Quantitative Structure-Pharmacokinetic Relationships Derived on Antibacterial Sulfonamides in Rats and Its Comparison to Quantitative Structure-Activity Relationships

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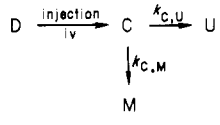
Quantitative structure-pharmacokinetic relationships have been derived for a series of substituted 2-sulfapyridines. Pharmacokinetic parameters, such as elimination rate constant (k_e), clearance (Cl), and protein-binding constant (K_{assoc}), have been determined in rats. The observed variation is statistically significant, explained by changes in the lipophilic (ΔR_m), electronic (pK_a), and steric effects (I, E_s) of the substituents. The obtained correlations are discussed with respect to the previously derived correlations for the antibacterial activity of these compounds. A scale up of the results opens up the possibility of a rational synthesis of highly active sulfonamides with special pharmacokinetic properties because lipophilicity influences strongly the pharmacokinetic properties, whereas no influence on the degree of antibacterial effect is observed. Steric substituent influence is opposite on specific binding to bacterial enzymes and unspecific binding to serum proteins.

Drug action proceeds in several branched or consecutive reaction steps and several equilibria can be involved. Each of the steps can be rate determining. A quantitative structure-activity analysis using in vivo data to explain the observed variation in the parameters of biological activity by changes in certain physicochemical properties may result in a statistically significant equation. We have, however, to realize that our model equations do not give an answer on the true nature of the processes behind it, that they are only mathematical statistical descriptions, and that meaningful interpretation seems difficult. In a simplifying way, we may assume that three main processes are involved in drug action: a pharmaceutical, a pharmacokinetic, and a pharmacodynamic process. The *pharmaceutical* processes, i.e., the amount and rate of dissolution and absorption, are mainly influenced by the drug formulation, besides certain structural properties of the drug molecule. The *pharmacokinetic* processes are determined by the action of the macroorganism on the drug. The drug is distributed (diffusion of the drug molecules into tissues and various body compartments) and bound to membranes and serum proteins, as well as metabolized and excreted. The *pharmacodynamic* process, finally, is the interaction between the specific receptor and those drug molecules which have reached the receptor. If the structural influence on these three main processes is analyzed separately, more meaningful answers may be expected. A scale up of the QSAR results obtained from the various biological systems and processes may improve our understanding of drug action and the rational design of new drugs.

The pharmacokinetic parameters and their variation on structural changes can be followed in animals or humans. In the case of antibacterial drugs as the sulfonamides (SA), a separate analysis of structural dependency of the pharmacodynamic effects—the antibacterial action (minimal inhibitory concentration, MIC)—can be performed outside of the host organism in a bacterial culture or even in isolated enzyme systems.[1-3]

Structure-Pharmacokinetic Relationships. The enormous impact of pharmacokinetic properties on the SA dosage needed for therapy with bacteriostatically acting SA can strikingly be demonstrated by the results of Krüger-Thiemer.[4] In this paper, it has been shown, in spite of the fact that the MIC values ("pharmacodynamic effect") for the SA studied are almost identical, that a large variance in the maintenance dose for a successful therapy is found. This demonstrates the importance of the pharmacokinetic properties in drug design.

Scheme I[a]



[a] $k_{C,U}$ = first-order rate of elimination (unchanged drug); $k_{C,M}$ = first order rate of elimination (metabolized drug); k_e = overall rate of elimination = $k_{C,U} + k_{C,M}$

The following pharmacokinetic parameters might be changed by structural modifications of the drug molecule: (1) rate of absorption (k_a), (2) apparent volume of distribution (V_c), (3) rate (k_m) and type of metabolism, (4) protein binding (K_{assoc}), and (5) rate (k_e) and type of elimination. Previous analyses of quantitative structure-pharmacokinetic relationships on SA are only of limited value for different reasons. The number of studied compounds was small,[5-7] the series was heterogenous[8-11] or the analysis was only of qualitative nature,[12] and/or the pharmacokinetic parameters were taken from various nonhomogenous literature data.

Physicochemical Parameters. The series of sulfapyridines used in this study are listed in Table I, together

(1) G. H. Miller, P. H. Doukas, and J. K. Seydel, *J. Med. Chem.*, **15**, 700 (1972).
(2) L. Bock, G. H. Miller, K.-J. Schaper, and J. K. Seydel, *J. Med. Chem.*, **17**, 23 (1974).
(3) J. K. Seydel, *Top. Infect. Dis.*, **1**, 25 (1974).
(4) E. Krüger-Thiemer, *Chemotherapy*, **10**, 61, 129 (1965/66).
(5) J. K. Seydel and E. Wempe, *Arzneim.-Forsch.*, **21**, 187 (1971).
(6) J. K. Seydel, in "Drug Design", Vol. 1, E. J. Ariens, Ed., Academic Press, 1971, p 343.
(7) J. K. Seydel, G. H. Miller, and P. H. Doukas, in "Medicinal Chemistry", P. Pratesi, Ed., Butterworths, London, 1973, p 139.
(8) T. Fujita, *Adv. Chem. Ser.*, **6**, 80 (1973).
(9) T. Fujita, *J. Med. Chem.*, **15**, 1049 (1972).
(10) H. Aaron and J. J. Boyle, *Cancer J. Physiol. Pharmacol.*, **42**, 809 (1964).
(11) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, **12**, 428 (1964).
(12) J. Rieder, *Arzneim.-Forsch.*, **13**, 81 (1963).

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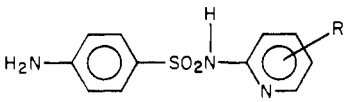
Table I. Physicochemical Data of the Sulfapyridine Derivatives Studied



no.	R	mp, °C	ΔR_m	log k_r	pK_a	α	E_s (ortho)
1	H	188-191	0.00	-0.661	8.54	0.00	0.00
2	5-CH_3	217-219	0.39	-0.413	8.95	-0.17	0.00
3	6-CH_3	118-121	0.07	-0.675	9.20	-0.07	0.00
4	4-CH_3	237-238	0.18	-0.688	9.01	-0.07	0.00
5	4,6-$(CH_3)_2$	235-238	0.44	-0.436	9.54	-0.14	0.00
6	5-Cl	210	1.09	0.072	7.04	0.23	0.00
7	5-Br	194-199	1.11	0.154	6.98	0.23	0.00
8	5-NO_2	225	0.20	-0.279	5.56	0.78	0.00
9	5-$N(CH_3)_2$	208-213	0.68	-0.280	9.21	-0.83	0.00
10	3-CH_3	225-226	0.19	-0.581	8.74	-0.17	-1.24
11	3-Cl	211	0.33	-0.359	6.80	0.23	-0.97
12	3-CN	207-211	-0.36	-0.863	5.25	0.66	-0.51
13	3-OCH_3	214-215	0.12	-0.605	8.07	-0.27	-0.55
14	3-OC_2H_5	195	0.25	-0.305	8.20	-0.24	-0.55
15	3-CH_3, 5-Br	230-234	1.06	0.244	7.19	0.06	-1.24
16	3-CH_3, 5-Cl	258	0.90	0.128	7.26	0.06	-1.24
17	3,5-Cl_2	225-228	0.96	0.262	5.98	0.46	-0.97
18	3,5-Br_2	233-239	1.17	0.377	5.78	0.46	-1.16
19	3-CN, 5-CH_3	204-207	-0.03	-0.546	5.35	0.49	-0.51

Table II. Correlation Matrix for the Physicochemical Parameters

	ΔR_m	pK_a	I	$E_s - 2$	log k
ΔR_m	1.00				
pK_a	-0.11	1.00			
I	-0.06	-0.48	1.00		
$E_s - 2$	-0.21	0.37	-0.89	1.00	
log k	0.95	0.349	0.17	0.40	1.00

with the determined physicochemical parameters (see Experimental Section). The electronic influence of the substituents on the parent molecule can be expressed by σ Hammett or by the pK_a of the acid dissociation constants. The change in lipophilicity is expressed as ΔR_m or by the logarithm of the retardation factor obtained from high-pressure liquid chromatography (LC). The steric influence can be described by Taft E_s values or in this special case by an indicator variable I, having the value 1 for ortho-substituted derivatives and 0 for all other derivatives. The colinearity matrix for the various physicochemical parameters applied (substituent constants) is given in Table II.

Rate of Elimination (k_e or $t_{50\%}$). The elimination rate constants have been derived from blood-level data after an iv injection of about 50 mg of SA/kg to male Sprague–Dawley rats using an open one-compartment body model with first-order elimination (see Scheme I). The injection time was about 1 min (see Experimental Section). The elimination includes metabolism and excretion. The iv application eliminates possible influence of structural modifications on rate (k_a) and amounts of absorption. It is known that SA are eliminated totally by renal clearance (unmetabolized and metabolized fractions). This behavior simplifies data analysis. The concentrations of unchanged and total drug have been followed as a function of time after injection, and $k_e = k_{C,U} + k_{C,M}$ or $k_{C,U}$ has been determined by computerized fitting to the exponential curves plotting C_U, the concentration of unmetabolized drug, or C_{total} after hydrolysis of the N^4 metabolites against t. An example is given in Figure 1.

Stepwise regression analysis to explain the observed variation in k_e was performed separately for para-, meta-, and ortho-substituted or ortho,para-substituted 2-sulfapyridines (Tables III and IV). The most significant equations obtained were eq 1 and 2:

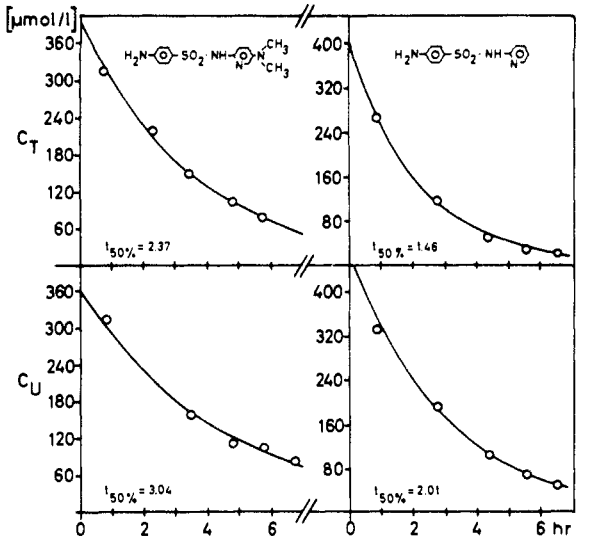


Figure 1. Determination of the overall elimination rate constant, k_e (C_T), and elimination rate constant, $k_{C,U}$ (C_U), for unmetabolized SA in rats. k_e values are listed for all SA studied in Table III and $k_{C,U}$ values are in Table IV.

para- and meta-substituted derivatives

$$\log k_e = -(0.58 \pm 0.1)\Delta R_m + (0.31 \pm 0.03)pK_a - 3.03 \quad (1)$$
(5.5) (9.7)

$$n = 9,\ r = 0.98,\ s = 0.12,\ F = 88.9$$

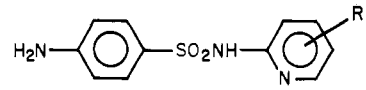
ortho- and ortho,para-substituted derivatives

$$\log k_e = -(0.83 \pm 0.12)\Delta R_m + (0.16 \pm 0.05)pK_a - 1.53 \quad (2)$$
(6.98) (3.26)

$$n = 10,\ r = 0.94,\ s = 0.19,\ F = 29.6$$

In the equations, n gives the number of derivatives studied, r is the regression coefficient, s the standard error of estimate, the number in parentheses is the Student's t test, and F is the decision statistics of the F test of significance. For both series of compounds, the rate of elimination, k_e,

Table III. Pharmacokinetic Parameters of Various Substituted 2-Sulfapyridines



no.	R	overall rate of elimination, h^{-1}: k_e (obsd)	range obsd	k_e (calcd)[b]	biol half-life, h: $t_{50\%}$	total clearance, mL min^{-1}: Cl_T (obsd)	Cl_T (calcd)[c]	app vol of distribut, mL: V_c	affinity constant for protein binding, L/mol: K_{assoc} (obsd)	K_{assoc} (calcd): eq 7 and 30	eq 32
1	H	0.47	(0.437-0.503)	0.49	1.47	1.79	1.5	229	4 592	5 825	5 774
2	5-CH_3	0.294	(0.269-0.319)	0.30	2.36	1.15	0.95	234	9 183	7 242	7 276
3	6-CH_3	0.369	(0.386-0.352)	0.61	1.88	1.04	1.87	168.6	5 129	4 949	4 940
4	4-CH_3	0.581	(0.575-0.588)	0.46	1.19	1.38	1.41	142.8	6 606	5 856	5 848
5	4,6-$(CH_3)_2$	0.577	(0.489-0.666)	0.38	1.20	1.76	1.18	183	5 495	6 206	6 266
6	5-Cl	0.032	(0.028-0.042)	0.032	21.65	0.099	0.11	185.5	25 703	26 996	27 220
7	5-Br	0.028	(0.025-0.031)	0.030	24.75	0.116	0.10	247.1	a	27 624	28 314
8	5-NO_2	0.042	(0.038-0.046)	0.071	16.50	0.135	0.23	192.8	20 417	19 656	19 138
9	5-$N(CH_3)_2$	0.333	(0.292-0.374)	0.21	2.08	0.62	0.66	111.2	8 511	8 732	8 857
10	3-CH_3	0.538	(0.537-0.538)	0.71	1.29	1.17	1.20	131	1 622	2 342	2 317
11	3-Cl	0.08	(0.065-0.096)	0.19	8.66	0.20	0.35	147.5	13 441	5 108	5 113
12	3-CN	0.48	(0.377-0.583)	0.30	1.44	0.77	0.51	96.7	1 820	4 377	4 453
13	3-OCH_3	0.544	(0.545-0.542)	0.57	1.27	0.91	0.96	100.3	2 399	2 730	2 715
14	3-OC_2H_5	0.496	(0.428-0.558)	0.48	1.4	0.81	0.82	98.3	4 169	2 979	2 957
15	3-CH_3, 5-Br	0.085	(0.062-0.108)	0.067	8.15	0.20	0.12	142.6	5 888	9 118	9 043
16	3-CH_3, 5-Cl	0.085	(0.075-0.095)	0.092	8.15	0.195	0.17	137.9	a	7 619	7 563
17	3,5-Cl	0.032	(0.03-0.034)	0.042	21.65	0.05	0.08	99.2	12 706	12 314	12 319
18	3,5-Br	0.032	(0.03-0.034)	0.026	21.65	0.048	0.05	90.3	13 800	16 136	16 132
19	3-CN, 5-CH_3	0.318	(0.314-0.322)	0.017	2.18	0.47	0.31	89.7	10 965	5 826	5 906

[a] K_{assoc} not determined (concentration range studied too small). [b] Calculated by eq 4. [c] Calculated by eq 7.

Table IV. Metabolic and Renal Clearance for Some Sulfapyridines

no.	renal clearance, mL·min^{-1}: Cl_R (obsd)	Cl_R (calcd)[a]	metabolic clearance, mL·min^{-1}: Cl_M (obsd)	Cl_M (calcd)[b]	unchanged drug $k_{C,U}$, h^{-1}
1	1.33	1.53	0.46	0.43	0.35
5	1.28	0.97	0.48	0.39	0.42
6	0.06	0.06	0.04	0.05	0.02
7	0.04	0.06	0.07	0.04	0.01
9	0.43	0.48	0.19	0.24	0.23
17	0.05	0.05	0.03	0.03	0.03
18	0.03	0.03	0.02	0.02	0.02

[a] Calculated by eq 21. [b] Calculated by eq 24.

decreases with increasing lipophilicity and increasing acidity of SA.

A regression analysis on the combined data set shows a drop in significance of the correlation (eq 3).

para-, meta-, and ortho-substituted derivatives

$$\log k_e = \underset{(7.1)}{-(0.79 \pm 0.11)\Delta R_m} + \underset{(5.18)}{(0.18 \pm 0.0355)pK_a} - 1.78 \quad (3)$$

$$n = 19,\ r = 0.92,\ s = 0.22,\ F = 43.5$$

$$\log k_e = \underset{(8.21)}{-(0.77 \pm 0.09)\Delta R_m} + \underset{(6.7)}{(0.23 \pm 0.03)pK_a} + \underset{(2.74)}{(0.26 \pm 0.096)I} - 2.8 \quad (4)$$

$$n = 19,\ r = 0.95,\ s = 0.18,\ F = 43.4$$

If, however, the ortho substitution is accounted for by an indicator variable I (1 for ortho-substituted, 0 for meta- or para-substituted derivatives) or by the steric substituent constant E_s, for ortho substitution a slight increase in significance is observed (eq 4). A differentiation between I and E_s is not possible because of the significant intercorrelation between the two variables ($r = 0.89$) (Table II). Throughout this study the indicator variable I has, therefore, been used to describe the additional "ortho effect".

If log k, the retardation factor from high-pressure LC, is used instead of ΔR_m to describe the lipophilic influence of the substituent, a similar regression equation is obtained (eq 5).

$$\log k_e = \underset{(8.62)}{-(1.0 \pm 0.11)} \log k_r + \underset{(5.14)}{(0.18 \pm 0.03)pK_a} + \underset{(3.49)}{(0.32 \pm 0.09)I} - 2.55 \quad (5)$$

$$n = 19,\ r = 0.95,\ s = 0.17,\ F = 47$$

More than 90% in the variation of the overall elimination rate k_e can be explained by the lipophilic and electronic influences of the substituents. An additional "steric" influence of ortho substituents can possibly be considered (eq 4 and 5). The decrease in k_e with increasing lipophilicity and in ionization can be explained by either one of the following factors or a combination: (1) increase in protein binding (see structure–protein binding relation) which decreases the glomerularly filtered amount; (2) change in apparent volume of distribution V_c; (3) increase in nonionic tubular absorption due to an increase in the lipophilicity; (4) decrease in rate of metabolism ($k_e = k_{C,U} + k_{C,M}$).

Clearance. Clearance is closely related to both the rate of elimination and the volume of distribution. The apparent volume of distribution of the central compartment, V_c, is a proportionality factor relating the administered dose, D_0, to the extrapolated apparent initial plasma concentration, c_p^0, at $t = 0$ (eq 6). The clearance of a drug

$$V_c = \frac{D_0}{c_p^0} \quad (6)$$

is its rate of elimination determined with respect to the total plasma volume which is cleared in a certain time

Table V. Clearance of SA at Different Urinary pH Values

compounds	no. of determinats n	pK_a	R_m	renal clearance, $mL \cdot g^{-1} \cdot min^{-1}$	inulin clearance, $mL \cdot g^{-1} \cdot min^{-1}$
			2-sulfapyridine		
urine pH normal	12	8.54	0.00	1.39 ± 0.04	1.4 ± 0.1
urine pH acidic	16			0.66 ± 0.01[a]	1.2 ± 0.1
urine pH alkaline	10			1.24 ± 0.05[a]	1.3 ± 0.1
			3-chlorosulfapyridine		
urine pH normal	12	6.80	0.33	0.08 ± 0.005	1.3 ± 0.1
urine pH acidic	8			0.05 ± 0.003[a]	1.4 ± 0.1
urine pH alkaline	8			0.12 ± 0.01[a]	1.2 ± 0.1
			5-chlorosulfapyridine		
urine pH normal	12	7.04	1.09	0.03 ± 0.001	1.4 ± 0.12
urine pH acidic	10			0.015 ± 0.0006[a]	1.4 ± 0.13
urine pH alkaline	12			0.05 ± 0.001[a]	1.4 ± 0.12

[a] Significance of deviation from clearance value under normal pH conditions is $p < 0.05$.

interval (min^{-1}). Total clearance, Cl_T, from the central compartment can be described by eq 7. The total

$$Cl_T = k_e V_c \quad (7)$$

clearance is the sum of the renal clearance, Cl_R, and the extrarenal clearance which may be assumed to be proportional to the metabolic clearance Cl_M in the case of SA because the metabolites are also renally excreted. Metabolic clearance may be determined by eq 8, however, only

$$Cl_M = Cl_T - Cl_R \quad (8)$$

under the assumption that the relative volume of distribution for sulfapyridines and their main metabolites, N^4-acetylsulfapyridines, are comparable.

For some of the SA studied, the concentration in the central blood compartment has therefore been determined before and after hydrolysis of the N^4-acetyl metabolites, and $k_{C,U}$ was determined. k_m was obtained from the difference $k_m = k_e - k_{C,U}$, and Cl_R and Cl_M were calculated. The data are summarized in Table IV.

It is obvious that k_e and $k_{C,U}$ are colinear, pointing to the fact that the rate of elimination ($k_{C,U}$) and the rate of metabolism (k_m) do depend on the variation of the same physicochemical properties, especially on lipophilicity. This is quantitatively described in eq 9a and 9b. The

$$\log k_{C,U} = -(1.41 \pm 0.31)\Delta R_m - 0.09 \quad (9a)$$
$$(4.53)$$
$$n = 7, r = 0.89, s = 0.33, F = 20.5$$
$$\log k_{C,U} = (0.39 \pm 0.1)pK_a - 4.15 \quad (9b)$$
$$(3.93)$$
$$n = 7, r = 0.87, s = 0.37, F = 15.5$$

regression equation for k_e for these seven compounds are eq 10a and 10b.

$$\log k_e = -(1.26 \pm 0.27)\Delta R_m - 0.03 \quad (10a)$$
$$(4.74)$$
$$n = 7, r = 0.90, s = 0.28, F = 22.5$$
$$\log k_e = (0.37 \pm 0.06)pK_a - 3.82 \quad (10b)$$
$$(5.74)$$
$$n = 7, r = 0.93, s = 0.24, F = 33.6$$

A functional relationship between elimination, volume of distribution, and protein binding has already been postulated by Martin,[13] and mathematical models have been developed.[14] Therefore, attempts have been made to explain quantitatively the variation of Cl_T, Cl_R, and Cl_M by variance in physicochemical properties of the drug molecules.

For this purpose, a detailed analysis has been performed for three 2-sulfapyridines (compounds 1, 6, and 11, Table I). Drug solutions have been infused with a rate of 1.9 mL/h, and concentrations have been determined simultaneously as a function of time in plasma (c_p) and urine (c_u) of rats. Clearance has been calculated using eq 11,

$$Cl = \frac{c_u V}{c_p} \quad (11)$$

where c_u is the concentration in urine and c_p in plasma and V is the urine volume per minute.

In addition, *p*-aminohippuric acid (PAH) and inulin clearance were determined. Inulin clearance is considered to describe glomerular filtration rate (GFR) and is 1–1.5 mL/min per kidney in healthy rats. Normal PAH clearance corresponds to renal plasma flow (RPF) and is 3 mL/min per gram of kidney, i.e., 6 mL/min per kidney. A pH dependency of the clearance would give strong evidence for the contribution of tubular reabsorption processes on clearance.

The pH of urine was varied by application of NH_4Cl (pH 5–6) and by $NaHCO_3$ (pH 7.8). The observed clearance rates are summarized in Table V, together with the values observed under normal pH conditions (pH 6.8). For 2-sulfapyridine ($pK_a = 8.54$), a significant change in Cl_R is observed under acidic conditions. The drug is nearly totally un-ionized under these conditions and the tubular reabsorption process increased. In general, however, the clearance of 2-sulfapyridine (1.79 mL min^{-1}) can be explained by glomerular filtration; clearance and inulin clearance are comparable (Table V). For the more lipophilic drugs (3-chloro- and 5-chloro-2-sulfapyridine, R_m = 0.33 and 1.09), renal clearance is much smaller than inulin clearance, due to higher protein binding (see Table III) and a significant contribution of reabsorption. The clearance rate is almost doubled if the fraction ionized is increased by alkaline pH. This is not in contradiction to eq 4, where k_e decreases with increasing ionization. Equation 11 describes the elimination rate from the plasma, and the protein binding is increasing with increase in ionization, thus decreasing glomerular filtration. These results are in agreement with earlier observations on the pH dependency of renal elimination rates of SA.[15]

(13) B. K. Martin, *Nature (London)*, 207, 274 (1965).

(14) E. Krüger-Thiemer, *Arzneim.-Forsch.*, **16**, 1431 (1966).

(15) L. Dettli and P. Spring, *Farmaco*, **23**, 795 (1968).

The agreement between clearance data obtained from plasma data and from urinary data is obvious (Tables III and V). A stepwise regression analysis to explain the variation in clearance values ($Cl_T = k_e V_c$) due to influence of substituents resulted in eq 12–17.

meta and para-substituted 2-sulfapyridines

$$\log Cl_T = -(0.88 \pm 0.34)\Delta R_m + 0.15 \quad (12)$$
$$(2.57)$$

$$n = 9,\ r = 0.7,\ s = 0.4,\ F = 6.59$$

$$\log Cl_T = (0.34 \pm 0.07)pK_a - 3.02 \quad (13)$$
$$(4.69)$$

$$n = 9,\ r = 0.87,\ s = 0.28,\ F = 22$$

$$\log Cl_T = -(0.59 \pm 0.12)\Delta R_m + (0.28 \pm 0.037)pK_a - 2.28 \quad (14)$$
$$(4.86) \qquad (7.56)$$

$$n = 9,\ r = 0.97,\ s = 0.13,\ F = 58.3$$

ortho- and ortho,para-substituted 2-sulfapyridines

$$\log Cl_T = -(0.79 \pm 0.19)\Delta R_m - 0.16 \quad (15)$$
$$(4.11)$$

$$n = 10,\ r = 0.82,\ s = 0.3,\ F = 16.9$$

$$\log Cl_T = (0.19 \pm 0.12)pK_a - 1.86 \quad (16)$$
$$(1.56)$$

$$n = 10,\ r = 0.48,\ s = 0.46,\ F = 2.43$$

$$\log Cl_T = -(0.79 \pm 0.1)\Delta R_m + (0.19 \pm 0.04)pK_a - 1.51 \quad (17)$$
$$(7.57) \qquad (4.46)$$

$$n = 10,\ r = 0.96,\ s = 0.16,\ F = 38.4$$

In both series the total clearance is decreasing with increasing lipophilicity and increasing with increasing basicity. The influence of pK_a, however, is smaller in the ortho-substituted series. A combination of both series results also in a regression equation of high significance (eq 18). The decrease in Cl_T with increasing lipophilicity

$$\log Cl_T = -(0.74 \pm 0.08)\Delta R_m + (0.22 \pm 0.02)pK_a - 1.73 \quad (18)$$
$$(9.44) \qquad (8.89)$$

$$n = 19,\ r = 0.96,\ s = 0.15,\ F = 95$$

can be explained by the increase in protein binding, thus decreasing V_c (Table III). At the same time, the rate of reabsorption increases for more lipophilic compounds. The dependency of pK_a is not so easily understood. Cl_T increases with increase in pK_a, i.e., increase of the un-ionized fraction which should increase the tubular reabsorption and therefore decreases Cl_T.

The similarity of eq 1–3 and 14, 17, and 18 is obvious, as k_e is the decisive factor and V_c varies only in range of 50 to 240 mL. With respect to the total extra- and intracellular volume of a rat, this variation is, however, a large one.

We have to point again to the fact that these pharmacokinetic parameters are connected in a rather complex way. As already discussed, the protein binding increases with increasing ionization, and an increase in protein binding decreases V_c and, therefore, Cl_T. Another argument is that Cl_T is the sum of Cl_R and Cl_M, and the pK_a dependency of Cl_T may arise from a change in Cl_M as a function of pK_a.

The different weight of pK_a on the variation of Cl_T in the two series studied (eq 14 and 17) may arise from the different ranges of pK_a values covered compared to the normal urinary pH in rats (6).

From separate analysis of renal (Cl_R) and metabolic (Cl_M) clearance for seven SA where the total and unmetabolized drug concentration has been determined in plasma, eq 19–24 were obtained.

$$\log Cl_R = (-1.57 \pm 0.26)\Delta R_m + 0.43 \quad (19)$$
$$(6.08)$$

$$n = 7,\ r = 0.94,\ s = 0.27,\ F = 37.0$$

$$\log Cl_R = (0.44 \pm 0.08)pK_a - 4.1 \quad (20)$$
$$(5.02)$$

$$n = 7,\ r = 0.91,\ s = 0.32,\ F = 25.3$$

$$\log Cl_R = (-0.97 \pm 0.15)\Delta R_m + (0.23 \pm 0.04)pK_a - 1.78 \quad (21)$$
$$(6.41) \qquad (5.34)$$

$$n = 7,\ r = 0.99,\ s = 0.10,\ F = 134.7$$

$$\log Cl_M = (-1.17 \pm 0.26)\Delta R_m - 0.108 \quad (22)$$
$$(4.53)$$

$$n = 7,\ r = 0.89,\ s = 0.27,\ F = 20.5$$

$$\log Cl_M = (0.35 \pm 0.06)pK_a - 3.66 \quad (23)$$
$$(5.90)$$

$$n = 7,\ r = 0.93,\ s = 0.22,\ F = 34.8$$

$$\log Cl_M = (-0.59 \pm 0.18)\Delta R_m + (0.22 \pm 0.05)pK_a - 2.25 \quad (24)$$
$$(3.24) \qquad (4.32)$$

$$n = 7,\ r = 0.98,\ s = 0.13,\ F = 55.8$$

A similar dependency of Cl_R and Cl_M on pK_a and R_m is obtained. The two parameter equations have to be considered with caution, as the number of compounds is too small and as already stated a significant intercorrelation between R_m and pK_a is observed for this limited series of compounds ($r = 0.74$). In separate studies (unpublished data), it was demonstrated that the rate of acetylation for these SA using rat liver preparations is, indeed, decreasing with increasing lipophilicity and decreasing pK_a, as shown for Cl_M.

Protein Binding. Protein binding has an important influence on various pharmacokinetic parameters and on the therapeutic dose necessary. The bound fraction of the drug is not available for antibacterial action of the drugs.[16,17] Previous analysis of the structural dependency of protein binding of SA have mostly dealt with homologous series, where only changes in lipophilicity occured. The variance was explained by log P or π.[5,7,18,19] The answer to the question whether hydrophobic or ionic forces[9,20] or a combination of these forces[21] are responsible for protein binding of SA depends on the series of SA studied. For the ionic binding to serum albumin, an appreciable degree of ionization at the N^1 atom is necessary.[5,12,22] In the series studied this precondition is fulfilled. The association constant, K_{assoc}, of the serum–drug

(16) A. N. Anton, *J. Pharmacol.*, **10**, 425 (1966).
(17) E. Krüger-Thiemer, E. Wempe, and M. Töpfer, *Arzneim.-Forsch.*, **15**, 1309 (1965).
(18) W. Scholtan, *Arzneim.-Forsch.*, **18**, 505 (1968).
(19) C. Hansch and C. W. Deutsch, *J. Med. Chem.*, **8**, 705 (1965).
(20) T. Fujita and C. Hansch, *J. Med. Chem.*, **10**, 991 (1967).
(21) A. Ågren, R. Elofsson, and S. O. Nilsson, *Acta Pharm. Suec.*, **8**, 475 (1971).
(22) R. Elofsson, S. O. Nilsson, and B. Kluczykowska, *Acta Pharm. Suec.*, **8**, 465 (1971).

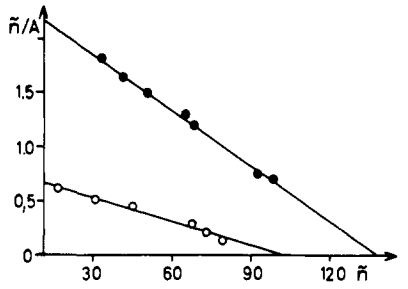


Figure 2. "Scatchard plot" for the estimation of the association constant, K_{assoc}: (●) 3,5-dibromosulfapyridine; (○) 4-methylsulfapyridine. K_{assoc} values are summarized in Table IV. $\bar{n} = (A_{tot} - A)/P$ is the average number of drug molecules bound to an albumin molecule, A is the concentration of unbound drug, A_{tot} is the total drug concentration, and P is the protein concentration.

complex has been studied in fresh rat serum using an ultracentrifugation technique. The concentration of free drug as a function of total drug concentration was determined, and K_{assoc} was calculated from the slope of a Scatchard plot (see Experimental Section). An example is given in Figure 2 for a strongly bound SA (3,5-dibromo-2-sulfapyridine) and a weakly bound SA (4-methyl-2-sulfapyridine).

The derived K_{assoc} values are summarized in Table III, column 8. The obtained correlations are described by eq 25–30.

meta- and para-substituted 2-sulfapyridines

$$\log K_{assoc} = \underset{(2.06)}{(0.479 \pm 0.24)}\Delta R_m - 2.25 \quad (25)$$

$$n = 8,\ r = 0.64,\ s = 0.23,\ F = 4.3$$

$$\log K_{assoc} = -\underset{(3.58)}{(0.16 \pm 0.03)}pK_a - 0.65 \quad (26)$$

$$n = 8,\ r = 0.82,\ s = 0.17,\ F = 12.8$$

$$\log K_{assoc} = \underset{(4.4)}{(0.40 \pm 0.08)}\Delta R_m - \underset{(6.1)}{(0.15 \pm 0.02)}pK_a - 0.95 \quad (27)$$

$$n = 8,\ r = 0.97,\ s = 0.07,\ F = 46.1$$

ortho- and ortho,para-substituted derivatives

$$\log K_{assoc} = \underset{(1.94)}{(0.43 \pm 0.22)}\Delta R_m - 2.43 \quad (28)$$

$$n = 9,\ r = 0.59,\ s = 0.33,\ F = 3.8$$

$$\log K_{assoc} = -\underset{(1.54)}{(0.149 \pm 0.096)}pK_a - 1.25 \quad (29)$$

$$n = 9,\ r = 0.50,\ s = 0.35,\ F = 2.4$$

$$\log K_{assoc} = \underset{(2.21)}{(0.42 \pm 0.19)}\Delta R_m - \underset{(1.87)}{(0.14 \pm 0.08)}pK_a - 1.45 \quad (30)$$

$$n = 9,\ r = 0.77,\ s = 0.28,\ F = 4.3$$

In both series the degree of protein binding depends on lipophilicity and pK_a. Protein binding is increasing with increase in lipophilicity and ionization. The result is in agreement with earlier publications,[19,21] where it has been postulated that ionic forces are involved in protein binding of SA.

A combination of both series in a regression analysis results in a decrease of significance (eq 31).

$$\log K_{assoc} = \underset{(2.64)}{(0.42 \pm 0.16)}\Delta R_m - \underset{(1.45)}{(0.07 \pm 0.05)}pK_a - 1.82 \quad (31)$$

$$n = 17,\ r = 0.64,\ s = 0.28,\ F = 4.9$$

The deviation of ortho-substituted SA from regression line $\log K_{assoc}/R_m$ or π has already been described[5,7] (see negative sign for E_s or I, respectively) and has been used for a rational design of new SA with low protein binding. It is interesting to note that there is an opposite effect of ortho substitution on the affinity of SA to the specific receptor, the dihydropteroic acid synthetase[1] (eq 34). If ortho substitution is considered by an indicator variable, eq 32 with increasing significance is obtained.

$$\log K_{assoc} = \underset{(3.7)}{(0.42 \pm 0.11)}\Delta R_m - \underset{(3.8)}{(0.15 \pm 0.04)}pK_a - \underset{(3.9)}{(0.44 \pm 0.11)}I - 0.95 \quad (32)$$

$$n = 17,\ r = 0.85,\ s = 0.19,\ F = 11.7$$

Scale Up of QSAR Information Obtained in Biological Systems of Different Biological Complexity. Previous work from our laboratory on QSAR of SA using *E. coli* cultures as test organism has resulted in a highly significant correlation between minimum inhibitory concentration (MIC)[6,23-25] or growth kinetic data (k_{50})[24,26] and the electronic (ppm, pK_a) and steric nature of the substituents in a series of sulfapyridines and sulfabenzenes (eq 33). The same series of SA has also been tested in

$$\log \text{MIC} = \underset{(9.49)}{0.68}pK_a - \underset{(1.32)}{0.11}I - 4.80 \quad (33)$$

$$n = 18,\ r = 0.93,\ s = 0.17,\ F = 45$$

a cell-free folate-synthesizing enzyme system. The variance in the concentration needed to cause 50% inhibition of folate synthesis (I_{50}) could be explained by eq 34.[1-3,7] The

$$\log I_{50} = \underset{(14.92)}{0.46}pK_a - \underset{(8.97)}{0.32}I - 2.46 \quad (34)$$

$$n = 18,\ r = 0.97,\ s = 0.08,\ F = 136.41$$

coefficients of the independent variables in eq 33 and 34 are almost identical. This result favors the interpretation that the rate-determining step in both systems is the same. It excludes cell-wall permeation as being rate determining. This argument is valid as long as the pK_a of the SA is ≥ 6.5.[1] For compounds with a $pK_a \leq 6.5$, a cut off in the linear regression is observed for the whole cell system (MIC); i.e., there is no further increase in antibacterial activity (MIC) with $pK_a \leq 6.5$. Changes in lipophilicity had no influence on the antibacterial activity. The variance is explained by pK_a; i.e., increase in pK_a and ortho substitution favor the affinity to the target enzyme and, therefore, augment the antibacterial activity. If these equations are compared with the equations from the regression analysis of the pharmacokinetic parameters discussed above, important differences are observed.

All of the partly interrelated pharmacokinetic parameters, such as association constant for protein binding (K_{assoc}; eq 32), clearance (eq 18), and elimination rate (eq 4), depend mainly on the lipophilic properties and only to a small degree on pK_a; the influence of I (E_s) in the case of protein binding is inverse (eq 32 and 34). It is therefore possible to design SA with high antibacterial activity according to eq 33 and 34 and with special pharmacokinetic properties (eq 35), for instance, a long or short biological

(23) J. K. Seydel, *Mol. Pharmacol.*, 2, 259 (1966).
(24) J. K. Seydel, *J. Med. Chem.*, 14, 724 (1971).
(25) A. Cammarata, R. C. Allen, J. K. Seydel, and E. Wempe, *J. Pharm. Sci.*, 59, 1496 (1970).
(26) E. R. Garrett, J. B. Mielck, J. K. Seydel, and H. J. Kessler, *J. Med. Chem.*, 12, 740 (1969).

half-life and smaller protein binding (eq 31). In rats, eq 35 was obtained ($t_{50\%} = \ln 2/k_e$; Table III). A comparison

$$\log t_{50\%} = \underset{(8.22)}{(0.77 \pm 0.09)R_m} - \underset{(6.71)}{(0.23 \pm 0.03)pK_a} - \underset{(2.76)}{(0.27 \pm 0.09)I} + 2.12 \quad (35)$$

$$n = 19,\ r = 0.95,\ s = 0.18,\ F = 43.4$$

to the situation in humans would be of great interest. However, only 4 of these 19 compounds have yet been studied. Only one independent variable can therefore be used in regression analysis. Equation 36 is obtained.

$$\log t_{50\%} = \underset{(3.52)}{(0.75 \pm 0.21)R_m} + 1.13 \quad (36)$$

$$n = 4,\ r = 0.93,\ s = 0.20,\ F = 12$$

The same four derivatives tested in rats:

$$\log t_{50\%} = \underset{(5.27)}{(1.0 \pm 0.19)R_m} + 0.37 \quad (37)$$

$$n = 4,\ r = 0.97,\ s = 0.18,\ F = 28$$

With all caution necessary it seems reasonable to assume that the biological half-life of SA in humans is comparably dependent on the lipophilicity of the SA as in rats.

Experimental Section

Sulfonamides. N^1-Phenyl- and N^1-pyridylsulfonamides have been prepared by standard methods which have been described elsewhere.[24]

Acid Dissociation Constants (pK_a). The pK_a values of the acid NH group of the sulfapyridines were determined spectrophotometrically according to the method outlined by Albert and Sergeant[27] and Yoshioka et al.[28] and are listed in Table I.

R_m Values. R_m values were determined according to Biagi et al.[29] by reversed-phase thin-layer chromatography on paraffin oil coated (5% paraffin/hexane) silica gel GF_{254} plates. The compounds were dissolved in acetone and 5 μL of the solution was spotted on the plates. An aqueous mobile phase was used (0.1 M phosphate buffer, pH 5.0) in various proportions with acetone. The experimental R_{m_0} values were calculated by regression analysis as the R_m with buffer alone being the mobile phase. The R_m values are corrected for ionization if necessary. ΔR_m is defined as $\Delta R_m = R_{m_x} - R_{m_H}$, where R_{m_H} is the R_m value of the reference compound and R_{m_x} the value of the substituted derivative.[30]

Determination of Retardation Factor, Log k. Retardation time for the studied SA have been derived from high-performance liquid chromatography (LC) using a Waters 6000 (Waters Associates, Milford, Mass.) with an U6K universal injector and a Model 440 UV absorbance detector at 280 nm. Commercial columns, LiChrosorb RP_{18}, 250 × 4.6 mm (Knauer prepacked, Knauer, West Berlin) were used. The eluent was a mixture of 0.1 M phosphate buffer, pH 5, and methanol in the ratio 45:55 (v/v). The flow rate of eluent was 2 mL/min, and the column inlet pressure 200 bars. Computations were carried out with a Spectra Physics Minigrator. The relative retention time was calculated according to Chen[30] as

$$k = (t_R - t_0)/t_0 \quad (38)$$

where t_R and t_0 are the retention time of the substance under investigation and the hold-up time of an unretained tracer (in this case sulfanilic acid), respectively. The logarithm of the retardation factor, log k, has the same physicochemical meaning as R_m. The colinearity for the studied SA derivatives is described by eq 39.

$$\Delta R_m = (0.78 \pm 0.06) \log k - 0.65 \quad (39)$$

$$n = 19,\ r = 0.95,\ s = 0.12,\ F = 163$$

Determination of Rate of Elimination, k_e. A sodium salt solution of SA was infused into the tail vein of male Sprague–Dawley rats within 1 min. The dosage administered was 50 mg/kg. After injection, blood samples were taken in certain time intervals from the tail artery. After centrifugation, the plasma was immediately transferred to storage tubes, and total concentration and concentration of the unmetabolized SA were determined before and after hydrolysis of SA according to a modified Bratton and Marshall procedure; k_e was determined in accordance with a one-compartment body model using a computerized curve-fitting program of Heinzel (Dr. Karl Thomae GmbH, Biberach, West Germany) (see Table III and Figure 1). The apparent volume of distribution, V_c, was calculated from the known doses and c_0 (ordinate intercept of the plot): $V_c = D/c_0$.

Statistical Analysis. Statistical analysis was performed with a Wang Computer 700 B or 2000 VP. For all regression equations, the number of data points or number of compounds used is n, the correlation coefficient is r, and the standard deviation is s; F and Student's t test are given. In case of multiple regression analysis, the intercorrelation has been checked. The resulting matrix is given in Table II.

Binding Constants, K_{assoc}, to Rat Serum Proteins. To freshly prepared rat serum, various amounts of 2-sulfapyridines have been added. The concentration range studied was 100–600 μmol/L in dilution steps of 100 μmol/L. These solutions were transferred to centrifuge tubes containing 300 μL each and centrifuged for 10 h at 45 000 rpm at 37 °C in a Beckman ultracentrifuge L 50 (g = 150 000).[31] From the uppermost protein-free layer, 50 μL was taken and the concentration of unbound drug determined photometrically using the method of Bratton and Marshall.[32] Controls have been centrifuged to correct for sedimentation of unbound drug. Total drug concentration in rat serum had been determined before centrifugation. The bound fraction was obtained from the difference ($C_{bound} = C_{total} - C_{free}$). The association constant was calculated according to Scatchard[33] (Table III). The variance in K_{assoc} for repeated determinations was 15%.

(27) A. Albert and A. B. Sergeant, "Ionization Constants of Acids and Bases", Methuen and Co., London, 1962.
(28) M. Yoshioka, K. Hamamoto, and T. Kubota, *Nippon Kagaku Zasshi*, **84**, 412 (1963).
(29) G. L. Biagi, A. N. Barbarao, M. C. Guerra, and M. F. Gamba, *J. Chromatogr.*, **44**, 195 (1965).
(30) B.-K. Chen and C. Horváth, *J. Chromatogr.*, **171**, 15 (1979).
(31) H. Büttner and F. Portwich, *Arzneim.-Forsch.*, **11**, 1133 (1961).
(32) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 544 (1939).
(33) G. Scatchard, *Ann. N.Y. Acad. Sci.*, **51**, 660 (1949).