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Studies on Chemical Carcinogens. XXI.[1] Quantitative Structure-Mutagenicity Relationship among Substituted Styrene Oxides

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The quantitative structure-mutagenicity relationship among 8 substituted styrene oxides, *p*-methyl-, *p*-butyl, *p*-phenyl, *p*-chloro, *m*-chloro, *p*-cyano, *p*-nitro, and the parent styrene oxide, was examined. Mutagenic and cytocidal activities were correlated with the reactivity of the epoxide (Hammett's σ), the van der Waals volume of the molecule (V_W), and/or the partition parameter (π). Multiple regression analyses revealed that mutagenic capacity could be well described by a linear combination of σ and log V_W and that cytocidal capacity depended only on π of the derivatives, as already reported by Sugiura *et al.*

Keywords——mutagenicity; styrene oxides; van der Waals volume; Hammett's σ; regression analysis; structure-activity relationship; partition coefficient

Since most genetic damage is considered to be initiated through chemical alterations of desoxyribonucleic acid (DNA), much attention has been paid to the quantitative relationship between the chemical properties of compounds and their genetic toxicity, such as mutagenicity and carcinogenicity.[2-9] Sugiura *et al.* recently reported a reasonably good correlation of mutagenicity with electrophilic reactivity at the benzylic site of the epoxide moiety of some substituted styrene oxides.[2,3] Laird and Parker had shown that, in the reaction of some *m*- and *p*-substituted styrene oxides with benzylamine in ethanol, nucleophilic ring-opening of the epoxide proceeded at both sites, α-CH and β-CH_2, of the epoxide moiety, following S_N2 kinetics, and that the ρ-values of these reactions had opposite signs; -1.15 for the reaction at α-CH and $+0.87$ for that at β-CH_2.[10] Sugiura *et al.* examined the mutagenicity of some of these derivatives in *Salmonella typhimurium* TA100 and *E. coli* WP2uvrA and found that their mutagenicity was correlated linearly with the σ-values of the substituents.[2,3] Thus, an electron-donating group at the *p*-position of the styrene oxide increased the mutagenic capacity and conversely, an electron-withdrawing group decreased it. These results led them to the conclusion that the mutagenic capacity of this class of compounds dependent only on the electrophilic reactivity toward nucleophiles at the benzylic site (α-CH), regardless of the reactivity at the β-carbon of the epoxide. They also concluded that LD_{30} toward microorganisms was correlated linearly with the partition coefficient, although it appeared to have no effect on the mutagenicity. Their papers[2,3] prompted us to report here the results which have so far been obtained in our laboratory with regard to the quantitative structure-activity relationship (QSAR) of mutagenic styrene oxides. The results reported here represent a significant extension of the work of Sugiura *et al.*

Experimental

Compounds——Styrene oxide,[11] and its *p*-methyl,[12] *p*-chloro,[12] and *p*-nitro[13] derivatives were prepared by the methods described in the literature. All the other styrene oxides used were synthesized by hydrogenation of *p*-substituted phenacyl bromides[14] to the corresponding bromohydrines, followed by their dehydrobromination to the corresponding styrene oxides.[11-13]

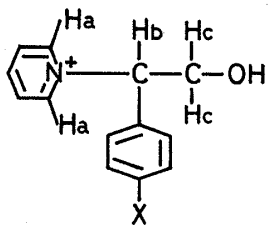
***p*-Butylstyrene Oxide**——To 100 ml of a methanol solution of 4.0 g of *p*-butylphenacyl bromide was added in portions 2 g of $NaBH_4$ under stirring at room temperature. The reaction mixture was stirred for 30 min, then diluted with water and extracted with CH_2Cl_2. The solvent of the extract was evaporated off, and the

residue was stirred with 150 ml of aqueous 1 N NaOH for 2 h. The reaction mixture was extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on a neutral silica gel (100 g) column and eluted with 5% ether in hexane. The main fraction of the eluate was concentrated and distilled under reduced pressure. The yield of the colorless oil thus obtained was 50%. bp 130°C (1 Torr). *Anal.* Calcd for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found: C, 81.93; H, 9.37.

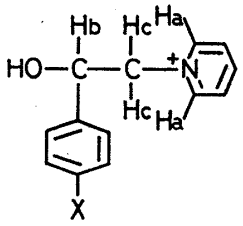
***p*-Phenylstyrene Oxide**——The *p*-phenyl derivative was prepared from *p*-phenylphenacyl bromide in the same manner as described above. mp 96—97°C. *Anal.* Calcd for $C_{14}H_{12}O$: C, 85.68; H, 6.16. Found: C, 86.36; H, 6.07.

***p*-Cyanostyrene Oxide**——The *p*-cyano derivative was prepared from *p*-cyanophenacyl bromide in the same manner as described above. mp 34—35°C. *Anal.* Calcd for C_9H_7NO: C, 74.47; H, 4.86; N, 9.65. Found: C, 74.71; H, 4.69; N, 9.87.

Ring-Opening Reaction with Pyridine——Styrene oxide or a derivative (about 100 mg) was dissolved in 50 ml a 3: 2 v/v mixture of acetone and 1/15 M phosphate buffer (pH 6.0), and 3 ml of pyridine was added. The reaction mixture was warmed at 60°C for a sufficient period for completion of the reaction; 17 h for *p*-methyl, 23 h for *p*-phenyl, 44 h for non-substituted, 65 h for *p*-cyano, 69 h for *p*-chloro, and 70 h for the *p*-nitro derivative. After completion of the reaction, the solution was evaporated to dryness under reduced pressure. The residue was dissolved in 3 ml of D_2O and evaporated again. This residue was dissolved in about 1 ml of 20% DCl again. The proton magnetic resonance (PMR) spectrum of the solution thus obtained was measured with a JEOL MH-100 spectrometer. The product ratio of product α to product β (see Chart 1) was determined by integrations of the appropriate signals on the spectrum. The chemical shifts of the protons of each product are shown in Chart 1.



product α



product β

	Ha	Hb	Hc	Ha	Hb	Hc
X: –H	9.08	NA	4.48	8.84	5.30	4.49
$-CH_3$	9.08	6.18	4.58	8.85	5.46	4.98
$-C_4H_9$	9.07	NA	4.39	8.88	5.40	4.96
$-C_6H_5$	8.91	6.09	4.48	8.74	5.38	4.85
–Cl	8.94	6.11	4.56	8.74	5.40	4.92
–CN	9.02	6.27	4.60	8.78	5.50	4.95
$-NO_2$	9.10	NA	NA	8.91	5.60	5.04

(NA: not assigned.)

Chart 1. Proton Chemical Shifts of Products α and β with Reference to DSS in D_2O Solutions

Table I. Partition Parameter estimated from Retention Time on High Performance Liquid Chromatography and Hydrophobicity Parameter from the van der Waals Volume, V_w

Substituent	t_R (min)[a]	π_{HPLC}[b]	π_{llst}[c]	V_w(Å^3)[d]	$\log[V_{w(X)}/V_{w(H)}]$[e]
None	5.40	0.000	0.00	108.3	0.000
p-Methyl	9.34	0.238	0.56	123.7	0.058
p-Butyl	50.55	0.972	(2.48)	169.9	0.196
p-Phenyl	32.88	0.784	1.96	177.7	0.215
p-Chloro	11.53	0.330	0.71	124.8	0.062
m-Chloro	11.48	0.328	0.71	124.8	0.062
p-Cyano	3.66	−0.169	−0.57	126.0	0.066
p-Nitro	5.95	0.042	−0.28	128.1	0.073

a) Retention times. See "Experimental".
b) π_{HPLC}=log (t_R of substituted derivative/t_R of styrene oxide)
c) The π values listed in the table of Appendix II of ref. 20. The value in parentheses for the *p*-butyl derivative was obtained from the correlation equation between π_{HPLC} and π_{llst}.
d) Calculated by using the values reported by Moriguchi *et al.*,[21] uncorrected for branching.
e) $V_{w(X)}$ and $V_{w(H)}$ are the van der Waals volumes of x-substituted derivative and parent styrene oxide, respectively.

Determination of Retention Times on High Performance Liquid Chromatography——Chromatography was carried out with a JASCO TWINCLE type high performance liquid chromatography (HPLC) apparatus with an incorporated ultraviolet (UV) detector, equipped with a 4.6 mm$\phi \times$250 mm JASCO Finepak SIL C_{18} column. The retention time was determined at 23°C by using 60% methanol as a developing solvent. The retention time was calculated by subtracting the solvent retention time from the observed retention time. The results are shown in Table I.

Assay for Mutagenicity on *Salmonella trphimurium* TA100——The mutation test was carried out as described by Ames *et al.*[15] with a slight modification using *S. typhimurium* TA100. The bacteria cells were incubated in liquid nutrient broth (0.8% Difco nutrient broth plus 0.6% NaCl) at 37°C for 10 h. To 0.2 ml of this culture (about 2×10^9 cells/ml), 0.7 ml of 0.25 M sodium phosphate buffer (pH 7.4) and 0.1 ml of dimethyl sulfoxide containing an appropriate amount of a test compound were added. The reaction mixture was gently shaken at 37°C for 60 min, then added to 3 ml of phosphate buffer and centrifuged at 3000 rpm for 20 min. The cells were suspended in 0.5 ml of the buffer and layered on minimal agar medium (1.5% Difco Bacto-agar in Vogel-Bonner Medium E[16] with 0.4% glucose) together with 2.0 ml of top agar (0.5% agar containing 1/10 volume of 0.5 mM histidine and 0.5 mM biotin) in an 86 mmϕ Petri dish. The plated dishes were incubated at 37°C for 2 d and the numbers of colonies were counted. For measurement of surviving cells, the reaction mixture was diluted 10^2- to 10^4-fold with phosphate buffer, and 0.1 ml of this diluted mixture was layered on a nutrient agar plate together with the top agar and incubated at 37°C for 1 d. The mutation frequency was calculated as $[(M-M_0)/N]$, where M and M_0 are the numbers of mutants per ml of the reaction mixture of the test compound and those of the control (dimethyl sulfoxide), respectively, and N is the number of surviving cells per ml of the reaction mixture.

Results

The derivatives of styrene ouide used in this study are those having at the *p*-position a cyano, nitro, phenyl, or butyl group, in addition to those having a *p*-methyl, *p*-chloro, or *m*-chloro group which Sugiura *et al.* had previously examined.[2,3]

Hammett's Relation in the Reaction of the Styrene Oxides with Pyridine

We attempted to confirm Hammett's relation in the nucleophilic ring-opening of the styrene oxides used in the present study in a simple way, as follows. Thus, the styrene oxides were treated with pyridine in phosphate buffer containing 60% acetone at 60°C. The two types of products, product α (P_α) and product β (P_β) shown in Chart 2, were quantitatively analyzed by PMR spectroscopy after completion of the ring-opening reaction. The results are summarized in Table II. Since both reactions are known to follow S_N2 kinetics, the product ratio, $[P_\alpha]/[P_\beta]$, is equal to k_α/k_β, where $[P_\alpha]$ and $[P_\beta]$ are the molar concentrations of the products from the reactions at the α-CH and the β-CH_2, respectively, and k_α and k_β are the pseudo-first order rate constants for nucleophilic attacks on the α-CH and β-CH_2, respectively.[17] Provided that Hammett's relation holds in the data space studied here, the following equation should be satisfied.

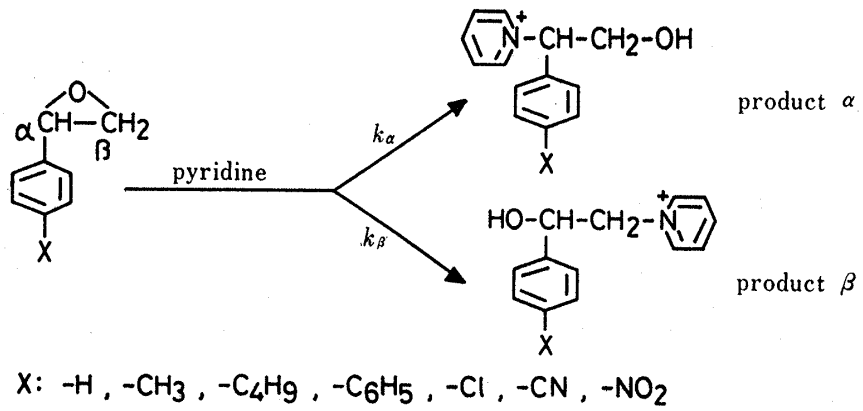


Chart 2. Epoxide Ring Opening Reaction with Pyridine in Phosphate Buffer (pH 6.0) and Acetone

Table II. Ratios of Pseudo-First Order Rate Constants, k_α to k_β, for Ring-Opening Reactions of Substituted Styrene Oxides with Pyridine in Acetone–Phosphate Buffer (pH 6.0) at 60°C

Substituent	Hammett's σ[a]	Product ratio (%)		k_α/k_β[b]
		at α-CH	at β-CH_2	
p-Methyl	−0.17	46.4	53.6	0.866
p-Butyl	−0.16	46.2	53.8	0.859
p-Phenyl	−0.01	40.0	60.0	0.667
(None)	0.00	38.9	61.1	0.637
p-Chloro	0.23	23.1	76.9	0.300
m-Chloro	0.37	(not examined)		
p-Cyano	0.66	12.0	88.0	0.136
p-Nitro	0.78	9.0	91.0	0.099

a) Cited from Appendix II of ref. 20.
b) The terms "k_α and k_β" are the pseudo-first order rate constants for the ring-opening reactions at α-CH and β-CH_2 of the oxides, respectively. They were calculated from the product ratio.

$$\log[k_{\alpha(X)}/k_{\beta(X)}] = (\rho_\alpha - \rho_\beta)\sigma_X + \log[k_{\alpha(H)}/k_{\beta(H)}]$$

The data were subjected to regression analysis. Equation 1 thus obtained explains 99% ($=r^2$) of the variance in the relative rate constants between α and β carbons of the epoxide (Fig. 1).

$$\log[k_\alpha/k_\beta] = -1.052(\pm 0.121)\sigma - 0.224 \qquad \text{Eq. 1}$$
$$n = 7;\ r = 0.995;\ r^2 = 0.990;\ S = 0.0445$$

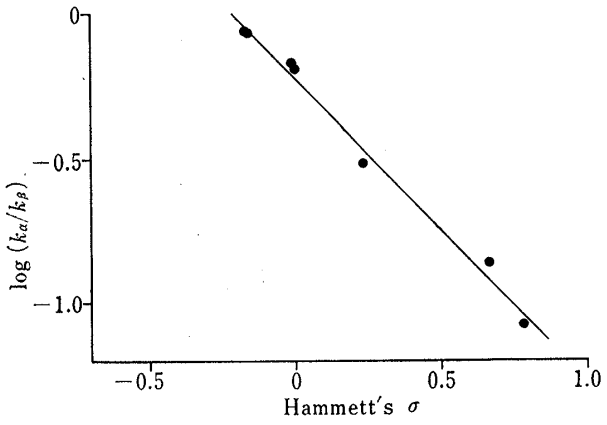


Fig. 1. Plots of log (k_α/k_β) *versus* Hammett's σ

The values, $(\rho_\alpha - \rho_\beta)$ and $\log[k\alpha_{(H)}/k\beta_{(H)}]$, were obtained from Eq. 1 as −1.052 and −0.224, respectively. It is, therfeore, concluded that the linear correlation between the reactivity at α-CH and Hammett's σ, as proposed by Sugiura *et al.*, is applicable to the present extended range of derivatives.

Partition Parameters estimated from Retention Times of the Epoxides in High Performance Liquid Chromatography (HPLC)

In order to estimate the contribution of hydrophobic nature to QSAR, the π values,[18] expressed as $\log[P_X/P_H]$ where P_X and P_H are the partition coefficients of X-substituted and unsubstituted styrene oxides, respectively, were estimated from the retention times of the derivatives in HPLC.[19] The values thus obtained will be designated as π_{HPLC} in this paper. The results are shown in Table I, which also shows π_{list} values which are those given in the literature as values generally applicable to substituted benzene derivatives.[20] The π_{HPLC} and π_{list} values are fairly well correlated.

Mutagenicity and LD_{50} of the Epoxides on *Salmonella typhimurium* TA100

The compounds were assayed for their mutagenicity by using *S. typhimurium* TA100. Concentrations of the compounds required to induce a mutation frequency of 10^{-6} were taken as a measure of the mutagenicity of each compound. In addition, the mutation frequency at the dose of 1 mM, MF(mM), and that at LD_{50}, MF(LD_{50}), were also taken into consideration.

TABLE III. Mutagenicity of Styrene Oxides on *Salmonella typhimurium* TA100

Substituent	LD_{50} (M)	Mutation frequency at 1 mM	Mutation frequency at LD_{50}	Concentration for 10^{-6} mutation frequency (M)
None	5.70×10^{-3}	1.2×10^{-6}	100.0×10^{-7}	8.5×10^{-4}
p-Methyl	1.85×10^{-3}	6.7×10^{-6}	135.0×10^{-7}	2.2×10^{-4}
p-Butyl	0.075×10^{-3}	24.0×10^{-6}	11.0×10^{-7}	0.65×10^{-4}
p-Phenyl	0.125×10^{-3}	120.0×10^{-6}	110.0×10^{-7}	0.15×10^{-4}
p-Chloro	0.84×10^{-3}	2.0×10^{-6}	16.5×10^{-7}	5.6×10^{-4}
m-Chloro	0.55×10^{-3}	1.7×10^{-6}	9.3×10^{-7}	6.3×10^{-4}
p-Cyano	$9.50\times1.^{-3}$	1.0×10^{-6}	180.0×10^{-7}	11.4×10^{-4}
p-Nitro	2.00×10^{-3}	4.7×10^{-6}	130.0×10^{-7}	3.6×10^{-4}

The dose-response curves of mutation frequency versus does were linear on a logarithm scale in all the cases examined. All the experimental data shown in Table III are the mean values from triplicate experiments.

Correlation

The correlations were tested for statistical significance by multiple linear regression analysis. The analyses were carried out on an NEAC ACOS S-700 computer. The 95% confidence limit is given in parentheses in each equation and the number of the compounds used (n), the correlation coefficient (r), the contribution ratio (r^2), and standard deviation (S) are also given.

Relationship between LD_{50} and π_{HPLC}

The correlation of LD_{50} with π_{HPLC} is given by Eq. 2 and the plots are shown in Fig. 2.

$$\log(1/LD_{50}) = 1.863(\pm0.390)\pi_{HPLC}+2.421 \qquad \text{Eq. 2}$$
$$n = 8;\ r = 0.979;\ r^2 = 0.958;\ S = 0.164$$

Thus, this equation euplains 96% of the variance in LD_{50} of the derivatives examined. Sugiura's conclusion was thus proved to be applicable to the wider range of compounds used in the present work.

It is worth noting that the van der Waals volume of a molecule, as proposed by Moriguchi *et al.*,[21] does not seem to be correlated with LD_{50}. Thus, $\log[V_{W(X)}/V_{W(H)}]$, where $V_{W(X)}$ and $V_{W(H)}$ are the van der Waals volumes of X-substituted and unsubstituted styrene oxides, respectively, could not replace π_{HPLC} to provide a satisfactory correlation.

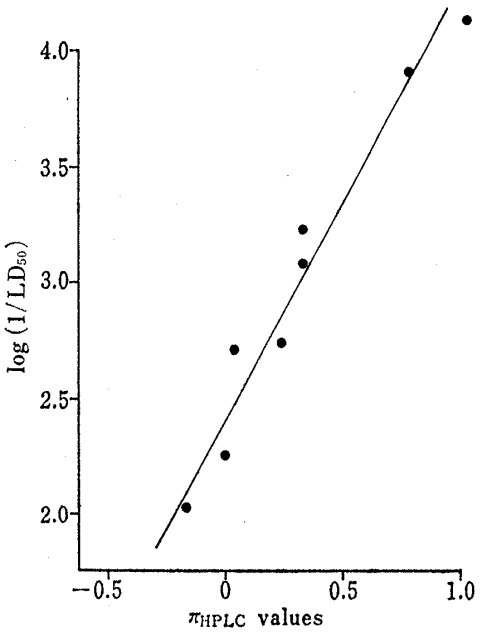


Fig. 2. Plots of log $(1/LD_{50})$ *versus* π_{HPLC}

Correlation of Mutagenicity with Hammett's σ, the van der Waals Volume, and/or π_{HPLC}

The terms log $(1/C)$ where C is the molar concentration of a chemical required to induce 10^{-6} mutation frequency, log [MF(mM)] where MF (mM) is the mutation frequency at the dose of 1 mM of the chemical, and log [MF(LD_{50})] where MF (LD_{50}) is the mutation frequency at

LD_{50} of the chemical were correlated with Hammett's σ, log $[V_{W(X)}/V_{W(H)}]$, and/or π_{HPLC}. The data were subjected to multiple linear regression analysis. Tables IV-VI summarize the coefficients of the variables, the 95% confidence limits, r, r^2, and S for each regression equation. In all the correlation procedures, *p*-nitrostyrene oxide was excluded, because it is possible that this derivative is metabolized to the corresponding *p*-amino derivative, which is known to be very reactive in the ring-opening reaction. Therefore, the number of the compounds (n) was seven. The correlations found in the data space including the *p*-nitro derivative were more or less worse than those shown in the tables in most regression analyses.

Table IV. Regression Equations of Concentration of Seven Substituted Styrene Oxides Required for 10^{-6} Mutation Frequency as Functions of Hammett's σ, Molecular Volume, and/or Partition Parameter

$$\log(1/C) = A\sigma + B \log[V_{W(X)}/V_{W(H)}] + C\,\pi_{HPLC} + \text{constant}$$

Equation	A	B	C	Constant	r	r^2	S
(3)	−1.380(±2.046)	0	0	3.771	0.613	0.375	0.596
(4)	0	7.839(±4.181)	0	2.852	0.907	0.823	0.317
(5)	0	0	1.464(±1.007)	3.071	0.858	0.737	0.387
(6)	−0.779(±0.887)	6.830(±3.406)	0	3.049	0.964	0.929	0.225
(7)	−0.341(±1.954)	0	1.309(±1.482)	3.171	0.867	0.751	0.420
(8)	0	5.729(±9.786)	0.478(±1.932)	2.882	0.917	0.842	0.335
(9)	−1.010(±1.441)	8.763(±9.034)	−0.505(±2.102)	3.077	0.970	0.940	0.238

Values in parentheses after the coefficients, A, B, and C, are the 95% confidence limits.

Table V. Regression Equations of Mutation Frequency at 1 mM for Seven Substituted Styrene Oxides

$$\log[\text{MF(mM)}] = A'\sigma + B' \log[V_{W(X)}/V_{W(H)}] + C'\,\pi_{HPLC} + \text{constant}$$

Equation	A'	B'	C'	Constant	r	r^2	S
(10)	−1.561(±2.321)	0	0	−5.095	0.612	0.374	0.675
(11)	0	8.876(±4.731)	0	−6.136	0.907	0.823	0.359
(12)	0	0	1.640(±1.174)	−5.881	0.849	0.721	0.451
(13)	−0.880(±1.009)	7.737(±3.870)	0	−5.913	0.963	0.928	0.255
(14)	−0.405(±2.277)	0	1.456(±1.724)	−5.763	0.858	0.737	0.489
(15)	0	6.822(±11.248)	0.465(±2.221)	−6.107	0.915	0.837	0.385
(16)	−1.201(±1.555)	10.432(±9.747)	−0.704(±2.267)	−5.875	0.973	0.946	0.256

Values in parentheses after the coefficients, A', B', and C', are the 95% confidence limits.

Table VI. Regression Equations of Mutation Frequency at LD_{50} for Seven Substituted Styrene Oxides as Functions of Hammett's σ, Molecular Volume, and/or Partition Parameter

$$\log[\text{MF}(LD_{50})] = A''\sigma + B'' \log[V_{W(X)}/V_{W(H)}] + C''\,\pi_{HPLC} + \text{constant}$$

Equation	A''	B''	C''	Constant	r	r^2	S
(17)	0.089(±2.113)	0	0	−5.346	0.048	0.002	0.615
(18)	0	−1.231(±7.990)	0	−5.218	0.174	0.030	0.606
(19)	0	0	−0.699(±1.385)	−5.087	0.501	0.251	0.533
(20)	−0.022(±2.677)	−1.259(±10.267)	0	−5.213	0.175	0.031	0.678
(21)	−0.730(±2.580)	0	−1.031(±1.952)	−4.873	0.593	0.352	0.554
(22)	0	7.780(±13.608)	−2.039(±2.688)	−5.344	0.736	0.541	0.466
(23)	−1.718(±0.840)	12.943(±5.252)	−3.711(±1.227)	−5.012	0.985	0.970	0.139

Values in parentheses after the coefficients, A'', B'', and C'', are the 95% confidence limits.

Discussion

As Sugiura *et al.* had already reported,[2,3] the lethal effect of substituted styrene oxides was well correlated only with hydrophobicity, expressed by π_{HPLC}. This was so even with the wider range of derivatives that we used. It is of interest that the magnitude of the lethal effect of these derivatives is not dependent upon the reactivity of the epoexide, although the presence of the epoxide must be essential for the toxicity toward microorganisms. The *p*-nitro derivative, which was excluded from the calculation of the mutagenicity correlation, could be incorporated into this correlation, probably due to the negligible contribution of the reactivity to the lethal effect.

With regard to the mutagenic capacity of this class of compounds (Table III), in contrast to the previous conclusion,[2,3] Hammett's substituent constant, σ, is not the only correlating factor, but either of the factors relating to molecular volume or hydrophobicity also seems to participate in mutagenic activity. Thus, equations 6, 9, 13, and 16 account for 93—95% of the variance in the mutagenic capacity of derivatives, whereas equation 3 or 10, which involves the term σ alone, explains 38% of the variance. Equations 4 or 11 and 5 or 12, which involve the van der Waals volume and the hydrophobicity alone, respectively, account for 82% and 71% of the variance, respectively. The molecular volume and the hydrophobicity might be related to each other in QSAR, at least for compounds without ionic or polar substituents. In any cases, the molecular volume or hydrophobicity seems to be a more important factor determining mutagenic capacity than the reactivity of the epoxide. In the data space studied here, the most significant correlation is that involving σ and V_w (Eq. 6 or 13). Incorpor-

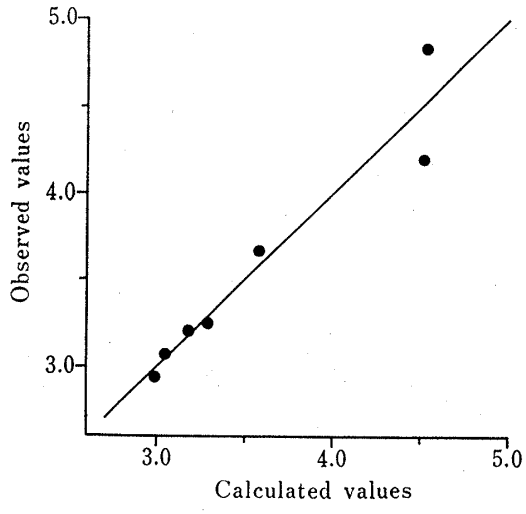


Fig. 3. Plots of the Observed log $(1/C)$ *versus* the Values calculated by Means of Eq. 6.

C is the molar concentration required to induce 10^{-6} mutation frequency.

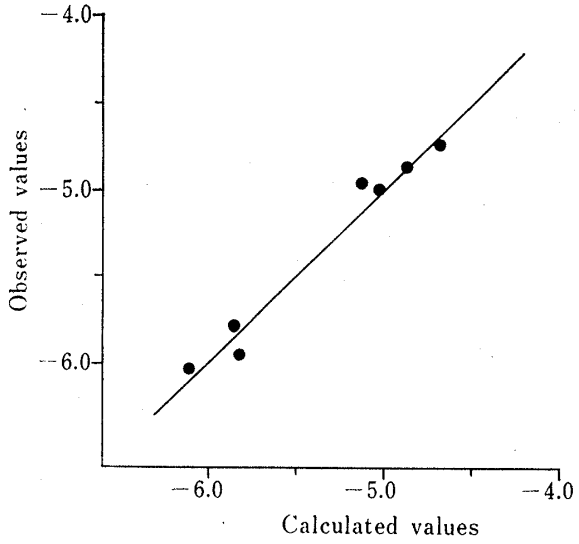


Fig. 4. Plots of the Observed log [MF(LD_{50})] *versus* the Values calculated by Means of Eq. 23.

MF (LD_{50}) is the mutation frequency at LD_{50}.

TABLE VII. *T*-Values of Each Parameter in Regression Equations

Equation	σ	$\log[V_{w(X)}/V_{w(H)}]$	π_{HPLC}	Constant
(6)	−2.438	5.575		13.562
(9)	−2.230	3.085	−0.764	12.953
(13)	−2.423	5.558		−23.144
(16)	−2.459	3.404	−0.988	−22.925
(23)	−6.500	7.805	−9.622	−36.154

ration of π_{HPLC} (Eq. 9 or 16) does not seems to improve the correlation to an appreciable extent, the *t*-test showing a negligible contribution of π_{HPLC} (Table VII). Equation 6 provides the calculated log $(1/C)$ values, which are fairly close to the observed values (Fig. 3). An interesting correlation was found with regard to the mutation frequency at LD_{50}, which might be a measure of relative efficiency of mutagenic and lethal effects of the chemicals. Thus, as seen in Table VI, Eq. 23 gives a correlation which includes all 3 parameters, whereas all the other equations do not show significant correlations. The values calculated by Eq. 23 are very close to the observed ones, as can be seen in Fig. 4.

The results obtained in the present study suggest that the mutagenicity of this group of compounds is enhanced by increases in (i) the electrophilic reactivity of the α-CH carbon of the epoxide and (ii) the van der Waals volume of the molecule, and that the mutation frequency at LD_{50} is enhanced by increases in (i) the electrophilicity, (ii) the molecular volume, and (iii) the hydrophilicity of the compounds. However, a more careful study is needed for elucidation of the molecular mechanism of the mutagenesis, although our correlation presented here is more general than that reported by Sugiura *et al.* From a chemical viewpoint, in conclusion, it is worth emphasizing that the chemical reactivity may be quantitatively correlated with mutagenic capacity, taking appropriate physicochemical properties of the mutagens into consideration.

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