Anal.—Calcd. for $C_6H_8N_6O_{18}$: N, 18.59. Found: N, 18.50.

Hexabenzoate—Prepared by treating the substance with benzoyl chloride in presence of pyridine, m.p. 148° (corrected).

Benzaldehyde Condensation Product—The condensation (4) of D-mannitol (100 mg.) with benzaldehyde (200 mg.) was more satisfactorily achieved using phosphorus pentoxide (100 mg.) or concentrated sulfuric acid (2 drops), than with concentrated HCl. After shaking the mixture for 15 min., the product was washed with dilute sodium carbonate solution, water, and ether and crystallized from ethanol. The condensation product melted at 220° (corrected) (*cf.* m.p. 223–224°) (4).

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Observations Concerning the Correlation of *In Vitro* Sulfonamide Activity with pKa and the Hammett Values

By ARTHUR CAMMARATA and RICHARD C. ALLEN*

Linear correlations of the *in vitro* bacteriostatic activity of sulfonamides with pKa provide little evidence in support of there being a definite pKa which a sulfonamide must possess in order to exhibit maximum activity.

Bell and Roblin (1) first reported a correlation between the *in vitro* bacteriostatic activity of a series of sulfonamides and their pKa values. They summarized their proposition in the statement: "the more negative the SO_2 group of a sulfonamide type compound, the greater the bacteriostatic activity of the compound . . . . .; the correlation between acid dissociation (pKa) and the activity is shown to be directly associated with the negative character of the SO_2 group." Seydel, Krüger-Thiemer, and Wempe (2, 3) obtained the polarizability of the SO_2 group by infrared spectrophotometric measurement of the S-O force constant. They reported that the SO_2 polarizability had no relation to the *in vitro* bacteriostatic activity of the sulfonamides, and the hypothesis of Bell and Roblin is thus cast into doubt. However, the experimental data which led to the Bell and Roblin hypothesis—namely, the correlation of activity with pKa is widely accepted (4, 5). Implications which are derived from the data of Bell and Roblin, and which are considered to be valid are: (*a*) a sulfonamide must possess a pKa which lies within a definite pKa region (6.0–7.5) in order to exhibit maximum activity; (*b*) sulfonamides having a pKa which lies to either side of this region exhibit decreasing activities.

Seydel (6) has recently shown that the *in vitro* bacteriostatic activities for an homologous series of N^1-phenylsulfanilamides are linearly correlated with their respective Hammett σ values. The correlation obtained provided a line of negative slope. In the present report, the authors present other correlations, obtained from the data of Bell and Roblin (1) and supplemented by the data of Seydel, Krüger-Thiemer, and Wempe (2, 3, 6, 7), whose activities correlate linearly with both pKa and with σ values[1] and for which lines of both positive and negative slopes are obtained. In light of these data, it is pointed out that there is little evidence to support the implications derived from the Bell and Roblin correlation; *i.e.*, that a sulfonamide must have a *definite* pKa (in the range of 6.0–7.5) in order to exhibit maximum activity.

If one classifies the activity-pKa data of Bell and Roblin in terms of homologous series it is noted that: (*a*) in the region where pKa = 6–11, the Bell and Roblin "curve" actually consists of a number of incomplete homologous series, each of which describes a straight line of negative slope; (*b*) in the region where pKa = 2–6, a limited number of homologous compounds can be found (we were able to find no more than three) to which a line of positive slope can be ascribed. In these terms, for the maximum in the Bell and Roblin curve to constitute a true maximum, it would be expected that one set of homologous series would afford lines of positive slope (pKa 2–6), another set of homologous series would afford lines of negative slope (pKa 6–11), and each set would intercept at the accepted maximum.

A search of the literature was made in an attempt to find results which would provide lines of positive slope to substantiate the Bell and Roblin maximum. Only a very limited number of antibacterial activity and pKa data could be found for use in this study. The compiled data (Table I) are presented graphically in Fig. 1; the curved dashed line is that originally reported in the work of Bell and Roblin (1). From the information at hand, only one very incomplete series, the 2-sulfanilamido-(substituted) thiadiazoles, could be said to support the ascending portion (pKa 2–6) of the Bell and Roblin plot.

Two other series appear to cast some doubt on the validity of the Bell and Roblin correlation; the N^1-(substituted) methylsulfanilamide series, which give a line of positive slope and which lies at the

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[1] Results obtained by the use of the Hammett σ value should also be obtained when pKa is used, since pKa is related to σ by the equation: $pKa = -\rho\sigma + pKa^\circ$, where ρ is Hammett's reaction constant and pKa° is the pKa of a reference member of the homologous series.

TABLE I—ACTIVITY-pKa CORRELATIONS FOR FAMILIES OF SULFONAMIDES

	pKa	$C_R \times 10^5$ (M)[a,b]	Ref.
N^1-(Substituted) Phenylsulfanilamides			
Substituents			
4-NO_2	6.8	0.8	(2)
4-CH_3NHSO_2	7.3	0.2	(2)
4-H_2N-SO_2	7.85	0.5	(1)
3-H_2N-SO_2	8.23	2.0	(1)
4-Cl	8.6	3.2	(2)
H	9.60	3.0	(1)
3-CH_3	9.74	5.0	(1)
4-CH_3	9.82	5.0	(1)
2-CH_3	9.96	10.0	(1)
4-NH_2	10.22	5.0	(1)
Substituted N^1-Benzoylsulfanilamides			
meta and/or *para* Substituents			
H	4.57	0.3	(1)
4-$(CH_3)_2CH$	4.70	0.4	(3)
4-CH_3	4.7	0.4	(3)
4-CH_3CH_2	4.7	0.24	(3)
3-CH_3	4.75	0.4	(3)
4-$CH_3CH_2CH_2$	4.76	0.67	(3)
3,4-CH_3	4.95(4.86)	0.4(0.36)	(2, 3)
4-$(CH_3)_2CH$—O	4.9	0.4	(2)
3-CH_3, 4-CH_3O	4.9	0.56	(3)
4-NH_2	5.20	0.5	(1)
ortho Substituents			
2,4,5-CH_3	5.1	0.8	(3)
2,4-CH_3	5.1	2.25	(3)
2-CH_3	4.90	2.68	(3)
2,5-CH_3	5.05	2.68	(3)
2,4,6-CH_3	5.1	about 6.4	(3)
2,3,4,5,6-CH_3	5.25	about 51.2	(3)
2-Sulfanilamido (Substituted) Thiadiazoles			
Substituents			
H	4.77	0.6	(1)
5-CH_3CH_2	5.1	0.2	(2)
5-CH_3	5.45	0.2	(1)
N^1-(Substituted) Methylsulfanilamides			
$CON(CH_2CH_3)_2$	10.1	409.6	(2)
H	10.77	30.0	(1)
	10.88	20.0	(1)
CH_2OH	10.92	50.0	(1)
$SO_3^-NH(CH_2CH_2OH)_3$[c]	7.6	51.2	(2)
2-Sulfanilamido (Substituted) Pyridines			
5-Br	7.15	0.5	(1)
H	8.43	0.6	(1)
5-NH_2	8.47	0.6	(1)
2-Sulfanilamido (Substituted) Pyrimidines			
H	6.48	0.08	(1)
4-CH_3	7.06	0.2	(1)
4,6-CH_3	7.37	0.3	(1)
4-NH_2	7.44	20.0	(1)

[a] Minimum inhibitory concentration for bacteriostasis of *E. coli*. [b] The actual concentration was employed in evaluating $\log 1/C_R$. [c] This is the only ionic substituent in this series, which may, in part, account for its departure from the line.

very end of the Bell and Roblin curve (pKa 10–11); and, the *ortho*-substituted members of the N^1-benzoylsulfanilamide series, which yields an almost vertical line bisecting the Bell and Roblin curve at about pKa 5. These last two series have not been considered as reliable test series in our study, for there is reason to believe that the mechanism of action for these compounds may differ from that of the other series considered.

Figure 2 shows the correlation of activity with σ for the N^1-benzoylsulfanilamides.[2] It demonstrates a definite break in the line when the transition is made from *meta* and *para* substituted derivatives to the *ortho* substituted ones. If the analogy can be made between the Hammett equation and the equation which correlates activity with σ:

$$\log 1/C_R = \alpha\sigma + \log 1/C_R^\circ$$

[2] Hammett values were obtained from Jaffé's compilation (8); *ortho* values are those given by Bray and Barnes (9).

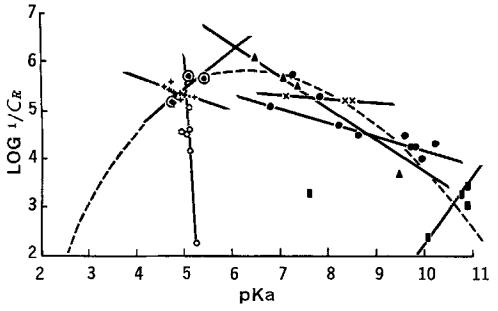

Fig. 1—Key: ⊙, *2-sulfanilamido (substituted) thiadiazoles;* +, meta *and/or* para-*substituted* N^1-*benzoylsulfanilamides;* O, ortho-*substituted* N^1-*benzoylsulfanilamides;* ▲, *2-sulfanilamido (substituted) pyrimidines;* ×, *2-sulfanilamido (substituted) pyridines;* ●, N^1-*(substituted) phenylsulfanilamides;* ■, N^1-*(substituted) methylsulfanilamides.*

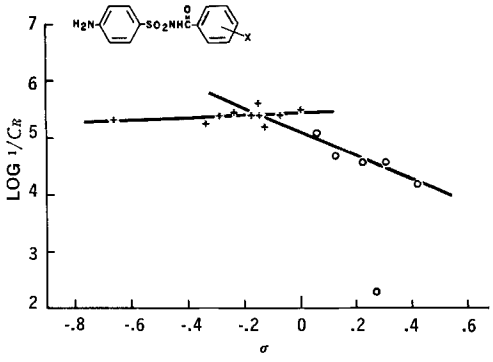

Fig. 2—Key: +, meta *and/or* para-*substituted* N^1-*benzoyl-sulfanilamides;* O, ortho-*substituted* N^1-*benzoyl-sulfanilamides.*

where α defines the "intrinsic" activity which may be associated with a given homologous series, and where log $1/C_R°$ is an arbitrary reference activity, then the break in the line could be interpreted as a possible change in the mechanism of action. This would follow from similar considerations of the Hammett equation in relation to chemical reaction mechanisms (10).

It has been shown (11) that sulfonamides bearing an N^1-aromatic substituent are capable of binding at an enzyme site through each of their aromatic rings. Since all the series considered in this study possess an N^1-aromatic ring except the N^1-(substituted) methylsulfanilamides, the latter is not considered a reliable test series. It is quite conceivable that binding differences, coupled with diminished pKa values, may account for the divergent pattern in activities exhibited by *ortho*-substituted N^1-benzoyl-sulfanilamides.

The linear correlation of *in vitro* bacteriostatic activity with pKa, which is observed in this study for homologous series of sulfonamides, provides little evidence in support of there being a definite pKa which a sulfonamide must possess in order to exhibit maximum activity. Rather, from the data at hand, it appears that many sulfonamides lying outside of the "prescribed" Bell and Roblin maximum, notably those containing electron withdrawing substituents on an N^1-aromatic ring, are potentially capable of high *in vitro* activities, provided they can penetrate the bacterial cell wall.

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Gas Chromatography of Alkyl Ether Derivatives of *p*-Hydroxybenzoate Esters

By MERRILL WILCOX

A procedure for the separation and estimation of mixtures of the four lower normal alkyl *p*-hydroxybenzoates as derivatives is described. The esters were converted to phenyl alkyl ethers by means of diazoalkanes. Diazoethane, diazo-*n*-propane, diazoisobutane, and diazo-*n*-butane, when catalyzed with 0.007 per cent boron trifluoride, alkylated essentially quantitatively in 30 min. at room temperature. Variable yields were noted in the absence of the catalyst for reaction times as long as 18 hr. Diazomethane did not alkylate quantitatively when catalyzed in the same manner. Derivatives prepared from mixtures of the four esters were resolved on the gas chromatograph regardless of the diazoalkane used.

THE ALKYL *p*-hydroxybenzoate esters are important food and pharmaceutical preservatives. Lach and Sawardeker (1) list several reviews of the role of these esters as preservatives. They also review the general methods of analysis which have been published. An additional general method of analysis has recently appeared (2). Higuchi *et al.* (3) developed an assay using column chromatography and U.V. spectrophotometry which could be used to resolve mixtures of the esters. Lach

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