

Reaction Mechanism in Aromatic Heterocyclic Compounds. X.¹⁾ Kinetics of the Reaction of Carcinogenic 4-Nitroquinoline 1-Oxide and Its Derivatives with Sodium Ethoxide

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Basic kinetic data, rate constants, activation energies, and entropies of activation were elucidated for the reactions of 22 kinds of 4-NQO derivatives with sodium ethoxide in anhydrous ethanol by spectroscopic-method. Correlations existed between the rate constants and carcinogenicities, and half-wave reduction potentials. Hammett parameters were applied to the rate constants, and a linear correlation existed in 6- and 7-substituted derivatives. Interactions of 4-nitro group and 3- or 5-position substituents retarded the rate, but for 2- or 8-position, only bulky substituents such as methyl group interacted with the N-oxide group.

A potent carcinogen, 4-nitroquinoline 1-oxide (4-NQO), synthesized first by Ochiai and co-workers³⁾ in 1943, has been found to be carcinogenic in 1957 by Nakahara and co-workers.⁴⁾ Since 4-NQO has a simple chemical structure and a strong activity as a carcinogen, many studies have been undertaken to elucidate the structure-carcinogenicity relationship.⁵⁻¹⁴⁾

It was suggested in earlier time that, for the carcinogenicity of this type of compounds, 4-nitro group and N-oxide group are essential and the nucleophilic reactivity at 4-position might be closely related.^{4,6,8)} From this point of view, several investigations have been made, such as the reactivities to nucleophilic reagents,^{7,10)} calculation of quantum-chemical

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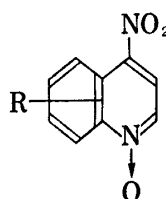
reactivity coefficients,⁹⁾ and the measurement of half-wave reduction potentials by polarography.¹³⁾

Kinetic studies on the reactivity of 4-nitro group have been made only by Okamoto and Itoh¹⁰⁾ with five kinds of 4-NQO derivatives and two related compounds, but no definite conclusion has yet been drawn.

The present study employed 22 kinds of 4-NQO derivatives and their basic kinetic data were evaluated. The reactions were carried in anhydrous ethanol with sodium ethoxide as a nucleophilic reagent, using a quartz cell of ultraviolet spectroscopy, held in a newly developed isotherm chamber, as the reaction chamber.

From a continuous and direct measurement of changes in absorption of the reaction solution, reaction rate constants, and energies and entropies of activation were determined.

TABLE I. Materials for Kinetics



R-	mp (°C)	Synthesis	Carcinogenicity
H-	154—155	(a)	(n)
2-CH ₃ -	156—157	(b)	(o)
3-CH ₃ -	172—173	(c)	(p)
5-CH ₃ -	179—180	(c)	(p)
6-CH ₃ -	189—191	(d)	(p)
7-CH ₃ -	167—168	(c)	(p)
8-CH ₃ -	155—156	(c)	(p)
3-CH ₃ O-	195—200	(e)	(p)
6-CH ₃ O-	209—210	(f)	
2-Cl-	159—160	(f)	
3-Cl-	160—161	(c)	(p)
5-Cl-	150—151	(c)	(p)
6-Cl-	199—200	(d)	(o, p)
7-Cl-	222—225	(g)	(p)
6,7-DiCl-	192—193	(c)	(p)
6-F-	147—148	(h)	(q)
8-F-	161—162	(i)	
3-Br-	160	(j)	(p)
6-CO ₂ Na-	over 300	(k)	(r, s)
5-NO ₂ -	261—262	(l)	(p)
6-NO ₂ -	221—223	(m)	(q)
8-NO ₂ -	220—221	(l)	(p)

a) All melting points were measured on Yanagimoto micromelting point apparatus and not corrected.

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(n) ref. 4 (o) ref. 6 (p) ref. 12
(q) Y. Kawazoe, unpublished data (r) ref. 11 (s) ref. 14

Experimental

Materials—Materials for the kinetic measurements are listed in Table I.

Solvent and Reagent—Anhydrous ethanol was prepared by refluxing 99% ethanol with sodium, and taking the median fraction on distillation. For the preparation of the ethoxide reagent, freshly cut sodium lumps were transferred rapidly into a convenient volume of anhydrous ethanol, and resulting solution was stored in a glass stoppered flask protected from moisture and carbon dioxide. The ethoxide reagent was titrated with 0.1N or 0.02N HCl solution to determine the factor after adding about 2.5 volumes of water, phenolphthalein being used as the indicator.

Ultraviolet (UV) Spectra—The UV spectra of 4-NQO derivatives, and their 4-ethoxy compounds or reaction solutions are largely classed into four types. Typical patterns of each type are presented in Fig. 1. Beer's law was proved to hold in the region of concentration for the kinetic measurements.

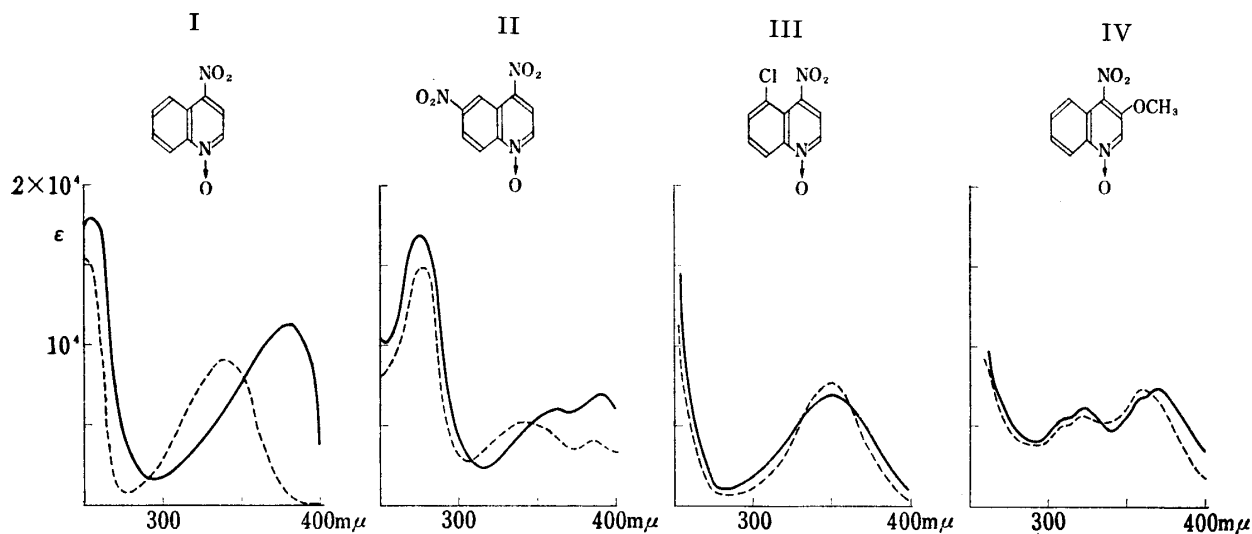


Fig. 1. Typical Patterns of UV Spectra

— NO₂ - - - OEt
 type I: 4-NQO, its 5-NO₂ deriv., and all 2-, 6-, 7-, and 8-substituted derivs. except 6-NO₂ deriv.
 type II: 6-NO₂ deriv.
 type III: All 3- and 5-substituted derivs. except 3-OCH₃ deriv.
 type IV: 3-OCH₃ deriv.

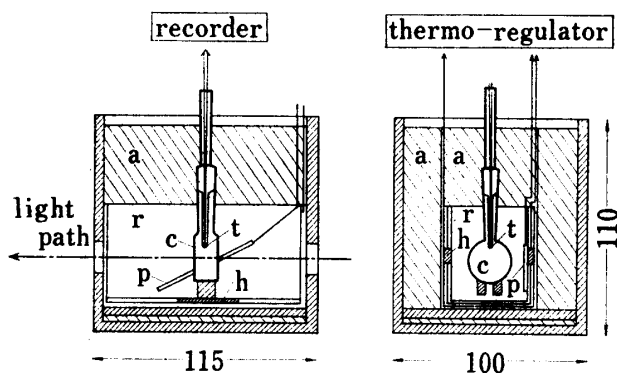


Fig. 2. Sectional Diagram of Isotherm Chamber for Kinetics (unit in mm)

a: adiabatic layer c: reaction cell h: heater
 p: platinum resistance r: radiator board t: thermistor
 This chamber was placed in the sample chamber of Cary Model 11 spectrometer.

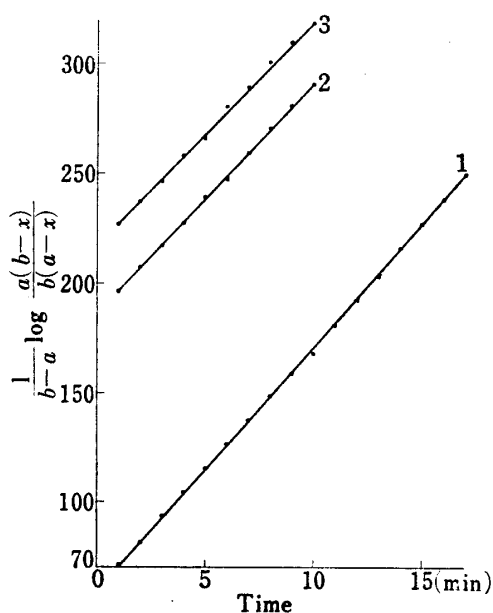


Fig. 3. Plots of $[1/(b-a) \log [a(b-x)/b(a-x)]]$ vs. Time (4-NQO)

a: concn. of reactant = $1.646 \times 10^{-4}M$
 b: concn. of reagent = $2.030 \times 10^{-2}M$ (1),
 $1.000 \times 10^{-2}M$ (2), $4.864 \times 10^{-4}M$ (3)

Apparatus—Reactions for the kinetic measurements were carried out in the UV cell held in a sample chamber of Cary Model 11 spectrometer, and a newly developed isotherm chamber to control the reaction temperature. The isotherm chamber is shown in Fig. 2. Current for the heater was controlled by the temperature of copper radiator board with platinum resistance, both connected to an oscillatory auto-regulatory thermometer. The reaction temperature was measured directly in the reaction solution with a thermistor and recorded *via* a bridge circuit. Polystyrol foam was used for adiabatic layer.

Rate Measurement by Spectroscopic Method—As shown in Fig. 3, the reaction was proved to follow the second order reaction kinetics and, in the presence of excess reagent, rate constant was measured as a pseudo-first order reaction kinetics. Rate constant, k , was derived as $k = (2.303/bt) \log(a/a-x)$, where a

TABLE II. Typical Kinetic Runs (i) Optical Densities of Reaction Solutions of 6-Methyl-4-NQO with Sodium Ethoxide at Each Time

Time (min)	Optical densities (Et)				
	(1)	(2)	(3)	(4)	(5)
1	1.634	1.562	1.491	1.381	1.067
2	1.624	1.546	1.464	1.345	1.023
3	1.615	1.530	1.440	1.316	0.986
4	1.602	1.512	1.410	1.279	0.948
5	1.591	1.491	1.389	1.247	0.911
6	1.579	1.473	1.361	1.214	0.874
7	1.564	1.457	1.336	1.180	0.841
8	1.555	1.440	1.316	1.151	0.807
9	1.541	1.423	1.297	1.120	0.779
10	1.534	1.407	1.270	1.091	0.748
	0.013	0.013	0.013	0.012	0.012
Temp.	19.4	23.7	28.1	33.1	37.6

$a = [6\text{-methyl-4-NQO}] = 1.663 \times 10^{-4} M$
wavelength 380.0 m μ

$b = [\text{NaOEt}] = 1.720 \times 10^{-2} M$
optical path 10 mm

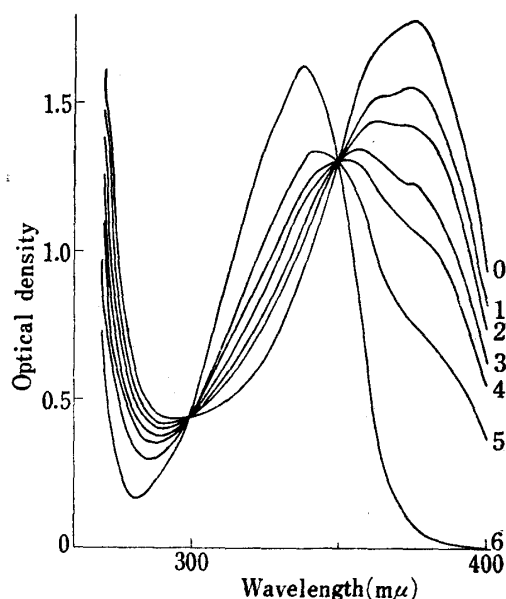


Fig. 4. Typical Kinetic Runs (ii)

UV spectra of reaction solution
0: initial time
1—5: immediately after each measurement
6: after infinite time
These spectra show that no by-product existed during these measurements.

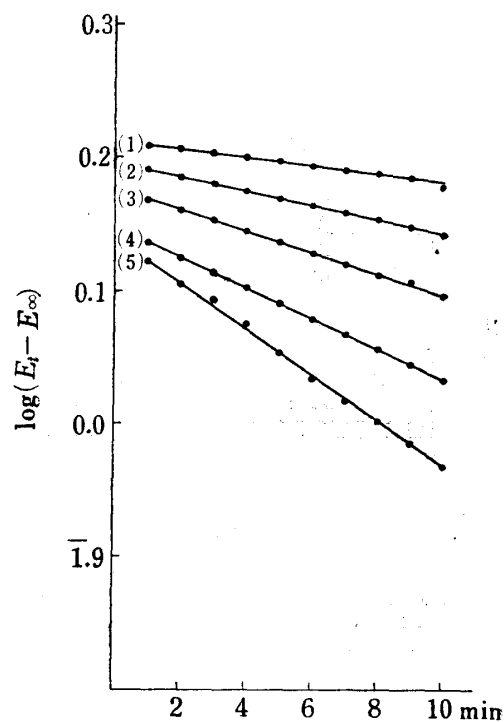


Fig. 5. Typical Kinetic Runs (iii)

plots of $\log(Et - E_{\infty})$ vs. time

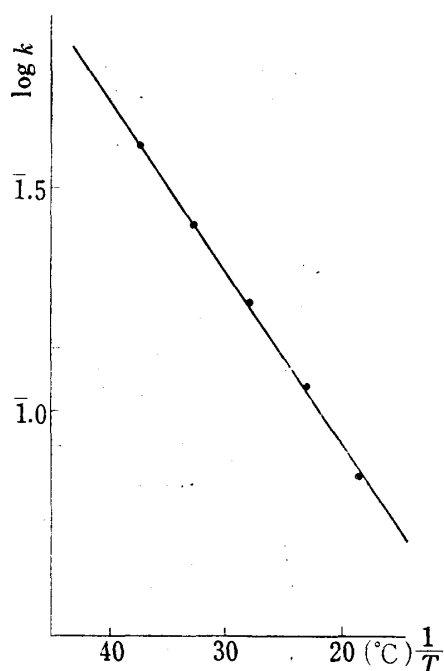


Fig. 6. Typical Kinetic Runs (iv)
plots of $\log k$ vs. $1/T$

and b are the initial concentrations of reactant and reagent, and x is the concentration of the product after t seconds. By Beer's law, the UV absorption at t second, E_t , is the sum of the absorptions of each component, then, $\log (E_\infty - E_t) = -(kbt/2.303) + \log (E_t - E_0)$, where E_0 , and E_∞ are the absorptions of the reaction solution at initial and infinite time respectively. Then rate constant was calculated as the slope of plots, $\log (E_\infty - E_t)$ vs. time, by use of the least square method. From these figures at each temperature, the line of plots, $\log k$ vs. $1/T$, was determined by the least square method, then rate constants, and entropies of activation at 40° , and activation energies were calculated by the equations, $\log k = -(E_a/2.303 RT) + \log A$, $\log A = (kT/h) + (\Delta S^\ddagger + R)/2.303 R$, where E_a and ΔS^\ddagger are the energy and entropy of activation, respectively.

To examine the influence of UV ray on measurement, especially of its wavelength, the rate constants were measured continuously at 380.0, 330.0, 260.0, 230.0, and 220.0 $m\mu$, and every 5 minutes at 380 $m\mu$ with 4-NQO, but no significant difference was found.

Typical examples of the rate constant measurements are described for 6-Me-4-NQO, in Table II, and in Fig. 4, 5, and 6.

Results and Discussion

Calculated rate constants and entropies of activation at 40° , and activation energies are listed in Table III. These rate constants are clearly correlated with the data of Okamoto and Itoh¹⁰ for five 4-NQO derivatives with thioglycolic acid as a reagent, in a mixed solution

TABLE III. Summary of Kinetic Data

Compound	$k_{(M^{-1}sec^{-1})}^{at 40^\circ}$	$E_a \pm S.D. (kcal)$	$S^\ddagger \pm S.D. (e.u.)^{at 40^\circ}$	Wavelength ($m\mu$)	Run. No.
6-NO ₂	18.46×10	—	—	390.0	3
6,7-DiCl	14.13	11.7 ± 0.9	-18.1 ± 3.0	380.0	3
8-NO ₂	12.72	—	—	390.0	4
2-Cl	8.43	14.1 ± 0.7	-11.4 ± 2.3	380.0	5
7-Cl	6.45	12.0 ± 0.7	-18.5 ± 2.2	380.0	5
6-Cl	5.12	14.8 ± 0.4	-10.3 ± 1.4	380.0	5
8-F	3.24	13.1 ± 0.3	-16.5 ± 1.1	380.0	4
6-F	2.33	16.1 ± 0.5	-7.6 ± 1.6	380.0	5
5-Cl	17.23×10^{-1}	14.5 ± 0.5	-13.2 ± 1.6	385.0	4
3-Cl	13.89×10^{-1}	13.2 ± 1.0	-17.9 ± 3.4	380.0	5
4NQO	11.27×10^{-1}	15.8 ± 0.4	-10.0 ± 1.3	380.0	12
3-Br	9.32×10^{-1}	14.0 ± 0.4	-16.2 ± 1.4	375.0	5
7-CH ₃	8.48×10^{-1}	17.0 ± 0.5	-6.7 ± 1.6	380.0	5
5-NO ₂	5.95×10^{-1}	—	—	380.0	6
6-CH ₃	4.91×10^{-1}	16.7 ± 0.5	-8.8 ± 1.5	380.0	5
5-CH ₃	4.10×10^{-1}	13.8 ± 0.2	-18.2 ± 0.7	375.0	5
6-CO ₂ Na	4.03×10^{-1}	17.4 ± 0.2	-7.0 ± 0.7	380.0	4
2-CH ₃	2.67×10^{-1}	14.6 ± 0.5	-16.6 ± 1.5	380.0	9
8-CH ₃	2.11×10^{-1}	16.6 ± 0.2	-10.6 ± 0.9	380.0	5
6-CH ₃ O	18.41×10^{-2}	15.3 ± 0.6	-15.0 ± 1.9	370.0	12
3-CH ₃	8.84×10^{-2}	10.1 ± 0.8	-33.1 ± 2.5	310.0	4
3-CH ₃ O	7.03×10^{-3}	14.3 ± 1.6	-24.7 ± 5.4	390.0	5

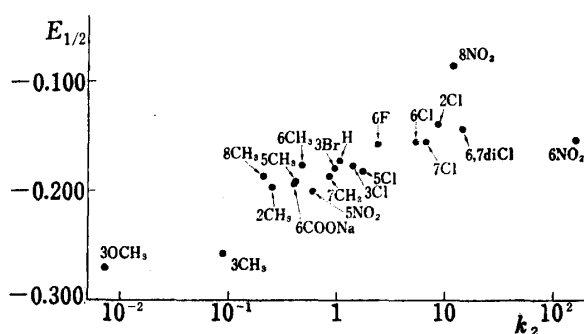


Fig. 7. Rate Constants and Reduction Potentials

of dioxan and water. They are also correlated with the half-wave reduction potentials in polarography, measured by Kawazoe and co-workers¹³⁾ with nearly the same materials as in the present work, as shown in Fig. 7.

This fact shows that both parameters represent the nucleophilicity of 4-position.

As shown in Fig. 8, the rate constants of carcinogenic derivatives are within a definite region, from 10^{-1} to $10 \text{ M}^{-1} \text{ sec}^{-1}$, except nitro derivatives, which have some uncertain points in the rate measurements by their low solubilities in a solvent and in biological assays. From this results, there might be a distinct correlation between carcinogenicity and reactivity of 4-NQO derivatives at 4-position.

Application of Hammett parameters to the rate constants was attempted with σ^*_{ortho} for 3-position, σ_{cata} and σ_{epi} ¹⁵⁾ for 6- or 7-position, and σ_{ana} (σ_5-1)¹⁶⁾ for 8-position, as shown in Fig. 9. For 3-position, the rate constants of 3-methyl and 3-methoxy derivatives, abnormally small in appearance, seem to be reasonable for *ortho*-substituent effect. The rates of 5-substituted derivatives are faster than that of 3-substituted derivatives, in spite of larger steric hindrance of 4-nitro group with 5-position substituent. It suggests that some effects such as steric acceleration effect or electronic effect on 4-nitro group and/or N-oxide group might play a significant role. In 6- and 7-substituted derivatives, differences in the rate constants are apparently determined by the electronic effect of substituents, and the position of substituents affected little. For 8-position, only the rate of methyl substituent deviated from the correlation to a smaller value than ordinary methyl substituent effect. This might be due to the interaction of methyl group with the N-oxide group. The same tendency was also observed for 2-position.

Energies and entropies of activation were calculated, as shown in Table III, but detailed discussion could not be made only from these data. Further investigations are in progress including spectral and physical properties.

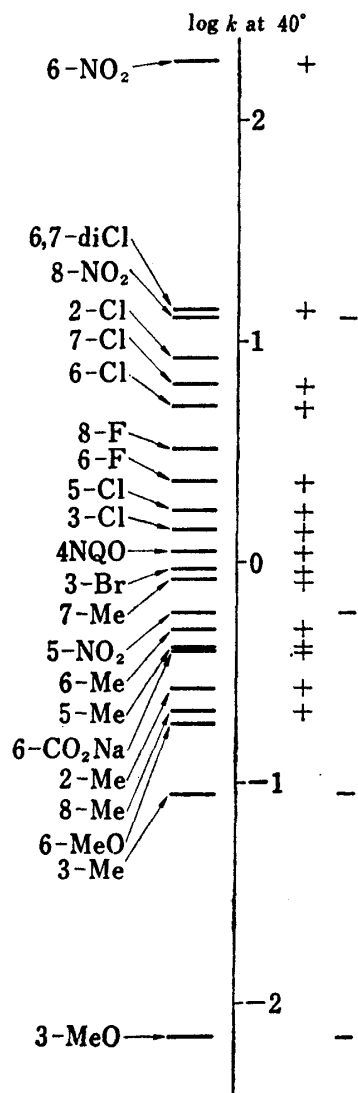


Fig. 8. Rate Constants and Carcinogenicity

+ : carcinogen
- : non-carcinogen

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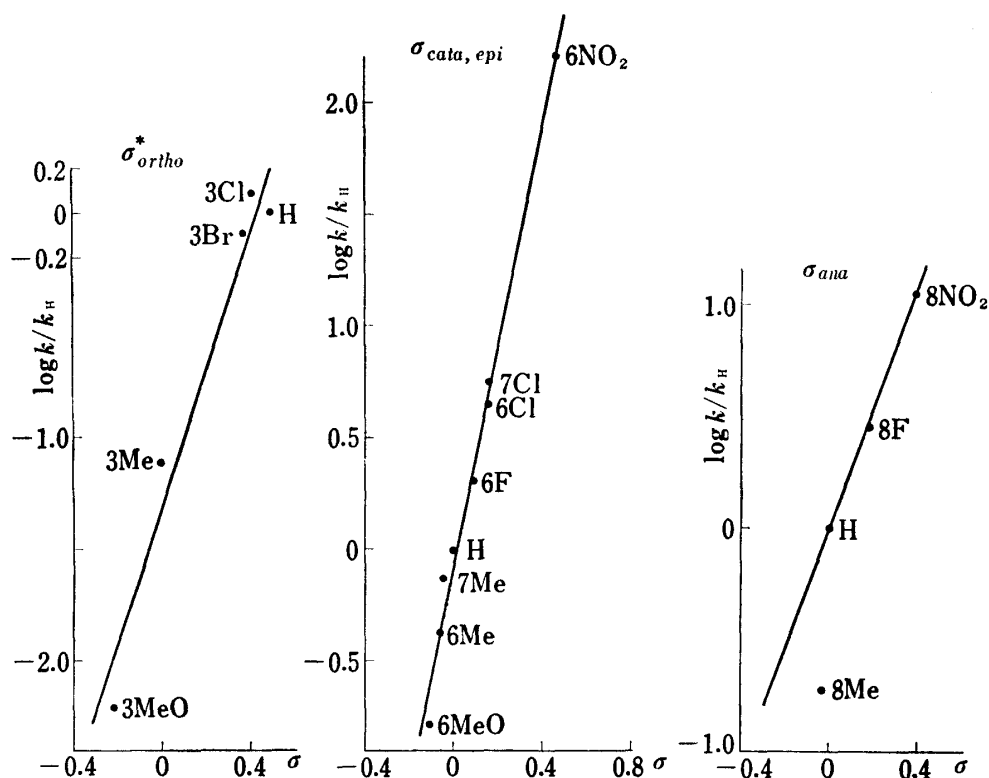


Fig. 9. Rate Constants and Hammett Parameters

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