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Studies on Absorption of Drugs. VII.[1] Absorption of Isomeric N^1-Heterocyclic Sulfonamides from the Rat Small Intestines and Relations between Physicochemical Property and Absorption of Unionized Sulfonamides[2]

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Absorption of isomeric N^1-heterocyclic sulfonamides was studied from the rat small intestines employing isomeric N-methyl sulfonamides in addition to usual antibacterial sulfonamides. The physicochemical properties of the compounds were studied in reference to their isomeric form in solution. Following Shepherd's method sulfapyridine and sulfathiazole were confirmed to exist as imido form about 84% and 100% respectively in aqueous solution.

N^1-methyl derivatives and ring N-methyl derivatives were adopted as a model of amido form and imido form respectively and the differences of their physicochemical properties and absorption characteristics from undissociated molecules of their parent compounds were discussed.

Also relations of physicochemical properties and absorption were persuaded following Hansch's method. And absorption rate constant of undissociated form of the compounds under study was likely to be related to partitioning to *n*-octanol and molecular weight.

Previously, it was reported[4] that undissociated and dissociated molecules were independently absorbed through the rat intestinal membrane in reference to antibacterial sulfonamides and the following equation was successfully confirmed.

$$A = R_m M + R_i I$$

where A is the amount of a drug absorbed in a certain time, M and I are amounts of the drug initially present in a perfusion solution in an undissociated and a dissociated form respectively, R_m and R_i are the absorption ratios of undissociated and dissociated drugs respectively.

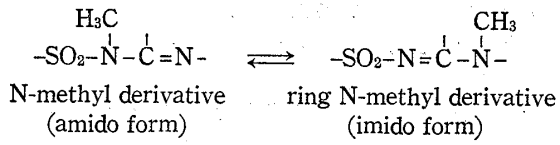
But many antibacterial sulfonamides have an N^1-heterocyclic structure. When nitrogen atom in the N^1-heterocyclic structure is conjugated with the nitrogen atom of the sulfamoyl group through the carbon atom, those sulfonamides show two types of isomeric form,[5,6] *i.e.*, amido form and imido form. They exist in equilibrium in a solution.

The isomers are expected to be different in terms of physicochemical properties and biological availability.

1) Part VI: T. Morishita, N. Yata, A. Kamada, and M. Aoki (the late), *Chem. Pharm. Bull.* (Tokyo), **19**, 1925 (1971).
2) Part of the present report was presented at the 91th Annual Meeting of the Pharmaceutical Society of Japan at Fukuoka, April, 1971.
3) Location: *Toneyama, Toyonaka, Osaka.*
4) T. Morishita, N. Yata, and A. Kamada, *Yakuzaigaku*, **31**, 187 (1971).
5) R.G. Shepherd, A.C. Bratton, and K.C. Blanchard, *J. Am. Chem. Soc.*, **64**, 2532 (1942).
6) T. Uno, K. Machida, K. Hanai, M. Ueda, and S. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **11**, 704 (1963).

The ultraviolet[5] and infrared[6] spectral features of structural isomers have been reported, but other physicochemical and biopharmaceutical studies have been left undone in reference to the isomers.

Presently a comparative study was made of physicochemical properties such as solubility in water or chloroform, oil-water partition, protein binding and of absorption following the perfusion method *in situ* in special reference to the two isomeric forms. Isomeric methyl derivatives were used as model isomeric forms.



Also a quantitative analysis was made on relations of the physicochemical properties of undissociated molecules to absorption with antibacterial sulfonamides and their isomeric methyl derivatives.

Result and Discussion

Parent hydrogen sulfonamides and their two isomeric methyl derivatives were listed in Table I. The methyl derivatives were synthesized by the method reported in the literature.[5,7,8]

Table I. Sulfonamides and Their Methyl Derivatives

Compound	Symbol
Sulfanilamide	S
N^1-Methylsulfanilamide	MS
N^1-Dimethylsulfanilamide	DS
Sulfapyridine	SP
N^1-Methyl-N^1-2-pyridyl-sulfanilamide (N^1-methylsulfapyridine)	MSP
1-Methyl-2-sulfanilamide-1,2-dihydropyridine (ring N-methylsulfapyridine)	ISP
Sulfathiazole	ST
N^1-Methyl-N^1-2-thiazolyl-sulfanilamide (N^1-methylsulfathiazole)	MST
3-Methyl-2-sulfanilamide-2,3-dihydrothiazole (ring N-methylsulfathiazole)	IST
Sulfamethoxazole	SMZ
N^1-Methyl-N^1-(5-methyl-3-isoxazolyl)sulfanilamide (N^1-methylsulfamethoxazole)	MSMZ
2,5-Dimethyl-3-sulfanilamide-2,3-dihydroisoxazole (ring N-methylsulfamethoxazole)	ISMZ

Isomeric Form of Undissociated Sulfonamides in Aqueous Solution

Shepherd, *et al.*[5] have reported the presence of the two isomeric forms in absolute ethanol in reference to N^4-acetylsulfapyridine, sulfapyridine and sulfathiazole. They also determined the amount of the isomer in the solution on an assumption that extinction coefficients of the two isomeric methyl derivatives are approximately the same as those of the corresponding hydrogen compounds. They also reported that N^4-acetylsulfapyridine and sulfathiazole contained about 60% and 90% of imido form respectively.

7) T. Sakai and S. Yamamoto, *Yakugaku Zasshi*, **58**, 683 (1938).
8) J. Walker, *J. Chem. Soc.*, **6**, 86 (1940).

Presently, likewise, a similar determination was made on sulfapyridine and sulfathiazole in acetate buffer in which they are present in an undissociated form.

The ultraviolet spectra of sulfapyridine and its isomeric methyl derivatives are shown in Fig. 1. The peak at 314 nm in Fig. 1 is assigned to the pyridone imine structure since it appeares in ISP and not in MSP. Likewise, the maximum at 259 nm in Fig. 2 shows the thiazolone imine structure.

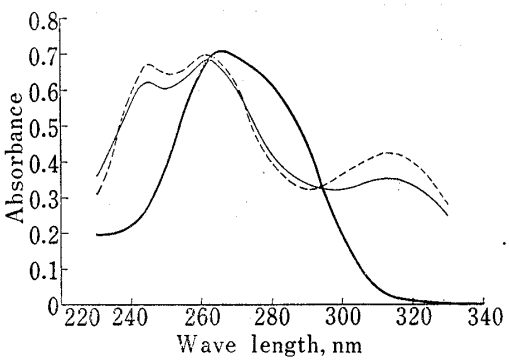


Fig. 1. Absorption Spectra of Sulfapyridine and Its Methyl Derivatives

concentration of each compound: 40 μM
——: SP, ------: ISP, ——: MSP

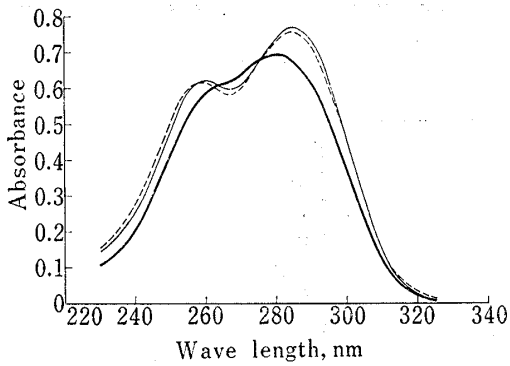


Fig. 2. Absorption Spectra of Sulfathiazole and Its Methyl Derivatives

concentration of each compound: 40 μM
——: ST, ------: IST, ——: MST

The amount of imido form of sulfapyridine and sulfathiazole was calculated from the follwing equation with the absorbance at 314 nm and 259 nm respectively.

$$\text{Imido form } \% = \frac{(\text{abs. of parent compd.}) - (\text{abs. of } N^1\text{–Me deriv.})}{(\text{abs. of ring N–Me deriv.}) - (\text{abs. of } N^1\text{–Me deriv.})} \times 100$$

The amount of imido form of sulfapyridine and sulfathiazole was accounted for about 84% and 100% respectively in the buffer solution in which they are present in an undissociated form (Table II). Sulfamethoxazole did not show a peak specific to imido form but it was considered to be present almost in amido form because its spectra closely resembled those of its N^1-methyl derivative.

Table II. Amount of Imido Form of Sulfapyridine and Sulfathiazole in Buffer

Compound	Absorbance[a)]	% of imido form
	at 314 nm	
SP	0.354	84.2
ISP	0.417	
MSP	0.018	
	at 259 nm	
ST	0.619	105
IST	0.617	
MST	0.577	

a) Each compound was dissolved in a concentration of 40 μM. Sulfapyridine and its derivatives were dissolved in pH 6 phosphate buffer with 0.2 ionic strength and sulfathiazole and its derivatives were dissolved in pH 4 acetate buffer with 0.2 ionic strength.

Low intestinal absorption of sulfapyridine and sulfathiazole compared to sulfamethoxazole may be partly subjected to their isomeric imido form in the solution, but the details will be discussed later.

Physicochemical Properties of Isomeric Form

Solubility in water and chloroform, oil-water partition and protein binding of isomeric methyl derivatives were summarized in Table III and IV in comparison with the data of their parent compounds.

Table III. Solubility of Sulfonamides in Water and Chloroform at 37±1°

Compound	Solubility, mM	
	Water	Chloroform
S	82.1	1.40
MS	94.5	70.0
DS	3.13	95.9
SP	2.09	2.86
MSP	4.74	>2630
ISP	3.69	5.53
ST[a)]	2.56	0.843
MST	1.15	1415
IST	0.569	3.15
SMZ[a)]	2.48	13.1
MSMZ	0.628	1000
ISMZ	7.59	29.7

a) Solubility in water was determined by continuousyl adjusting aqueous solution to pH 4 with 0.05 N HCl or 0.05N NaOH. Solubility of others in water was likewise determined at pH 6.

Table IV. Partition Coefficient between *n*-Octanol and Buffer at 37±1° and Protein Binding to Bovine Serum Albumin at 30±1°

Compound	Partition coefficient	Protein binding (%)
S	0.179	4.8
MS	0.977	10.4
DS	4.64	18.5
SP	1.06	16.1
MSP	20.1	36.2
ISP	0.265	13.1
ST[a)]	0.977	17.2
MST	42.5	70.0
IST	0.861	34.0
SMZ[a)]	7.55	30.3
MSMZ	56.9	73.3
ISMZ	0.333	2.9

a) ST and SMZ are measured at pH 4 (acetate buffer in partition coefficient and phosphate buffer in protein binding). Others are measured at pH 6 (phosphate buffer).

Solubility in chloroform of both isomeric methyl derivatives was higher than that of their parent compounds. But the degree of increase of solubility was less in ring N-methyl derivatives than in N^1-methyl derivatives. On the other hand solubility of sulfonamides in water was affected variously by methylation showing no definite trend.

The oil-water partition coefficient of both isomeric methyl derivatives was higher than that of their parent compounds. Ring N-methyl derivatives were less partitioned into the oil phase than N^1-methyl derivatives. These findings suggest that sulfonamides in an amido form are more partitioned into the oil phase than those in an imido form. A greater par-

titioning into the oil phase of sulfamethoxazole than of sulfapyridine or sulfathiazole seems to support the above suggestion because the later two compounds are mainely in an imido form in aqueous solution as described previously.

Hansch, *et al.*[9] reported significant relations between the binding to protein and the oil-water partition coefficient of the undissociated molecules of homologous compounds. Kakeya, *et al.*[10] also found good relation between the association coefficient in binding to bovine serum albumin of diuretic sulfonamide derivatives and their oil-water partition coefficient.

Presently the binding to bovine serum albumin of antibacterial sulfonamides and their isomeric methyl derivatives was studied in reference to their characteristics in oil-water partitioning following to Hansch's analysis.

Good relation between the bound per cent (β) and the logarithm of partition coefficient between *n*-octyl alcohol and buffer was observed as shown in the following equations (Fig. 3).

$$\beta = 18.55 + 23.92 \log P_0$$
$$n=12,\ r=0.883,\ s=10.914$$

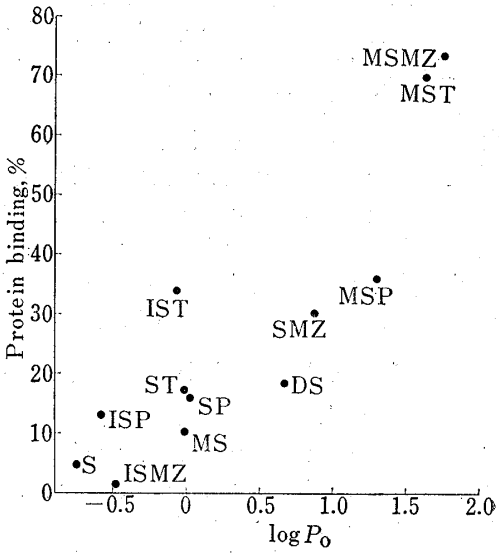


Fig. 3. Relations between Partition Coefficient (*n*-Octanol/Buffer) and Protein Binding of Undissociated Form of Sulfonamides and Their Methyl Derivatives

where n is the number of compounds, r the correlation coefficient and s the standard deviation.

So far as isomeric methyl derivatives were concerned, N^1-methyl derivatives showed an increased binding to bovine serum albumin over their parent hydrogen compounds. But there was no definite trend in the binding of ring N-methyl derivatives.

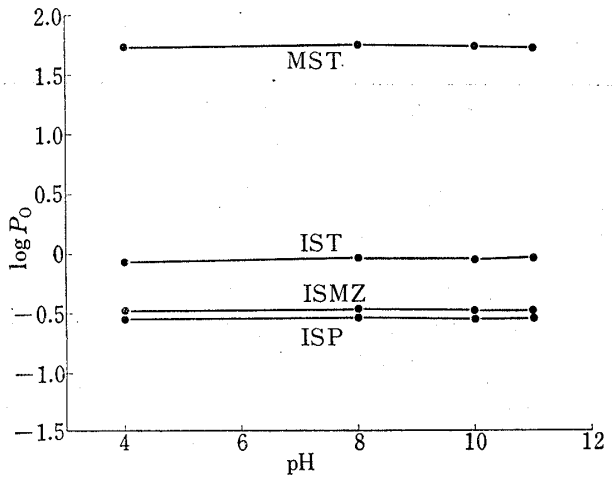


Fig. 4. Effect of Hydrogen Ion Concentration on the Partition Coefficient (*n*-Octanol/Buffer) of Isomeric Methyl Derivatives of Sulfonamides

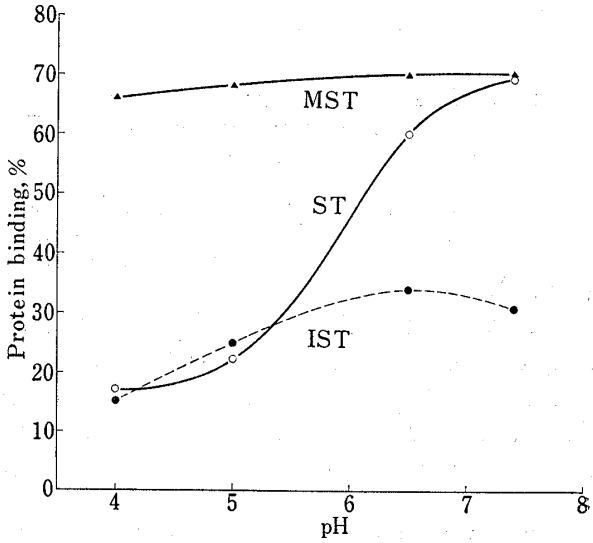


Fig. 5. pH-Profile of Protein Binding of Sulfathiazole and Its Methyl Derivatives

9) C. Hansch and A.R. Steward, *J. Med. Chem.*, **11**, 1 (1967).
10) N. Kakeya, M. Aoki, A. Kamada, and N. Yata, *Chem. Pharm. Bull.* (Tokyo), **17**, 1010 (1969).

Davis.[11] Nakagaki[12] and Yamazaki[13] reported that the binding of antibacterial sulfonamides to protein was increased with a decrease of the hydrogen ion concentration in a medium. Those binding characteristics were subjected to an increase of anionic form of sulfonamides. Both isomeric methyl derivatives are considered to exist as an undissociated form in aqueous solution of pH 4 to 8. The presence of undissociated form alone in this pH range was proved by the finding that the oil-water partition coefficient of methyl derivatives was not affected by pH (Fig. 4). With sulfathiazole and its methyl derivatives, effects of pH on binding to bovine serum albumin were studied (Fig. 5). Binding of the ring N-methyl derivative was considerably increased with an decrease of hydrogen ion concentration unexpectedly. But the reason is left unexplained.

Absorption of Isomeric Form from the Rat Small Intestines

Absorption *in situ* from the rat small intestines of undissociated sulfonamides and their methyl derivatives was summarized in Table V. N^1-Methyl derivatives showed more absorption than their parent compounds. But ring N-methyl sulfapyridine and ring N-methyl sulfamethoxazole were less absorbed than their parent compounds except for ring N-methyl sulfathiazole, which showed an increase. These findings may be explained by characteristics of those compounds in protein binding and oil-water partitioning.

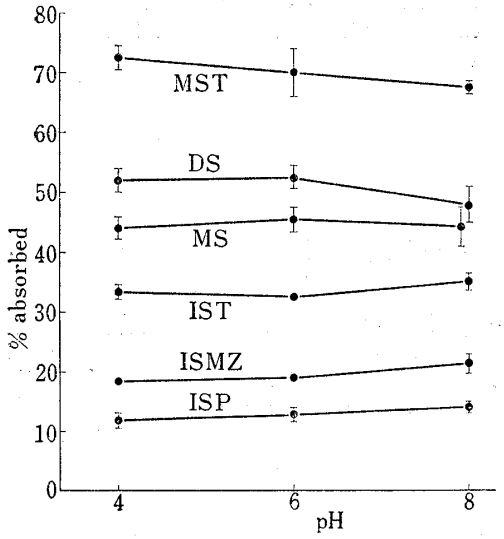


Fig. 6. Effect of Hydrogen Ion Concentration on Absorption of Methyl Derivatives of Sulfonamide

Table V. Absorption of Undissociated Sulfonamides from the Rat Small Intestines

Compound	% Abs. ± S.D.	Rate const., k_a (Hr^{-1})
S	22.7±1.4	0.258
MS	45.3±2.0	0.603
DS	52.3±2.1	0.740
SP	43.0±2.0	0.562
MSP	61.5±1.9	0.955
ISP	12.7±1.0	0.136
ST[a)]	16.8±2.4	0.184
MST	69.9±4.2	1.201
IST	32.7±0.3	0.396
SMZ[a)]	62.1±0.5	0.970
MSMZ	68.6±0.9	1.158
ISMZ	19.0±0.7	0.211

a) ST and SMZ at pH 4. others at pH 6

Absorption of both isomeric methyl derivatives was not influenced by the hydrogen ion concentration in a range of pH 4 to pH 8 (Fig. 6) as well as in oil-water partition characteristics (Fig. 4).

Methyl derivatives of sulfonamides are without antibacterial action[5] and their molecular sizes are larger than their parent sulfonamides, but they always exist as undissociated form in terms of sulfamoyl group. Therefore, for convenience's sake, either of two isomeric methyl derivatives can presently be taken as model molecule of undissociated molecule of the corresponding antibacterial sulfonamides, depending on the main form of undissociated molecules in aqueous solution.

N^1-Methyl derivatives showed more absorption than ring N-methyl derivatives in each sulfonamide. Thus the isomeric features of undissociated molecules of antibacterial sulfon-

11) B.D. Davis, *J. Clin. Invest.*, **22**, 753 (1943).
12) M. Nakagaki, N. Koga, and H. Terada, *Yakugaku Zasshi*, **83**, 586 (1963).
13) M. Yamazaki, M. Aoki, A. Kamada, and N. Yata, *Yakuzaigaku*, **27**, 40 (1967).

amide in aqueous solution will be reflected on their absorption through their physicochemical characteristics as found in case with the difference of absorption between N^1-methyl derivatives and ring N-methyl derivatives. For instance, undissociated molecules of sulfamethoxazole exist mainly as amido form whereas those of sulfapyridine and sulfathiazole exist as imido form. Therefore a higher absorption of undissociated sulfamethoxazole than that of other two compounds will be subjected to the physicochemical characteristics based on their isomeric feature.

Relations of Physicochemical Properties and Absorption

Absorption of sulfonamides has been qualitatively related to the hydrophobic characters of the compounds[14] but few quantitative studies have been so far available.

Hansch, *et al.*[15] have developed for the analysis of structure-biological activity relations. The methods has been followed by many studies including a modification made in reference to relation of structure to biological activity,[16] absorption,[17] metabolism[18] and excretion rate from the living body.[18]

Presently a similar quantitative analysis was made on relations of the physicochemical properties of undissociated molecules to absorption with antibacterial sulfonamides and their isomeric methyl derivatives.

In the previous section methyl derivatives of sulfonamides were dealt with as model of undissociated molecules of the corresponding sulfonamides for convenience's sake to explain a poorer distribution to oil phase and a less absorption of sulfathiazole and sulfapyridine than those of sulfamethoxazole. But the physicochemical properties of methyl derivatives differed from those of undissociated molecules of the corresponding parent sulfonamides. Therefore, it is considered that methyl derivatives may be also independent undissociated compounds having different physicochemical properties. Thus, in the following analysis, they were dealt with independent compounds.

Hansch, *et al.*[15] considered that the probability (A) of a molecule reaching a side of action in a given time interval could be related to the partition coefficient (P). They related probability (A) to log P as follows:

$$A = \mathrm{a} \cdot \exp\left(-\frac{(\log P - \log P_0)^2}{\mathrm{b}}\right) \qquad \text{Eq. 1}$$

where a and b are constants, log P_0 the ideal value for a substituent so that the sum of many free energy changes in the penetration process may be a minimum. Since log P_0 is a constant for a given type of parent molecule in a particular biological system, Eq. 1 becomes to Eq. 2.

$$\log A = -k_1 (\log P)^2 + k_2 (\log P) + k_3 \qquad \text{Eq. 2}$$

where k_1 (>0), k_2 and k_3 are constants. By regarding a probability of penetration to site of action as the probability of penetration from the intestinal tract to the blood stream, relations of absorption to the hydrophobic character of compounds can be likewise shown by Eq. 2. Thus the absorption can be parabolically related to log P. On the other hand Collander[19]

14) B.B. Brodie and C.M. Hogben, *J. Pharm. Pharmacol.*, **9**, 345 (1957); L.S. Schanker, B.B. Brodie, and C.M. Hogben, *J. Pharmacol. Exptl. Therap.*, **123**, 81 (1958); T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 421 (1964).

15) C. Hansch, R.M. Muir, T. Fujita, P.P. Maloney, F. Geiger, and M. Streich, *J. Am. Chem. Soc.*, **85**, 2817 (1963); C. Hansch and T. Fujita, *ibid.*, **86**, 1616 (1964).

16) T. Fujita, *J. Med. Chem.*, **9**, 797 (1966).

17) E.J. Lien, *Drug Intell. Clin. Pharm.*, **4**, 7 (1970).

18) T. Fujita and M. Yamazaki, "Absorption, Metabolism, and Excretion of Drugs," based on a symposium "The Second Symposium on Drug Metabolism and Action," ed. by K. Kakemi, Hirokawa, Tokyo, 1971, p. 283.

19) R. Collander, *Physiol. Plantarum*, **7**, 420 (1954). From C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).

indicated that the rate of movement of organic compounds through cellular material was linear with respect to log P. Then the relation may be shown by Eq. 3.

$$\log k = a(\log P) + b \qquad \text{Eq. 3}$$

In Eq. 2 and 3, P can be substituted by β or solubility in chloroform (S). β is a measure of hydrophobicity, *i. e.*, a degree of protein binding which is defined previously.[20] In the present analysis the distribution coefficient between *n*-octanol and buffer was used as P. With Eq. 2 or 3, constants such as k_1, k_2, k_3, a and b were obtained by the least squares analysis. In the following equations, n is the number of points used in the derivation of the constants by the method of least squares, r the correlation coefficient between the observed absorption rate constant and the calculated value with Eq. 2 or 3, and s the standard deviation of the observed values from the curve or line obtained by the least squares method. P_0 denotes the partition coefficient of the compound between *n*-octanol and buffer which each compound exists in an undissociated form. β denotes the per cent adsorbed on bovine serum albumin. k_a denotes the absorption rate constant which was calculated from the per cent absorbed in one hour.

$$\log k_a = 0.3397 \log P_0 - 0.4370 \qquad \text{Eq. 4}$$
$$n=12,\ r=0.8801,\ s=0.1658$$

$$\log k_a = 0.2226 \log S_c - 0.6332 \qquad \text{Eq. 5}$$
$$n=11,\ r=0.7163,\ s=0.2454$$

$$\log k_a = 0.5576 \log \beta - 1.0230 \qquad \text{Eq. 6}$$
$$n=12,\ r=0.7103,\ s=0.2456$$

In Eq. 5, N^1-methylsulfapyridine was excluded because solubility in chloroform is too high to measure adecuately.

The positive coefficient associated with log P_0, log S_c and log β means that the more lipophilic the compound the more rapidly the compound penetrates through the intestinal membrane. Eq. 4 shows the best dependence of absorption on the partition coefficient between *n*-octanol and buffer. In terms of log P_0, an analysis with Eq. 2 was made and the following equation was derived.

$$\log k_a = -0.0946 (\log P_0)^2 + 0.4396 \log P_0 - 0.3963 \qquad \text{Eq. 7 (Fig. 7)}$$
$$n=12,\ r=0.8950,\ s=0.1643$$

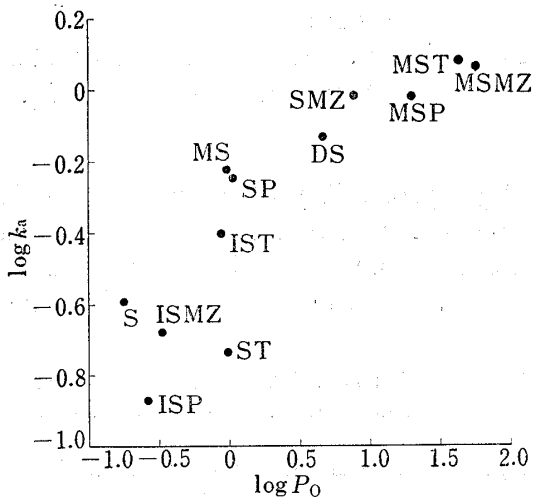


Fig. 7. Logarithmic Relations between the Absorption Rate Constant and the Partition Coefficient (*n*-Octanol/Buffer)

Table VI. Comparison of Observed and Calculated k_a with Eq. 8

Compound	log k_a	
	Obs.	Calcd.
S	−0.589	−0.533
MS	−0.220	−0.300
DS	−0.131	−0.087
SP	−0.250	−0.465
MSP	−0.020	−0.012
ISP	−0.867	−0.728
ST	−0.735	−0.492
MST	0.079	0.100
IST	−0.402	−0.546
SMZ	−0.013	−0.150
MSMZ	0.064	0.152
ISMZ	−0.676	−0.700

20) M. Yamazaki, N. Kakeya, T. Morishita, A. Kamada, and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **18**, 708 (1970).

The addition of the term of $(\log P_0)^2$ to Eq. 4 resulted in a slightly better correlation than that in Eq. 4. But the term of $(\log P_0)^2$ was not significant ($t_0=1.09$). Therefore, the term of $(\log P_0)^2$ can be excluded and a linear dependence of absorption on the partition coefficient can be used in the present study.

One parameter was taken up on an assumption that the absorption process is related to lipophilic character of compounds. But it does not necessarily follow that the lipophilic character related to the absorption process can be represented by only one parameter. So an analysis was made with two parameters of $\log P_0$, $\log M$ (M means the molecular weight of a compound) and $\log \beta$.

$$\log k_a = 0.3811 \log P_0 - 1.4019 \log M + 2.8859 \quad \text{Eq. 8}$$
$$n=12,\ r=0.9214,\ s=0.1428$$

$$\log k_a = 0.3894 \log P_0 - 0.1184 \log \beta - 0.3044 \quad \text{Eq. 9}$$
$$n=12,\ r=0.8836,\ s=0.1723$$

$$\log k_a = 0.6857 \log \beta - 1.6504 \log M + 2.7435 \quad \text{Eq. 10}$$
$$n=12,\ r=0.7727,\ s=0.2335$$

Molecular weight was adopted as a measure of the diffusibility of compounds in tissue fluids. In one parameter analysis, $\log M$ showed no significant correlation ($r=0.1960$). But a linear combination of $\log P_0$ and $\log M$ as shown in Eq. 8 revealed a better correlation than that in Eq. 4. A test indicates that Eq. 8 is significant at the 90% confidence ($t_0=2.11>t_{(9,\ 0.1)}$). A comparison of the observed values and calculated values with Eq. 4 and 8 was summarized in Table VI.

Experimental

Materials——Methyl derivatives of sulfonamides such ring N-methylsulfathiazole, N^1-methylsulfathiazole, ring N-methylsulfapyridine and N^1-methylsulfamethoxazole were synthesized following Shephered, *et al.*[5] Ring N-methylsulfamethoxazole was prepared following Sakai, *et al.*[7] N^1-Monomethyl and N^1-dimethylsulfanilamide were prepared following Walker.[8] Sulfanilamide, sulfapyridine, sulfathiazole and sulfamethoxazole on market were used with no further purification.

Solubility——Solubility in water or chloroform was routinely determined at $37\pm1°$. In case of sulfathiazole and sulfamethoxazole, solubility in water was determined by continuously adjusting aqueous solution to pH 4 with 0.05N HCl or 0.05N NaOH. Solubility of other sulfonamides in water was likewise determined at pH 6. Sulfonamides were routinely analysed by diazotization after proper dilution. Solubility in chloroform was determined as follows: One ml of chloroform solution at dissolution equilibrium was taken into a test tube. After evaporation of chloroform, residues were dissolved in a proper solvent. Sulfanilamide, sulfapyridine, sulfathiazole and sulfamethoxazole were dissolved in 1N NaOH. Methylsulfanilamide, ring N-methylsulfapyridine, ring N-methylsulfathiazole and ring N-methylsulfamethoxazole were dissolved in 1N HCl. Dimethylsulfanilamide, N^1-methylsulfapyridine, N^1-methylsulfathiazole and N^1-methylsulfamethoxazole were dissolved in EtOH. The solutions were properly diluted with deionized water and the concentrations of sulfonamide were routinely determined by diazotization.

Partition Coefficient——A buffer containing a sulfonamide of 200 μM was shaken with an equal volume of *n*-octanol at $37\pm1°$. After an equilibrium was established, the aqueous phase was separated and determined the concentration of sulfonamide, and then the partition coefficient was calculated. The buffer composition used was as follows: pH 4 acetate buffer, pH 6 and 8 phosphate buffer, pH 10 and 11 carbonate buffer. The ionic strength of each buffer was adjusted to 0.4 by adding NaCl.

Protein Binding——An equilibrium dialysis was used following Klotz, *et al.*[21] at $30\pm1°$. Bovine serum albumin (Fraction V, Armour Pharm. Co.) was dissolved in a phosphate buffer (ionic strength of 0.05) in a concentration of 5 w/v%. A Visking cellophane bag containing 5 ml of protein solution was immersed in 10 ml of the same buffer solution containing 150 μM of a sulfonamide, which was taken into a large test tube with a rubber stopper. Then, the tube was horizontally shaken (90 cycle/min) in a water bath kept at $30\pm1°$. After an equilibrium was established (requiring about 7 hours), sulfonamide in the outer solution was analysed. With a bag containing no protein, a similar experiment was made, then the per cent bound was calculated as follows:

21) I.M. Klotz, F.W. Walker, and R.B. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946).

$$\% \text{ bound} = \frac{\text{Concn. in control} - \text{Concn. in sample}}{\text{Concn. in control}} \times 100$$

Absorption——Absorption of sulfonamides from the rat small intestines was determined *in situ* with 0.9% NaCl solution of 200 μM sulfonamide whose pH was maintained by the continuous addition of 0.1N HCl or 0.1N NaOH as previously reported.[1] Three to four rats were used at each experiment. Perfusion was made for one hour and the absorption was calculated from the difference of the amounts of drugs in pre- and postperfusion. The absorption rate constant was calculated as follows:

$$k_a = \ln\left(\frac{100}{100 - \% \text{ Abs.}}\right)$$

This conversion proved to be appropriate from the findings that absorption of sulfonamides used in the present study followed the first order kinetics at least in one hour.