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Structure-Activity Correlations for N-Substituted N-Thioformylhydroxylamines on Bacterial Cells[1)]

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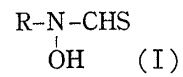
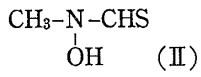
The quantitative correlations between the chemical structure and the antibacterial activity against *Staphylococcus aureus* 209P, *Bacillus subtilis* PCI-219, *Escherichia coli* NIHJ and *Salmonella typhimurium* of 19 N-substituted N-thioformylhydroxylamine derivatives have been studied by Hansch-Fujita's method. These compounds are analogues of an antibiotic thioformin, N-methyl N-thioformylhydroxylamine. The following parameters were used in this study; Hansch's π and chromatographic *Rm* values as hydrophobic parameters and pK_a value as an electronic parameter. As the result, in the N-substituted N-thioformylhydroxylamine series, the hydrophobic character of the molecule as expressed by π or *Rm* is closely correlated to the antibacterial activity of these compounds.

As reported previously,[1)] a series of N-substituted N-thioformylhydroxylamine derivatives (I), analogous compounds of an antibiotic thioformin (II), were active against numerous bacteria.



(I)



(II)

It would be interest to quantitatively investigate the structure-activity correlations between the chemical structure and the antibacterial activity of these compounds.

Hansch and Fujita[3)] have shown that in the members of a congeneric series the correlation between the substituent constants and the biological activity was generally expressed by the following equation.

$$\log 1/C = -a\pi^2 + b\pi + c\sigma + d\mathrm{Es} + e \qquad (1)$$

In the eq. 1, C is the molar concentration which gives a standard response in a standard time interval, π is the hydrophobic substituent constant which is defined as $\pi = \log P_X - \log P_H$, where P_X and P_H are the partition coefficient in *n*-octanol/water system of the substituted and parent compounds, respectively, σ is the electronic parameter on the reaction center, and Es is the steric constant. The constants a, b, c, d and e are regression coefficients determined by multiple regression analysis. Concerning the antimicrobial activities,[4)] correlation was usually well expressed by eq. 2 without a consideration of steric contribution.

$$\log 1/C = -a\pi^2 + b\pi + c\sigma + e \qquad (2)$$

According to the published paper,[4)] the hydrophobic character expressed by the partition coefficient log P or π was the most important factor relating to the antibacterial activity and

1) This forms Part V of "Antibiotic YC-73 of *Pseudomonas* Origin." Part IV: T. Miyagishima, T. Yamaguchi, and K. Umino, *Chem. Pharm. Bull.* (Tokyo), **22**, 2283 (1974). A part of this work was reported at the 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April, 1972.

2) Location: *2-2-50 Kawagishi, Toda-shi, Saitama.*

3) C. Hansch, R.M. Muir, T. Fujita, P.P. Maloney, F. Geiger, and M. Streich, *J. Am. Chem. Soc.*, **85**, 2817 (1963); C. Hansch, "Drug Design," Vol. I ed. by E.J. Ariens, Academic Press, New York, 1971, pp. 271—342; M.S. Tute, "Drug Design," Vol. III ed. by E.J. Ariens, Academic Press, New York, 1973, pp. 1—77.

4) E.J. Lien, C. Hansch and S.M. Anderson, *J. Med. Chem.*, **11**, 430 (1968).

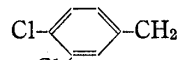
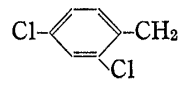
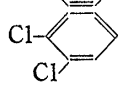
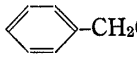
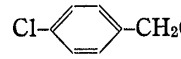
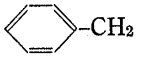
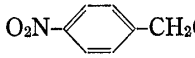
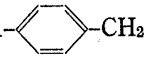
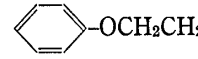
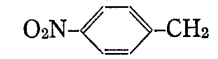
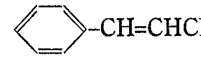
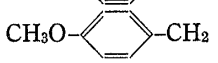
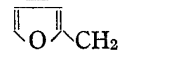
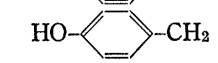
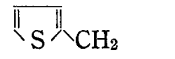
the electronic effect on the reaction center was also played a significant role in a several series of antibacterial agents. In this paper, Hansch–Fujita's method was applied to analyze the structure-antibacterial activity correlations of the N-substituted N-thioformylhydroxylamine ferric complexes using π and pK_a values as parameters at first. Hansch and Fujita have developed a rational quantitative analysis to know the structure-activity correlation by using a substituent constant π. However, the calculated π values cannot completely replace the partition coefficient P which can be obtained experimentally in n-octanol/water system. Intermolecular interactions such as electronic, hydrogen bonding and shielding effects[5] would cause the deviation between them. Instead of this parameter, several other experimental hydrophobic parameters which are linearly correlated with π-costant have been reported. For example, Boyce and Milborrow[6] utilized the Rm values which could be obtained by the reversed-phase thin-layer chromatography, and Bate-Smith and Westall[7] showed that Rm value was related to the partition coefficient and could be calculated from eq. 3.

$$Rm = \log\left(\frac{1}{Rf} - 1\right) \tag{3}$$

In this investigation, Rm values were introduced to express the hydrophobic character of the molecules in order to avoid the practical difficulties for determining the partition coefficient P. ΔRm value had been defined as $\Delta Rm = Rm_X - Rm_H$, where Rm_X was the Rm of a substituted derivative and Rm_H was that of N-phenyl N-thioformylhydroxylamine ferric complex (**4**), and correlation between π and ΔRm values was determined.

TABLE I. Parameter Values used in the Multiple Regression Analysis of N-Substituted N-Thioformylhydroxylamine Ferric Complexes

$$(R\text{-}\underset{\displaystyle O^-}{\underset{|}{N}}\text{-}CHS)_3Fe^{3+}$$

Compound No.	R	π	pK_a	Compound No.	R	π	pK_a
1	CH_3	0.5	7.55	**11**		4.15	8.86
2	CH_3CH_2	1.0	7.96				
3	$(CH_3)_2CH$	1.3	7.55	**12**		3.98	8.60
4		2.13	8.70				
5		3.42	8.20	**13**		3.15	7.15
				14		3.85	7.30
6		2.69	7.25	**15**		3.39	7.70
7		3.39	8.95	**16**		2.61	8.85
8		2.93	8.90	**17**		3.36	8.85
9		2.65	8.65	**18**		2.31	7.92
10		2.08	8.30	**19**		1.82	7.90

5) C. Hansch and S.M. Anderson, *J. Org. Chem.*, **32**, 2583 (1967); J. Iwasa, T. Fujita and C. Hansch, *J. Med. Chem.*, **8**, 150 (1965); D.J. Currie, C.E. Lough, R.F. Silver and H.L. Holmes, *Can. J. Chem.*, **44**, 1035 (1966).
6) C.B.C. Boyce and B.V. Milborrow, *Nature*, **208**, 537 (1965).
7) E.C. Bate-Smith and R.C. Westall, *Biochim. Biophys. Acta*, **4**, 427, (1950).

Materials and Methods

Thioformin Analogues——Thioformin analogues, N-substituted N-thioformylhydroxylamine ferric complexes, were synthesized as reported in the previous papers.[8]

Antibacterial Activity——The antibacterial activities of the test compounds were reported in earlier papers.[8] In this investigation, the minimum inhibitory concentrations (MIC) against *Staphylococcus aureus* 209P, *Bacillus subtilis* PCI-219, *Escherichia coli* NIHJ and *Salmonella typhimurium* were used. To calculate log $1/C$ values, the MIC in a weight basis (mcg/ml) was converted to a molar concentration basis (mole/ml).

Hydrophobicity——a) π Value: π values shown in Table I were calculated by the method outlined by Hansch, *et al.*[9]

b) *Rm* Value: *Rm* values were determined by reversed-phase thin-layer chromatography (TLC) as described by Biagi, *et al.*[10] A stationary nonpolar phase was obtained by impregnating the silica gel thin-layer sheet (Woelm) with 5% silicone oil (350 cs) in ethyl ether. The polar mobile phase was prepared by acetone and water in various proportion. The compounds to be tested were dissolved in acetone and spotted on the plate in randamized allocations in order to avoid any systematic error.

pK_a Value——pK_a values were measured on a potentiograph E 336 (Metrom Harison). Each thioformin analogue was dissolved in a 50% dioxane and 0.01N HCl mixture (2.0×10^{-3}M), and the solution was titrated with 0.02N NaOH standard solution at 25°.

Statistical Analysis——The structure-activity correlations were analyzed by means of multiple regression analysis on an IBM 360-15 computer system.

Results

The π and pK_a values of N-substituted N-thioformylhydroxylamine ferric complexes were shown in Table I.

The equations, shown in Table II which reflexed the structure-activity correlations, were obtained by multiple regression analysis of π, pK_a values and antibacterial activities of the

Table II. Antibacterial Activity Linearly Dependent on π and pK_a

Organism	Equation	$n^{a)}$	$r^{b)}$	$s^{c)}$	$F^{d)}$	Eq. No.
S. aureus 209P	$\log 1/C=-0.130\pi^2+0.304\pi-0.107pK_a+6.183$	19	0.926	0.167	30.20	4
	$\log 1/C=-0.123\pi^2+0.250\pi+5.396$	19	0.913	0.177	44.61	5
	$\log 1/C=-0.329\pi-0.077pK_a+6.574$	19	0.858	0.222	22.40	6
	$\log 1/C=-0.344\pi+5.981$	19	0.851	0.221	40.15	7
	$\log 1/C=-0.236pK_a+6.990$	19	0.361	0.393	2.55	8
B. subtilis	$\log 1/C=-0.177\pi^2+0.586\pi-0.124pK_a+6.119$	19	0.958	0.117	55.29	9
	$\log 1/C=-0.167\pi^2+0.523\pi+5.210$	19	0.937	0.137	57.95	10
	$\log 1/C=-0.274\pi-0.083pK_a+6.651$	19	0.803	0.235	14.52	11
	$\log 1/C=-0.289\pi+6.012$	19	0.792	0.233	28.56	12
	$\log 1/C=-0.215pK_a+6.997$	19	0.363	0.356	2.58	13
E. coli NIHJ	$\log 1/C=-0.043\pi^2-0.067\pi-0.104pK_a+5.763$	19	0.906	0.158	22.98	14
	$\log 1/C=-0.036\pi^2-0.119\pi+5.000$	19	0.888	0.166	29.85	15
	$\log 1/C=-0.278\pi-0.094pK_a+5.893$	19	0.895	0.161	32.37	16
	$\log 1/C=-0.295\pi+5.173$	19	0.880	0.166	58.44	17
	$\log 1/C=-0.228pK_a+6.245$	19	0.419	0.318	3.63	18
Sal. typhimurium	$\log 1/C=-0.069\pi^2-0.157\pi-0.124pK_a+5.602$	19	0.847	0.155	12.66	19
	$\log 1/C=-0.061\pi^2+0.094\pi+4.692$	19	0.800	0.169	14.27	20
	$\log 1/C=-0.179\pi-0.108pK_a+5.809$	19	0.798	0.170	13.99	21
	$\log 1/C=-0.194\pi+4.981$	19	0.760	0.178	23.22	22
	$\log 1/C=-0.194pK_a+6.036$	19	0.458	0.243	4.50	23

a) n: number of the tested compound
b) r: multiple correlation coefficient
c) s: standard deviation
d) F: values for statistical significance of the correlation

8) Reference 1; Y. Ito, K. Umino, T. Sekiguchi, T. Miyagishima and Y. Egawa, *J. Antibiotics*, **24**, 131 (1971).
9) A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
10) G.L. Biagi, A.M. Barbaro, M.F. Gamba and M.C. Guerra, *J. Chromatog.*, **41**, 371 (1969).

Table III. Observed and Calculated Values of log $1/C$ of N-Substituted N-Thioformylhydroxylamine Ferric Complexes

Compound No.	*S. aureus* 209P		*B. subtilis*		*E. coli* NIHJ		*Sal. typhimurium*	
	Obsd.[a]	Calcd.[b]	Obsd.[a]	Calcd.[c]	Obsd.[a]	Calcd.[d]	Obsd.[a]	Calcd.[e]
1	5.32	5.49	5.32	5.43	5.02	5.03	4.72	4.90
2	5.67	5.52	5.67	5.56	4.77	4.88	4.77	4.77
3	5.72	5.51	5.72	5.61	4.82	4.79	4.82	4.76
4	5.21	5.37	5.52	5.56	4.31	4.54	4.31	4.49
5	4.46	4.81	4.76	5.02	3.86	4.16	4.16	4.31
6	5.25	5.18	5.55	5.40	4.65	4.38	4.65	4.54
7	5.02	4.83	5.02	5.05	4.12	4.17	4.42	4.24
8	5.04	5.07	5.34	5.30	4.44	4.31	4.44	4.32
9	5.32	5.19	5.32	5.41	4.71	4.39	4.72	4.40
10	5.29	5.38	5.59	5.57	4.38	4.56	4.68	4.54
11	4.18	4.31	4.48	4.48	3.88	3.95	3.88	4.11
12	4.48	4.44	4.48	4.62	3.88	4.00	4.18	4.17
13	4.98	4.96	5.28	5.18	4.38	4.24	4.68	4.47
14	4.75	4.53	5.05	4.72	4.15	4.04	4.15	4.33
15	5.07	4.83	5.07	5.05	4.17	4.17	4.47	4.37
16	5.01	5.21	5.31	5.43	4.41	4.40	4.41	4.39
17	4.70	4.85	5.00	5.06	4.11	4.18	4.11	4.25
18	5.52	5.44	5.52	5.60	4.61	4.64	4.61	4.63
19	5.26	5.32	5.56	5.52	4.66	4.49	4.36	4.54

a) reference 8, *b*) calculated from eq. 5, *c*) calculated from eq. 10
d) calculated from eq. 17, *e*) calculated from eq. 22

test compounds. Among equations in Table II, eq. 5, 10, 17 and 22 statistically showed an excellent fit between the antibacterial activity and the hydrophobic parameter π. In Table III, the log $1/C$ values calculated from the above equations and the observed ones summarized on each bacteria and on each compound.

For *S. aureus* 209P and *B. subtilis* PCI-219, Gram-positive bacteria, a parabolic correlation exsisted between the hydrophobic character and the antibacterial activity, while for *E. coli* NIHJ and *Sal. typhimurium*, Gram-negative ones, linear correlation exsisted. The negative sign associated with π term means that the activity against Gram-negative bacteria increases linearly along with the increased hydrophilic character of the molecules. For these bacteria, the introduction of the π^2 term does not improve the correlation in a significant

Table IV. *Rf*, *Rm* and ΔRm values of N-Substituted N-Thioformylhydroxylamine Ferric Complexes

Compound No.	*Rf*	*Rm*	ΔRm
4	0.73	−0.43	0.00
5	0.09	1.00	1.43
6	0.56	−0.11	0.32
7	0.33	0.48	0.91
8	0.54	−0.07	0.36
9	0.63	−0.23	0.20
10	0.87	−0.83	−0.40
11	0.14	0.79	1.22
12	0.12	0.87	1.30
13	0.36	0.25	0.68
14	0.18	0.66	1.09
15	0.38	0.21	0.64

Table V. Antibacterial Activity Linearly Dependent on *Rm*

Organism	Equation	$n^{a)}$	$r^{b)}$	$s^{c)}$	$F^{d)}$	Eq. No.
S. aureus 209P	$\log 1/C=-0.371Rm^2-0.462Rm+5.147$	12	0.920	0.161	24.95	25
	$\log 1/C=-0.570Rm+5.044$	12	0.869	0.193	30.73	26
B. subtilis	$\log 1/C=-0.249Rm^2-0.539Rm+5.323$	12	0.917	0.170	23.86	27
	$\log 1/C=-0.611Rm+5.254$	12	0.896	0.180	40.76	28
E. coli NIHJ	$\log 1/C=-0.399Rm^2-0.280Rm+4.441$	12	0.890	0.144	17.21	29
	$\log 1/C=-0.395Rm+4.329$	12	0.782	0.187	15.75	30
Sal. typhimurium	$\log 1/C=-0.244Rm^2-0.268Rm+4.536$	12	0.776	0.185	6.80	31
	$\log 1/C=-0.338Rm+4.468$	12	0.723	0.192	10.95	32

a) n: number of the tested compounds *b*) r: multiple correlation coefficient
c) s: standard deviation *d*) F: values for statistical significance of the correlation

way. Judging from eqs. 8, 13, 18, 23 and others, electronic effect seemed not to relate to the antibacterial activity either alone nor in combination with π values.

Prior to adopt the *Rm* values for analysis, the relationship between ΔRm and π was analyzed. The *Rf* values determined by means of a reversed-phase thin-layer chromatographic method, the *Rm* values calculated from *Rf* and ΔRm values were listed in Table IV. These values were obtained with silicone oil and 55% acetone–water system, which was the best proportion, and the compounds of lower *Rm* values are more hydrophilic in nature than those characterized by higher *Rm* values. Since N-alkyl and N-heteroaralkyl N-thioformylhydroxylamines could not be clearly detected on TLC, the values were not presented here.

The eq. 24 relating π to ΔRm value indicated a good linear correlation between these parameters of N-substituted N-thioformylhydroxylamines.

$$\pi = 1.107\Delta Rm + 2.436 \qquad (24)$$
$$n=12,\ r=0.925,\ s=0.270$$

The equations which correlated the structure-activity relationship in respect of *Rm* values and log $1/C$ of N-aryl or N-aralkyl N-thioformylhydroxylamines obtained were calculated from the data shown in Tables III and IV by means of multiple regression analysis. They were illustrated in Table V. As evident from Table V, good correlations between *Rm* and antibacterial activities both for Gram-positive and -negative bacteria were indicated.

Discussion

The multiple regression analysis of the present work showed that the antibacterial activity of N-substituted N-thioformylhydroxylamines was greatly influenced by a lipophilic nature of these compounds, but not by an electronic effect.

The parabolic dependences of log $1/C$ on the π values of these derivatives for Gram-positive bacteria were presented. The optimum π values for *S. aureus* 209P and *B. subtilis* PCI-219 were 1.07 and 1.57, respectively.

On the other hand, linear correlations were for Gram-negative ones. The most active compound against Gram-negative organisms would be the most hydrophilic one. The absence of a parabolic dependence together with the negative sign of π terms was likely due to the lack of the more hydrophilic derivatives among the test compounds in the present analysis.

These correlations for Gram-positive and -negative bacteria are in agreement with the hypothesis of many investigations that the activity of antimicrobial compounds on bacteria mainly is varied with the penetrating ability of the molecule through the bacteria cell. For example, Lien, *et al.*[4] suggested the view that in antibacterial agent the compounds active against Gram-negative bacteria are more hydrophilic than those active against Gram-positive ones. The different lipophilic nature of the molecules against Gram-positive or negative

bacteria was assumed to be related to the different lipid composition of the cell wall. It is known that the cell wall of Gram-negative bacteria is richer in lipid than that of Gram-positive ones, so the lipophilic molecules might be retained by the cell wall of Gram-negative microorganisms more strongly than the hydrophilic ones.

As discussed in the introduction of this paper, the empirical parameter π is one of the most useful parameters which indicate the relevance to a hydrophobic effect for many substituents. However, when intramolecular interactions are possible, either because of polar and hydrogen functions or conformational flexibility, the calculated $\sum\pi$ values cannot be a complete substituent constant. Although good correlations were obtained by using this parameter, the *Rm* value obtained from the reversed-phase thin-layer chromatography was also a useful measure of the hydrophobic character. The excellent linear correlation between π and *Rm* in this experiment agreed with Collander's finding[11] that the logarithms of the partition coefficients of a compound in two different solvents were linearly related. As the results, this chromatographic method was proved to be an advantageous technique for determining partition coefficient because of easiness of the procedure and requirement of a small sample for test. Moreover, *Rm* values are rather important to display the information of the whole molecule.

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