designated π_0) and chloroform–water (designated π_c). A good linear correlation was obtained between π_0 and π_c. π_c could be expressed in terms of log S_w, solubility of unionized molecules in water, and log S_c, solubility in chloroform. Binding of sulfonamides to human plasma protein, rabbit plasma and rabbit blood was measured following an equilibrium dialysis method. A good correlation was obtained in any couple of the three measurements. The protein binding was found to be subjected to molecular weight of sulfonamides. Thus, an analysis was performed of the structure-activity relationship of sulfonamides between the bacteriostatic activity against *Escherichia coli* and the drug's physicochemical parameters including molecular weight.

Recently, Hansch, *et al.*[3] have found that the effect of substituent on the biological activity of a parent molecule can be interpreted by using two substituent constants, Hammet σ constant and hydrophobicity constant π. They assumed that the rate–limiting conditions for many biological responses to chemicals could be defined in two steps, *i.e.*, a random walk process of drug molecule to the active site where a biological response was originated and an interacting process of the molecule with the active site.[4] They developed a method for the correlation of biological activity and chemical structure using substituent constants and studied the structure–activity relationship of sulfonamides.[5] Hydrophobicity of sulfonamide derivatives was found to play a definite role on the biological activity of sulfonamides and an optimal hydrophobic character for the activity was deduced from the relationships.

Previously, we have studied the bacteriostatic activity of sulfonamides against *Escherichia coli*[6] and their physicochemical properties.[7] The present report concerns with an analysis of the structure-activity relationship of sulfonamides following the Hansch-Fujita method.

Results and Discussion

Physicochemical Parameters

The most widely and frequently used parameters for structure–activity studies in biochemical systems have been partitioning between oil and water and binding to protein. Before attempting to analyze a structure–activity relationship of sulfonamides, the two physico-

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4) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
5) T. Fujita and C. Hansch, *J. Med. Chem.*, **10**, 991 (1967).
6) M. Yamazaki, M. Aoki and A. Kamada, *Oyo Yakuri*, **2**, 210 (1968).
7) M. Yamazaki, M. Aoki, A. Kamada and N. Yata, *Yakuzakigaku*, **27**, 37, 40 (1967).

chemical parameters were related with water- and oil-solubility, acid dissociation constant and molecular weight.

Partition coefficient of sulfonamides between a phosphate buffer of pH 6.4 and chloroform or *n*-octyl alcohol was measured at 30°. A substituent constant π_x, which was introduced by Hansch,[3] was calculated with a correction for dissociation in the aqueous phase (pH 6.4).

$$\pi_X = \log P_X - \log P_H \quad (1)$$

where P_X and P_H are the partition coefficients of unionized molecules for a derivative with substituent X and a parent molecule H, respectively.

Binding of sulfonamides to human plasma protein, rabbit plasma and rabbit blood were measured as follows.

An equilibrium dialysis method following Klotz[8] was employed using a cellophane tube (Visking Company). Five ml of 5% phosphate buffer solution of the dried human plasma (Nippon Blood Bank) were taken into a cellophane bag. The bag was placed into a 30 ml test tube which contained 10 ml phosphate buffer solution of sulfonamide. The tube was kept at 10° for 3 days. To avoid a possible error due to an adsorption of sulfonamide to the cellophane membrane, a control experiment was performed with the same concentration of sulfonamide in the absence of protein. The phosphate buffer was pH 7.4 and ionic strength 0.05. The concentration of sulfonamide in the outer solution was measured following the Bratton-Marshall method.[9]

The binding of sulfonamide was calculated employing eq. 2.

$$\beta = \frac{C_0 - C_1}{C_0} \times 100 \quad (2)$$

where C_0 and C_1 are concentrations of sulfonamide in the outer solution for the contro lexperiment which contained no protein in the inner solution of the bag and for the experiment in the presence of protein, respectively. The results of 14 sulfonamides at $C_0 = 1 \times 10^{-4}$M are presented in Table I.

Table I. Physicochemical Parameters and Bacteriostatic Activity against *E. coli*

Substance	M.W.	pK_a	log S_w[a]	log S_c[b]	π_c[c]	π_o[d]	log K[e]	log 1/(CR)[f]
Sulfanilamide	172.2	10.45	−1.245	−2.879	0	0	2.433	2.824
Sulfacetamide	214.2	5.40	−1.453	−2.444	1.055	—	2.869	3.248
Sulfapyridine	249.3	8.37	−2.764	−2.485	1.443	0.677	2.980	4.102
Sulfathiazole	255.3	7.10	−2.836	−3.319	0.671	1.047	3.893	4.597
Sulfadiazine	250.3	6.15	−3.854	−3.310	1.620	0.568	3.248	4.337
Sulfamerazine	264.3	6.93	−3.208	−2.511	1.877	0.833	3.990	4.469
N-Sulfanilyl-3,4-xylamide	304.4	4.72	−4.538	−2.267	3.480	3.219	5.332	4.055
Sulfisoxazole	267.3	4.62	−3.377	−2.620	2.342	1.847	4.428	4.564
Sulfisomidine	278.3	7.38	−2.380	−2.788	0.846	0.402	4.039	4.502
Sulfaphenazole	314.4	5.91	−3.398	−2.001	2.852	2.274	5.036	4.450
Sulfamethoxypyridazine	280.3	7.05	−2.723	−1.854	2.071	1.097	4.437	4.570
Sulfadimethoxine	310.3	6.05	−4.131	−2.137	3.099	2.257	5.298	4.706
Sulfamethoxazole	253.3	5.81	−2.807	−2.171	1.915	1.580	3.965	4.634
Sulfamonomethoxine	280.3	6.03	−3.959	−2.580	2.110	1.549	4.265	4.943

a) solubility of sulfonamides in water (m mole/liter) as unionized molecules at 30°
b) solubility of sulfonamides in chloroform at 30°
c) Hydrophobicity constant which was derived from true partition coefficient between chloroform and water.
d) Hydrophobicity constant which was derived from true partition coefficient between *n*-octylalcohol and water.
e) stability constant of binding to human plasma protein at 10°
f) bacteriostatic activity against *E. coli*

8) I.M. Klotz, F.W. Walker and R.B. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946).
9) A.C. Bratton and E.K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

The association constant K and number of binding sites m were obtained by measuring C_1 at various concentrations of C_0 and calculated employing eq. 3.[10]

$$R/C_1 = K\mathrm{m} - K\mathrm{R} \tag{3}$$

where R is the number of moles of drug per mole of total protein and m, number of binding sites in a protein molecule.

Presently, the molecular weight of human plasma protein was assumed to be 69000 and m to be 0.86 which was experimentally estimated from the results of 5 sulfonamides.

Binding of sulfonamides to the rabbit plasma or blood was measured by placing a cellophane bag which contained 5 ml of plasma or blood into 15 ml of phosphate buffer solution of sulfonamide at pH 7.4 and 37°. The β values in Table II are presented at the concentration of $C_0 = 1 \times 10^{-4}$M.

Table II. β (%) of Sulfonamides

Substance	Human Plasma	Rabbit plasma	Rabbit Blood
Sulfanilamide	5.2	2.8	11.6
Sulfacetamide	12.6	11.3	10.8
Sulfapyridine	15.5	40.5	32.6
Sulfathiazole	54.5	67.8	57.8
Sulfadiazine	24.5	56.2	43.0
Sulfamerazine	59.2	73.3	59.3
N-Sulfanilyl-3,4-xylamide	96.0	92.7	80.7
Sulfisoxazole	77.7	87.6	72.1
Sulfisomidine	61.5	93.9	79.2
Sulfaphenazole	92.6	96.9	86.4
Sulfamethoxypyridazine	78.0	78.4	65.3
Sulfadimethoxine	95.7	93.6	81.4
Sulfamethoxazole	58.0	59.4	48.4
Sulfamonomethoxine	71.5	80.4	68.1

The solubility in a phosphate buffer at pH 7.4 and in chloroform was measured at 30° (Table I). Solubility in phosphate buffer is presented in terms of unionized molecules.

A good correlation was observed bewteen π_c and π_0 (Table I).

$$\pi_c = 0.551 + 0.989\pi_0 \tag{4}$$
$$\mathrm{n}=13,\ \mathrm{r}=0.832,\ \mathrm{s}=0.388$$

where n, r and s are the number of sulfonamides, correlation coefficient and standard deviation, respectively. The subscripts c and o designate chloroform and octyl alcohol, respectively.

It was interesting that π_c could be satisfactorily expressed in terms of log S_w, solubility of unionized molecules in water, and log S_c, solubility in chloroform.

$$\pi_c = 2.331 + 1.134 \log S_c - 0.770 \log S_w \tag{5}$$
$$\mathrm{n}=14,\ \mathrm{r}=0.973,\ \mathrm{s}=0.223$$

It suggests that the partition coefficient of sulfonamides is little influenced by change of the drug concentration in partition measurements, and a possible association of sulfonamide molecules in organic and aqueous phases can be ignored.

Binding of sulfonamides to protein changed considerably with the change of kind of proteins, but good correlations were obtained in any couple of three β's.

$$\beta_{\text{human-plasma}} = -6.478 + 0.956\,\beta_{\text{rabbit-plasma}} \tag{6}$$
$$\mathrm{n}=14,\ \mathrm{r}=0.919,\ \mathrm{s}=12.255$$

$$\beta_{\text{human-plasma}} = -10.573 + 1.193\,\beta_{\text{rabbit-blood}} \quad (7)$$
$$n=14,\ r=0.941,\ s=10.679$$

$$\beta_{\text{rabbit-plasma}} = -1.898 + 1.207\,\beta_{\text{rabbit-blood}} \quad (8)$$
$$n=14,\ r=0.991,\ s=4.015$$

A Plot of β as a function of log K was found to give a sigmoidal relationship for the human plasma. Thus, one can obtain eq. 9.

$$\log K = 3.811 + 1.110 \log \beta/(100-\beta) \quad (9)$$
$$n=14,\ r=0.999,\ s=0.012$$

Similarly, $\beta_{\text{rabbit-plasma}}$ and $\beta_{\text{rabbit-blood}}$ can be converted into log K for the human plasma employing the respective values of log $\beta/(100-\beta)$ for rabbit.

A least-squares fit of physicochemical parameters to log K was studied.

$$\log K = 2.580 + 0.792\,\pi_c \quad (10)$$
$$n=14,\ r=0.861,\ s=0.453$$

$$\log K = 2.979 + 0.840\,\pi_0 \quad (11)$$
$$n=13,\ r=0.774,\ s=0.411$$

$$\log K = 6.400 - 0.036\,pK_a \quad (12)$$
$$n=14,\ r=0.618,\ s=0.701$$

$$\log K = 1.843 - 0.713 \log S_w \quad (13)$$
$$n=14,\ r=0.757,\ s=0.582$$

$$\log K = 6.716 + 1.069 \log S_c \quad (14)$$
$$n=14,\ r=0.529,\ s=0.756$$

Struller[11] reported that the protein binding of sulfonamides gave an S-shaped relationship with drug's molecular weight. Presently, a good correlation was obtained between log K and molecular weight.

$$\log K = -1.761 + 0.0218\,\text{M.W.} \quad (15)$$
$$n=14,\ r=0.928,\ s=0.334$$

where M.W. is the molecular weight of sulfonamide derivative.

Binding to protein was significantly influenced by the molecular weight of sulfonamide. A similar analysis employing molecular weight was carried out for log $\beta/(100-\beta)$.

$$\log \beta/(100-\beta)_{\text{human-plasma}} = -5.032 + 0.020\,\text{M.W.} \quad (16)$$
$$n=14,\ r=0.930,\ s=0.293$$

$$\log \beta/(100-\beta)_{\text{rabbit-plasma}} = -5.384 + 0.022\,\text{M.W.} \quad (17)$$
$$n=14,\ r=0.966,\ s=0.221$$

$$\log \beta/(100-\beta)_{\text{rabbit-blood}} = -3.315 + 0.013\,\text{M.W.} \quad (18)$$
$$n=14,\ r=0.949,\ s=0.170$$

The correlation of log K to the molecular weight was improved by two-parameter expressions.

$$\log K = -0.739 + 0.0162\,\text{M.W.} + 0.265\,\pi_c \quad (19)$$
$$n=14,\ r=0.942,\ s=0.300$$

10) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).
11) Th. Struller, *Antibiot. Chemother.*, **14**, 179 (1968).

$$\log K = -0.980 + 0.0204\ \text{M.W.} - 0.061\ \text{p}K_a \quad (20)$$
$$n=14,\ r=0.932,\ s=0.324$$

$$\log K = -1.668 + 0.0211\ \text{M.W.} + 0.042 \log S_w \quad (21)$$
$$n=14,\ r=0.928,\ s=0.334$$

$$\log K = 0.198 + 0.0178\ \text{M.W.} + 0.348 \log S_c \quad (22)$$
$$n=14,\ r=0.931,\ s=0.333$$

It is considered that the binding of sulfonamide to protein is subjected to the interaction of *p*-aminobenzene sulfamoyl moiety to the active site of protein but the binding is enhanced by a substituted group of the drug suggesting the importance of a hydrophobic binding of the substituent or the drug molecule itself. A possible steric effect of the substituent on the binding to protein seems to be of secondary importance or remain constant with change of substituents.

Correlation of Partition Coefficient to Bacteriostatic Activity

Hansch, *et al.*[3] found the importance of partition coefficient or hydrophobicity of drug molecules to the analysis of structure–activity relationship of drugs. The correlation of bacteriostatic activity of sulfonamides against *E. coli* at pH 7.4 to π_c or π_0 were analyzed as follows:

$$\log 1/(\text{CR}) = 3.735 + 0.304\ \pi_c \quad (23)$$
$$n=14,\ r=0.507,\ s=0.501$$

$$\log 1/(\text{CR}) = 2.786 + 1.744\ \pi_c - 0.399(\pi_c)^2 \quad (24)$$
$$n=14,\ r=0.721,\ s=0.419,\ \pi_c=2.185$$

$$\log 1/(\text{HD}) = 3.820 + 0.908\ \pi_c \quad (25)$$
$$n=14,\ r=0.776,\ s=0.717$$

$$\log 1/(\text{HD}) = 3.228 + 1.826\ \pi_c - 0.258(\pi_c)^2 \quad (26)$$
$$n=14,\ r=0.812,\ s=0.661,\ \pi_c^\circ=3.538$$

$$\log 1/(\text{CR}) = 4.083 + 0.212\ \pi_0 \quad (27)$$
$$n=13,\ r=0.367,\ s=0.483$$

$$\log 1/(\text{CR}) = 3.496 + 1.350\ \pi_0 - 0.369(\pi_0)^2 \quad (28)$$
$$n=13,\ r=0.853,\ s=0.280,\ \pi_0^\circ=1.829$$

$$\log 1/(\text{HD}) = 4.092 + 1.043\ \pi_0 \quad (29)$$
$$n=13,\ r=0.776,\ s=0.717$$

$$\log 1/(\text{HD}) = 3.300 + 2.434\ \pi_0 - 0.434(\pi_0)^2 \quad (30)$$
$$n=13,\ r=0.867,\ s=0.590,\ \pi_0^\circ=2.804$$

where (CR) and (HD) are the concentrations of total and unionized molecules, respectively, and the subscript o designates the optimal hydrophobicity π of sulfonamides which are active at a minimum (CR) or (HD).

It was revealed that bifunctional equations were appropriate for the correlation between log 1/(CR) of log 1/(HD) and π better than monofunctional equations. The influence of sulfacetamide, which was excepted for the analysis of eqs. 27—30, on r and s was not significant for a correlation analysis with π_c. *n*-Octyl alcohol was recommendable as oil phase of partition measurements for a structure–activity analysis of sulfonamides, because of larger r and smaller s than those of π_c.

Effect of Protein-Binding on Bacterial Activity

Many workers have reported that the binding of drugs to proteins is important for drug activity. Klotz[12] stated that inhibition of a bacterial growth by sulfonamide is due to a

12) I.M. Klotz, *J. Am. Chem. Soc.*, **66**, 459 (1944).

reversible combination between the basic molecule or anion and an enzyme. He derived equations from the law of mass action for the combination. Fujita and Hansch[5] studied the effect of plasma protein binding of N^1-heterocyclic substituted sulfonamides on their bacteriostatic activity. Presently, a structure–activity analysis for 14 sulfonamides was made following the Hansch–Fujita method.

The bacteriostatic activity of sulfonamides against *E. coli* (Table I) was found to closely relate with log K, in a parabolic function.

$$\log 1/(\mathrm{CR}) = -4.341 + 4.106 \log K - 0.466(\log K)^2 \quad (31)$$
$$n=14,\ r=0.921,\ s=0.226,\ \log K^\circ = 4.404$$

where log K° is the optimal value for the bacteriostatic activity.

From eq. 15 and 31, one can derive an equation for the relation between log 1/(CR) and molecular weight.

$$\log 1/(\mathrm{CR}) = -7.132 + 0.0805\ \mathrm{M.W.} - 0.000139(\mathrm{M.W})^2 \quad (32)$$
$$n=14,\ r=0.892,\ s=0.262,\ \mathrm{M.W.}^\circ = 290$$

where M.W.^{0} is the optimal molecular weight of sulfonamides which are active at a minimum (CR).

Two-Parameter Analysis for The Structure-Activity Relationship

Presently, the importance of molecular weight of sulfonamides was well established. Thus a two-parameter analysis was performed with molecular weight used as one of the two parameters.

$$\log 1/(\mathrm{CR}) = -9.089 + 0.0760\ \mathrm{p}K_a + 0.0905\ \mathrm{M.W.} - 0.000155(\mathrm{M.W.})^2 \quad (33)$$
$$n=14,\ r=0.905,\ s=0.247,\ \mathrm{M.W.}^\circ = 292$$

$$\log 1/(\mathrm{CR}) = -7.051 - 0.140\ \pi_c + 0.0784\ \mathrm{M.W.} - 0.000128(\mathrm{M.W.})^2 \quad (34)$$
$$n=14,\ r=0.901,\ s=0.252,\ \mathrm{M.W.}^\circ = 306$$

$$\log 1(\mathrm{CR}) = -7.750 + 0.173 \log K + 0.0844\ \mathrm{M.W.} - 0.000154(\mathrm{M.W.})^2 \quad (35)$$
$$n=14,\ r=0.896,\ s=0.258,\ \mathrm{M.W.}^\circ = 274$$

$$\log 1/(\mathrm{CR}) = -1.420 + 1.085\ \mathrm{p}K_a - 0.0776(\mathrm{p}K_a)^2 + 0.00793\ \mathrm{M.W.} \quad (36)$$
$$n=14,\ r=0.848,\ s=0.308,\ \mathrm{p}K_a^\circ = 6.99$$

$$\log 1/(\mathrm{CR}) = 0.121 + 0.619\ \pi_c - 0.226(\pi_c)^2 + 0.0151\ \mathrm{M.W.} \quad (37)$$
$$n=14,\ r=0.900,\ s=0.253,\ \pi_c^\circ = 1.37$$

$$\log 1/(\mathrm{CR}) = -4.368 + 3.300 \log K - 0.407(\log K)^2 + 0.00857\ \mathrm{M.W.} \quad (38)$$
$$n=14,\ r=0.941,\ s=0.197,\ \log K^\circ = 4.05$$

From the present analysis, it may be concluded that an optimal molecular weight of sulfonamides which show a maximum bacteriostatic activity against *E. coli* is 290—300. Similarly, a structure–activity analysis was made for the results of Bell and Roblin,[13] Seydel[14] and Tsuruoka.[15]

Bell and Roblin — *E. coli* in a synthetic medium at pH 7:

$$\log 1/(\mathrm{CR}) = -6.884 + 0.0825\ \mathrm{M.W.} - 0.000139(\mathrm{M.W.})^2 \quad (39)$$
$$n=39,\ r=0.531,\ s=0.736,\ \mathrm{M.W.}^\circ = 297$$

13) P.H. Bell and R.O. Roblin, *J. Am. Chem. Soc.*, **64**, 2905 (1942).
14) J. Seydel, *Arzneim.-Forsch.*, **16**, 1447 (1966).
15) M. Tsuruoka, *Yakugaku Zasshi*, **71**, 350 (1951).

$$\log 1/(CR) = 0.0877 - 0.2263\ pK_a + 0.0446\ M.W. - 0.000072(M.W.)^2 \quad (40)$$
$$n=39,\ r=0.726,\ s=0.598,\ M.W.^\circ=309$$
$$\log 1/(CR) = 0.3309 + 1.705\ pK_a - 0.1311(pK_a)^2 - 0.000742\ M.W. \quad (41)$$
$$n=39,\ r=0.871,\ s=0.426,\ pK_a^\circ=6.50$$

Seydel — *E. coli* in Sauton's medium at pH 6.9—7.1:

$$\log 1/(CR) = 59.741 - 0.4211\ M.W. - 0.000804(M.W.)^2 \quad (42)$$
$$n=11,\ r=0.662,\ s=0.419,\ M.W.^\circ=262$$
$$\log 1/(CR) = 21.280 - 0.6608\ pK_a - 0.0769\ M.W. - 0.000139(M.W.)^2 \quad (43)$$
$$n=11,\ r=0.975,\ s=0.123,\ M.W.^\circ=277$$
$$\log 1/(CR) = 6.86 + 0.337\ pK_a - 0.0608(pK_a)^2 - 0.000940\ M.W. \quad (44)$$
$$n=11,\ 0.974,\ s=0.121,\ pK_a^\circ=2.77$$

Tsuruoka — *Shigella flexneri* Komagome B III in Koser's medium at pH 6.8—7.0:

$$\log 1/(CR) = -7.439 + 0.0890\ M.W. - 0.000159(M.W.)^2 \quad (45)$$
$$n=13,\ r=0.970,\ s=0.135,\ M.W.^\circ=280$$
$$\log 1/(CR) = -7.950 + 0.0323\ pK_a + 0.0907\ M.W. - 0.000161(M.W.)^2 \quad (46)$$
$$n=13,\ r=0.972,\ s=0.129,\ M.W.^\circ=282$$
$$\log 1/(CR) = -1.140 + 1.877\ pK_a - 0.1511(pK_a)^2 + 0.00120\ M.W. \quad (47)$$
$$n=13,\ r=0.871,\ s=0.273,\ pK_a^\circ=6.21$$

The above analysis also revealed that the bacteriostatic activity of sulfonamides is subjected to their molecular weight to some degree.

Many attempts have been made for the analysis of structure–activity relationship of drugs employing various physicochemical parameters. Generally, physicochemical parameters could not be obtained without experiments. Thus, a prediction of the activity of a new drug would be difficult without any information about physicochemical parameters of the new drug. Presently, the importance of molecular weight of sulfonamides is well established.