[Chem. Pharm. Bull. 18(4) 702—707 (1970)]

UDC 615.31.015.11 : 615.281.015.11

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

Masaru Yamazaki, Nobuharu Kakeya, Takashi Morishita, Akira Kamada and Masaru Aoki

Faculty of Pharmaceutical Sciences, Osaka University[3]

(Received October 8, 1969)

Based on the experimental observations that the concentrations of ionized and unionized molecules of sulfonamides at the bacteriostatis are linearly related to the drug's pK_a, equations of the relation of pK_a to bacteriostatic activity of sulfonamides against *Escherichia coli* are proposed. The optimal acid dissociation constant, pK_a^0, of sulfonamides which are active at a minimum concentration is calculated. pK_a^0 and the minimum bacteriostatic concentration are found to change with changes of pH of culture media.

Bell and Roblin[4] reported that the bacteriostatic activity of sulfonamides was a parabolic type of function of their acid strength and that the maximum potency of the activity was obtained, in a solution of pH 7, with drugs whose pK_a's were approximately 6.7. This correlation between the acidic dissociation constant and the bacteriostatic activity was interpreted in terms of the negative character of the SO_2 group in sulfonamide derivatives. Many attempts have been made to evaluate the inductive effects of substituted groups on the SO_2NH group of N[1]-substituted sulfonamides.[5-7]

In spite of these efforts and others,[8-10] the structure–activity study on sulfonamides still leave much to be pursued.

Recently, we have studied the bacteriostatic activity of sulfonamides against *Escherichia coli*[11] at various pH's and the acid dissociation constant of the drugs.[12] The present report concerns with an analysis of the change of pH of culture media on the bacteriostatic activity.

Results and Discussion

Bacteriostatic Activity against *Escherichia coli*

To provide consistent and reproducible results, an interpolating estimation was adopted for the determination of bacteriostatic activity of sulfonamides against *E. coli*. The method was reported in the previous paper.[11] But, for the convenience's sake, it is described again.

1) Part IX: N. Kakeya, N. Yata, A. Kamada and M. Aoki, *Chem. Pharm. Bull.* (Tokyo), **18**, 191 (1970).
2) Part of this work was reported at the 18th Kinki Branch Meeting of the Pharmaceutical Society of Japan at Kyoto, November 1968.
3) Location: *Toneyama, Toyonaka, Osaka.*

4) P.H. Bell and R.O. Roblin, *J. Am. Chem. Soc.*, **64**, 2905 (1942).
5) J. Seydel, *Arzneim.-Forsch.*, **16**, 1447 (1966).
6) A. Cammarata and R.C. Allen, *J. Pharm. Sci.*, **56**, 640 (1967).
7) S. Yamabe, *Chemotherapy*, **9**, 220 (1961).
8) P.B. Cowles, *Yale J. Biol. Med.*, **14**, 599 (1942).
9) D.J.N. Hossack, *Ann. Inst. Pasteur*, **103**, 866 (1962).
10) J.K. Seydel, *Mol. Pharmacol.*, **2**, 259 (1966).
11) M. Yamazaki, M. Aoki and A. Kamada, *Oyo Yakuri*, **2**, 210 (1968).
12) M. Yamazaki, M. Aoki, A. Kamada and N. Yata, *Yakuzaigaku*, **27**, 37, 40 (1967).

E. coli K 12 was cultivated in a broth at 37° for 24 hr. The growth rate was determined spectrophotometrically at 620 mμ. When an exponential growth rate was established, *E. coli* was separated by centrifugation and washed with saline solution. Suspension of *E. coli* was prepared in a saline solution to give an absorbancy of 0.31±0.01 at 620 mμ in a 1 cm path length cell. Culture media used for the bacteriostatic activity measurements was 1% phosphate buffer solution (0.1 of ionic strength) of vitamin free casamino acids (Difco Laboratories). Sulfonamides were dissolved in a culture medium at various concentrations. Ten ml of each solution was taken into glass tubes. The tubes were sterilized at 120° for 20 min. One drop (12.7±0.1 μl) of the *E. coli* suspension was inoculated into each solution. The growth of bacteria was measured spectrophotometrically at 620 mμ in a 1 cm path length cell after being shaken at 37° for 24 hr. Inhibitory concentration; (CR), was expressed in terms of a concentration with which absorbancy reached 0.3 at the end of an incubation period. Plots of absorbancy of the incubated suspensions against concentrations of sulfamethoxazole at pH 7.4 are presented in Fig. 1 where the CR-value of the drug is indicated by an arrow. The bacteriostatic activities of 14 sulfonamides

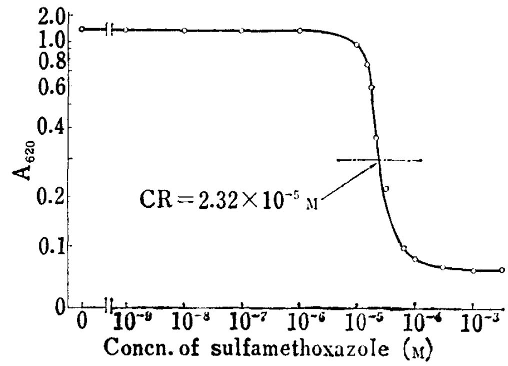

Fig. 1. Bacteriostatic Activity of Sulfamethoxazole at pH 7.4

CR of sulfonamides was presented as 50% inhibitory concentration which was graphically obtained from half of the difference of transmittance bewteen the two extreme plateau values. CR for sulfamethoxazole was 2.3×10^{-5}M. The 50% transmittance is about 0.3 of absorbancy.

Table I. Acid Dissociation Constant and Bacteriostatic Activity against *E. coli* at pH 7.4

No.	Substance	pK_a	log 1/(CR)
1	sulfanilamide	10.45	2.824
2	sulfacetamide	5.40	3.248
3	sulfapyridine	8.37	4.102
4	sulfathiazole	7.10	4.597
5	sulfadiazine	6.15	4.337
6	sulfamerazine	6.93	4.469
7	N-sulfanilyl-3,4-xylamide	4.72	4.055
8	sulfisoxazole	4.62	4.564
9	sulfisomidine	7.38	4.502
10	sulfaphenazole	5.91	4.450
11	sulfamethoxypyridazine	7.05	4.570
12	sulfadimethoxine	6.05	4.706
13	sulfamethoxazole	5.81	4.634
14	sulfamonomethoxine	6.03	4.943

Table II. Bacteriostatic Activity against *E. coli* at pH 6—7.4

Substance	Bacteriostatic activity			
	pH 6.0	pH 6.5	pH 7.0	pH 7.4
Sulfanilamide	2.00	2.10	2.45	2.82
Sulfapyridine	3.15	3.40	3.76	4.10
N-Sulfanilyl-3,4-xylamide	2.90	3.05	3.70	4.06
Sulfisomidine	3.55	3.85	4.25	4.50

at pH 7.4 and of 4 sulfonamides at pH's 7.4, 7, 6.5 and 6 are presented in Tables I and II, respectively.

Concentrations of Ionized and Unionized Molecules at CR

Bell and Roblin[4] found that the bacteriostatic activity of sulfonamides against *E. coli* in a synthetic medium at pH 7 increased with an increase of dissociation constant. But, further increase of the dissociation constant beyond 7 resulted in a decrease in the activity. It is reported that the maximum activity was observed in sulfonamides which ionized 50 per cent at the pH of culture media.

As to active form of sulfonamide for the bacteriostatis, Cowles[8] suggested that the negative ion is responsible for the bacteriostatic action, but is hardly penetrated to the site of action in the cell. Thus, there should be an optimal dissociation constant at which the balance of ionization is favorable for the penetration and interaction with the active site. Klotz[13] assumed that the bacteriostatic action is performed when a given amount of sulfonamide-enzyme complex is formed in the bacterial cell. He derived an equation of nonparabolic function of pK_a for the correlation of bacteriostatic activity.

Presently, the bacteriostatic activity of sulfonamides was separately considered in terms of ionized and unionized forms in the culture medium.

Concentrations of ionized and unionized molecules in the bacteriostatis are obtained employing acid dissociation constant, K_a, and hydrogen ion concentration, (H^+), of the culture medium.

$$(D^-)=\frac{(CR)}{1+(H^+)/K_a} \quad (1)$$

$$(HD)=\frac{(CR)}{1+K_a/(H^+)} \quad (2)$$

where (CR), (D^-), and (HD) are the concentrations of total, ionized and unionized molecules, respectively.

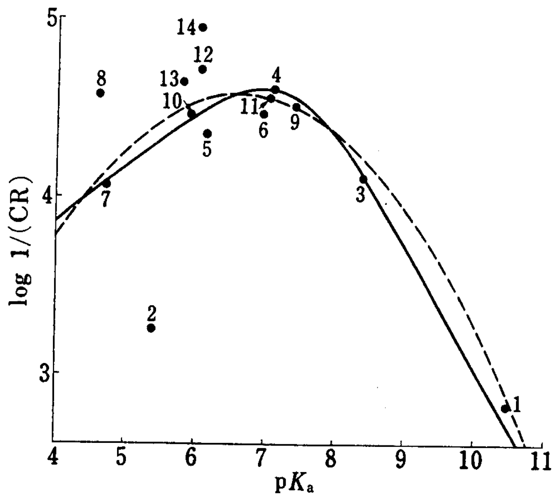

Fig. 2. Relationship between Bacteriostatic Activity and pK_a

Substituent numbers correspond to those in Table I.
——: $\log 1/(CR)=2.72-0.709\ pK_a-\log(3.98\times10^{-8}+K_a)$
n=14, r=0.781, s=0.366
------: $\log 1/(CR)=-0.490+1.520\ pK_a-0.115\ (pK_a)^2$
n=14, r=0.766, s=0.374

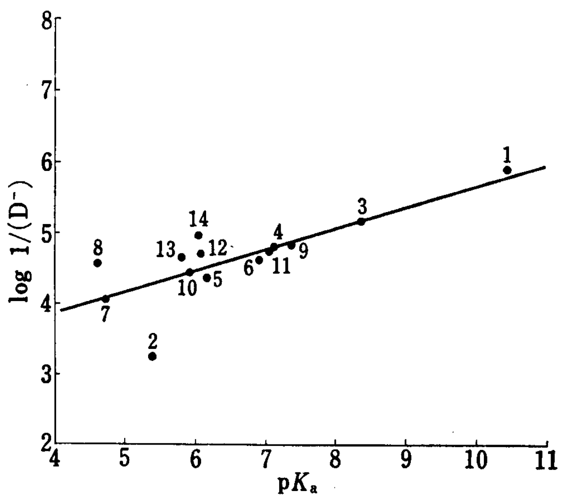

Fig. 3. Relationship between Bacteriostatic Activity of Ionized Molecule and pK_a

Substituent numbers correspond to those in Table I.
$\log 1/(D^-)=2.73+0.291\ pK_a$
n=14, r=0.769, s=0.368

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

The bacteriostatic activities in terms of log 1/(CR), log 1/(D^-) and log 1/(HD) are plotted against pK_a (Fig. 2, 3 and 4). It was interesting that, unlike a nonlinear relation of log 1/(CR) with pK_a (Fig. 2), log 1/(D^-) and log 1/(HD) were linearly correlated with pK_a (eq. 3 and 4; Fig. 3 and 4).

$$\log 1/(D^-)_{7.4}=2.73+0.291\,pK_a \quad (3)$$
$$n=14,\ r=0.769,\ s=0.368$$

$$\log 1/(HD)_{7.4}=10.12-0.709\,pK_a \quad (4)$$
$$n=14,\ r=0.947,\ s=0.367$$

where n, r and s are the number of sulfonamides, correlation coefficient and standard deviation, respectively. The subscript 7.4 designates the pH of the culture medium. Similarly, eq. 5 and 6 were derived excepting sulfacetamide.

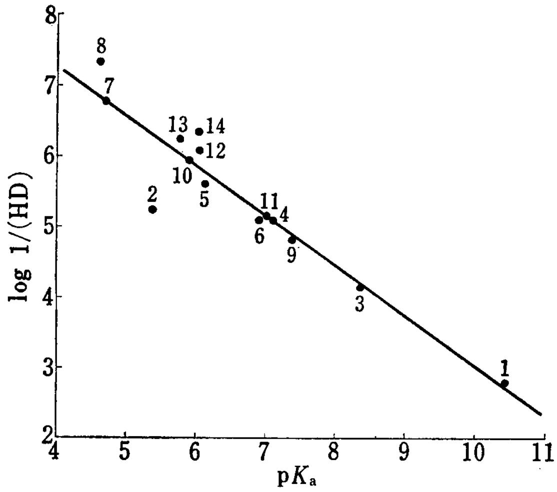

Fig. 4. Relationship between Bacteriostatic Activity of Unionized Molecule and pK_a

Substituent numbers correspond to those in Table I.
$\log 1/(HD)=10.12-0.709\,pK_a$
$n=14,\ r=0.947,\ s=0.367$

$$\log 1/(D^-)_{7.4}=3.11+0.245\,pK_a \quad (5)$$
$$n=13,\ r=0.876,\ s=0.208$$

$$\log 1/(HD)_{7.4}=10.51-0.755\,pK_a \quad (6)$$
$$n=13,\ r=0.985,\ s=0.207$$

Optimal pK_a of Sulfonamides for the Bacteriostatic Activity

From eq. 2, one can obtain eq. 7.

$$\log 1/(CR)=\log 1/(HD)-pH-\log\{(H^+)+K_a\} \quad (7)$$

From eq. 4 and 6, log 1/(HD) can be expressed in terms of pK_a. Thus, eq. 7 becomes

$$\log 1/(CR)=a\,pK_a+b-pH-\log\{(H^+)+K_a\} \quad (8)$$

where a and b are constants. In the present study at pH 7.4, eq. 8 becomes

$$\log 1/(CR)_{7.4}=2.72-0.709\,pK_a-\log(3.98\times10^{-8}+K_a) \quad (9)$$
$$n=14,\ r=0.781,\ s=0.366,\ pK_a^\circ=7.01$$

A solid line in Fig. 2 is what obtained from eq. 9.

A parabolic relation of log 1/(CR)—pK_a at pH 7.4 is expressed by eq. 10 (a dotted line in Fig. 2).

$$\log 1/(CR)_{7.4}=-0.490+1.520\,pK_a-0.115(pK_a)^2 \quad (10)$$
$$n=14,\ r=0.766,\ s=0.374,\ pK_a^\circ=6.61$$

It may be considered that eq. 9 was more appropriate for the activity-structure analysis than eq. 10 because of larger r and smaller s of eq. 9.

The optimal acid dissociation constant, pK_a°, of sulfonamides which are active at a minimum (CR) can be obtained by differentiating eq. 8 and 10 and setting $\partial\{\log 1/(CR)\}/\partial(K_a)=0$ and $\partial\{\log 1/(CR)\}/\partial(pK_a)=0$, respectively. Thus, one can obtain eq. 11 and 12.

$$pK_a^\circ=-\log(-a)+\log(1+a)+pH \quad (11)$$

$$pK_a^\circ=1.52/0.230 \quad (12)$$

In the present study, $pK_a°$ in a culture medium at pH 7.4 was 7.01 and 6.61 from eq. 11 and 12, respectively.

Klotz[13] theoretically derived a similar equation to eq. 8 assuming that the inhibitory action of sulfonamides to bacterial growth was a reversible interaction between drugs and protein.

The effects of ionization in culture media on the bacteriostatic activity were adopted for the analysis of the data of Bell and Roblin[4], Seydel[5] and Tsuruoka.[14]

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

$$\log 1/(D^-)_7 = 3.65 + 0.339\, pK_a \qquad (13)$$
$$n=39,\ r=0.871,\ s=0.393$$

$$\log 1/(HD)_7 = 10.65 - 0.661\, pK_a \qquad (14)$$
$$n=39,\ r=0.961,\ s=0.393$$

$$\log 1/(CR)_7 = 3.65 - 0.661\, pK_a - \log(1\times10^{-7} + K_a) \qquad (15)$$
$$n=39,\ r=0.893,\ s=0.393,\ pK_a°=6.71$$

$$\log 1/(CR)_7 = 0.261 + 1.67\, pK_a - 0.128(pK_a)^2 \qquad (16)$$
$$n=39,\ r=0.871,\ s=0.427,\ pK_a°=6.52$$

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

$$\log 1/(D^-)_7 = 4.55 + 0.239\, pK_a \qquad (17)$$
$$n=11,\ r=0.863,\ s=0.114$$

$$\log 1/(HD)_7 = 11.55 - 0.761\, pK_a \qquad (18)$$
$$n=11,\ r=0.985,\ s=0.114$$

$$\log 1/(CR)_7 = 4.55 - 0.761\, pK_a - \log(1\times10^{-7} + K_a) \qquad (19)$$
$$n=11,\ r=0.979,\ s=0.112,\ pK_a°=6.50$$

$$\log 1/(CR)_7 = 1.00 + 1.69\, pK_a - 0.142(pK_a)^2 \qquad (20)$$
$$n=11,\ r=0.972,\ s=0.129,\ pK_a°=5.95$$

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

$$\log 1/(D^-)_7 = 3.43 + 0.257\, pK_a \qquad (21)$$
$$n=13,\ r=0.804,\ s=0.285$$

$$\log 1/(HD)_7 = 10.43 - 0.743\, pK_a \qquad (22)$$
$$n=13,\ r=0.969,\ s=0.285$$

$$\log 1/(CR)_7 = 3.43 - 0.743\, pK_a - \log(1\times10^{-7} + K_a) \qquad (23)$$
$$n=13,\ r=0.864,\ s=0.285,\ pK_a°=6.54$$

$$\log 1/(CR)_7 = -0.701 + 1.86\, pK_a - 0.151(pK_a)^2 \qquad (24)$$
$$n=13,\ r=0.868,\ s=0.276,\ pK_a°=6.16$$

Similarly, Fujita and Hansch[15] reported that logarithmic plots of the activity of ionized and unionized molecules of sulfonamides against the dissociation constants are shown to be expressed by two straight lines, and the intersection of the two lines corresponds sometimes to the maximal activity for a series of sulfonamides. Presently, the intersection was found to correspond to the pH of culture media.

14) M. Tsuruoka, *Yakugaku Zasshi*, **71**, 350 (1951).
15) T. Fujita and C. Hansch, *J. Med. Chem.*, **10**, 991 (1967).

Effect of pH on the Bacteriostatic Activity

The bacteriostatic activity of sulfonamides decreased considerably with a decrease of pH of culture media.[11] The bacteriostatic activity of unionized molecules at pH's 7.4, 7, 6.5 and 6 was obtained from the results of sulfanilamide, sulfapyridine, N-sulfanilyl-3,4-xylamide and sulfisomidine (Table II). The constants a and b in eq. 8 were found to change linearly with changes of pH.

$$a = 0.986 - 0.227\ \mathrm{pH} \tag{25}$$

$$b = 10.56 + 2.769\ \mathrm{pH} \tag{26}$$

By inserting eq. 25 and 26 into eq. 8, one can obtain eq. 27

$$\log 1/(\mathrm{CR}) = -10.56 + 0.986\ \mathrm{p}K_a + 1.769\ \mathrm{pH} - 0.227(\mathrm{pH})(\mathrm{p}K_a) - \log\{(\mathrm{H}^+) + K_a\} \tag{27}$$

The correlation coefficient and standard deviation of eq. 27 for 4 sulfonamides and at 4 pH-conditions were 0.983 and 0.145, respectively. Here, it must be admitted that eq. 27 might be slightly changed since a small number of sulfonamides was used for the derivation of the equation. The bacteriostatic activity at pH 7.4 can be obtained by eq. 28.

$$\log 1/(\mathrm{CR})_{7.4} = 2.531 - 0.694\ \mathrm{p}K_a - \log(3.98 \times 10^{-8} + K_a) \tag{28}$$

$$n = 14,\ r = 0.775,\ s = 0.379,\ \mathrm{p}K_a{}^\circ = 7.04$$

The difference between eq. 9 and eq. 28 is not significant and it will be decreased by increasing the number of sulfonamides for the analysis.

$\mathrm{p}K_a{}^\circ$ at pH 6 to 7.4 can be estimated employing eq. 29 which is derived from eq. 11 and 25.

$$\mathrm{p}K_a{}^\circ = \mathrm{pH} - \log(0.227\ \mathrm{pH} - 0.986) + \log(-0.227\ \mathrm{pH} + 1.986) \tag{29}$$

The values of $\mathrm{p}K_a{}^\circ$ at pH 7.4, 7, 6.5 and 6 were 7.04, 6.82, 6.52 and 6.22, and the differences between pH and $\mathrm{p}K_a{}^\circ$ were 0.36, 0.18, -0.02 and -0.22, respectively, while Klotz[13] derived an equation for the difference between pH and $\mathrm{p}K_a$ and considered that the difference was constant irrespective of pH of culture media.

Maximum log 1/(CR)'s which were expected at pH 7.4, 7, 6.5, and 6 were 4.53, 4.31, 3.96, and 3.56, respectively. Bell and Roblin[4] reported a similar decrease in the maximum log 1/(CR) with a decrease of pH of culture media.

The present analysis, where the ionization of sulfonamides in culture media is emphasized, is able to describe the bacteriostatic activity of sulfonamides in terms of $\mathrm{p}K_a$ and the change of the activity with the change of pH of culture media. This analysis should help to predicting the bacteriostatic activity of sulfonamides and designing new sulfonamide drugs.