Summary

1-(N-Monosubstituted amido)pyridinium compounds derived from novel quaternary salts of pyridine, N-aminopyridinium compounds, were quite reactive to nucleophilic agents at 2- and 4-positions of their pyridine rings, especially at 4-position. The 1-(N-monosubstituted amido)pyridinium salts were reacted with cyanide ion to give N-monosubstituted amides and cyanopyridines, (predominantly 4-cyanopyridine), both in good yields. The reaction may involve dihydro-type intermediates and one intermediate 1-(N-methylacetamino)-4-cyano-1,4-dihydropyridine could be obtained stably.

By utilizing this reaction, it would be possible to introduce a cyano group at 4-position of various pyridine derivatives and to obtain various primary amines.

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138. Toshihiko Okamoto and Michiya Itoh : Reaction Mechanism in Aromatic Heterocyclic Compounds. V.*1 Kinetics of the Reaction of 4-Nitroquinoline 1-Oxide and Related Compounds with Thioglycolic Acid.*2

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The 4-nitro or 4-halo groups in quinoline 1-oxides are easily replaced by alkoxydes, phenoxydes or amines,[1] and these 4-substituents in quinoline are reactive toward nucleophlic reagents. Thus, some attempts have been made to correlate the reactivities of aromatic substitution reactions with the effects of heteroaromatic N-oxide groups.[2,3] 4-Nitroquinoline 1-oxide reacts readily with the SH groups of thioglycolic acid, of cystein and of glutathion to afford the corresponding sulfides of quinoline 1-oxide.[4]

On the other hand, 4-nitroquinoline 1-oxide has been known to be a skin cancer producing agent, and W. Nakahara, *et al.* have suggested that the carcinogenic action of this compound might be due to the nucleophlic reactivity at 4-position.[5]

In 4-nitroquinoline 1-oxide and its related compounds, H. Endo reported that there was a certain relationship between the chemical structure and carcinogenic activity.[6] But kinetic studies on the reactivities of these compounds have not been made, thus comparative data for the reactivities of 4-nitro groups in the 4-nitroquinoline 1-oxide and its related compounds are needed.

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*1 Part IV. This Bulletin, **11**, 514 (1963).

*2 A part of this work was reported at the 19th general meeting of the Japanese Cancer Association (Dec. 1960).

*3 Hongo, Bunkyo-ku, Tokyo (岡本敏彦, 伊藤道也).

1) E. Ochiai : J. Org. Chem., **18**, 534 (1953).
2) Collonna. Risalti : Gazzetta, **83**, 58 (1953); Reitsema : Chem. Rev., **43**, 58 (1948).
3) T. Okamoto, H. Hayatsu, Y. Baba : This Bulletin, **8**, 892 (1960).
4) T. Okabayashi : Yakugaku Zasshi, **73**, 936 (1953).
5) W. Nakahara, F. Fukuoka : Gann, **50**, 1 (1959).
6) H. Endo : *Ibid.*, **49**, 151 (1957).

For this purpose, the following 4-nitro compounds were employed, and the nucleophlic substitution reactions between 4-nitro groups of these compounds and SH group of thioglycolic acid were studied in several kinds of solvent system.

The reaction of the 4-nitro group with the SH group was proved to follow bimolecular reaction kinetics, reaction rate constants were determined, and energies and entropies of activation were calculated.

Experimental

Reactants and Products——Reactants : The following compounds were prepared by the methods known in the literatures, and recrystallized before the use. The melting points of these compounds showed good agreement with values in the literatures.

	m.p. (°C)
4-Nitroquinoline 1-oxide[7]	153～154
4-Nitroquinoline[8]	87
4-Nitro-7-chloroquinoline 1-oxide[9]	219 (decomp.)
4,8-Dinitroquinoline 1-oxide[10]	222～223 (〃)
4,6-Dinitroquinoline 1-oxide[10]	218 (〃)
4-Nitroquinaldine 1-oxide[11]	157
4-Nitropyridine 1-oxide[12]	159

These compounds were reacted easily with thioglycolic acid in EtOH-H_2O mixed system affording the corresponding 4-quinoline- or 4-pyridine-thioacetic acids. The structures of these products were confirmed by the IR and UV spectroscopy, and also by elementary analysis.

4-Quinolinethioacetic Acid 1-Oxide[13] **(I)**——Into the solution of 0.3 g. of 4-nitroquinoline 1-oxide in 200 ml. of 50% EtOH, 0.32 g. of thioglycolic acid was added. This solution was adjusted to pH 7 with 10% NaOH sol., and then heated for 2 hr. at 35°. After the reaction finished, this solution was acidified to pH 2.5～3.0 with *N* HCl sol., to afford crude white precipitate, 0.28 g. (yield. 78%). Colorless needles of m.p. 205～207° (decomp.) was obtained by recrystllization from MeOH containing (iso-Pr)$_2$O. *Anal.* Calcd. for $C_{11}H_9NO_3S$: C, 56.16; H, 3.83; N, 5.96. Found : C, 55.66; H, 3.66; N, 5.99.

4-Quinaldinethioacetic Acid 1-Oxide (II)——To the solution of 0.4 g. of 4-nitroquinaldine 1-oxide in 100 ml. 50% EtOH, 0.4 g. thioglycolic acid was added. After adjusting the reaction solution to pH 7 with 10% NaOH sol., this solution was heated at 70° for 3 hr., then the reaction mixture was treated as described previously to get 0.416 g. of crude product (85% yield.). The resulting product was recrystallized from H_2O containing Me_2CO to colorless needles of m.p. 209～210° (decomp.). *Anal.* Calcd. for $C_{12}H_{12}NO_3S$: C, 57.83; H, 4.41; N, 5.55. Found : C, 57.38; H, 4.17; N, 6.02.

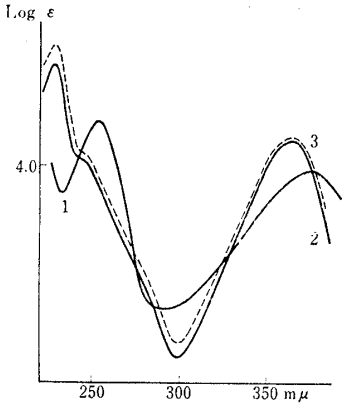
7-Chloro-4-quinolinethioacetic Acid 1-Oxide (III)——To 0.14 g. of 4-nitro-7-chloroquinoline 1-oxide, 0.2 g. of thioglycolic acid in 300 ml. 95% EtOH was mixed. By the same treatment as previously mentioned, crude product 0.12 g. was obtained (yield. 71.5%), and this was recrystallized from MeOH to afford colorless needles of m.p. 205～208° (decomp.). *Anal.* Calcd. for $C_{11}H_8NO_3SCl$: C, 48.8; H, 2,96; N, 5.18. Found : C, 48.25; H, 2.91; N, 5.29.

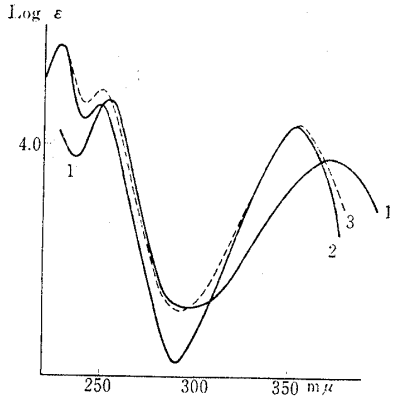
8-Nitro-4-quinolinethioacetic Acid 1-Oxide (IV)——To 4,8-dinitroquinoline 1-oxide 0.3 g. in 300 ml. 95% EtOH, thioglycolic acid 0.25 g. was mixed, and this mixture was adjusted to pH 7 with 10% NaOH sol. This solution was heated for 1 hr. at 35° to afford crude product 0.27 g. (yield. 75.4%). By the recrystallization from MeOH, light yellow needles was obtained, m.p. 185～188° (decomp.). *Anal.* Calcd. for $C_{11}H_8N_2O_5S$: C, 47.14; H, 2.86; N, 10.00. Found : C, 46.92; H, 2.86; N, 10.35.

6-Nitro-4-quinolinethioacetic Acid 1-Oxide (V)——This compound was prepared similarly from 4,6-dinitroquinoline 1-oxide (yield. 67.5%). Yellow needles was obtained by the recrystallization from MeOH, m.p. 195～198° (decomp.). *Anal.* Calcd. for $C_{11}H_8N_2O_5S$: C, 47.14; H, 2.85; N, 10.00. Found : C, 46.92; H, 2.85; N, 10.35.

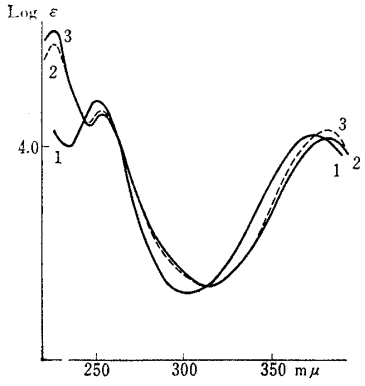
4-Quinolinethioacetic Acid (VI)——Into the solution of 0.5 g. of 4-nitroquinoline in 100 ml. EtOH, thioglycolic acid 0.7 g. was added. After adjusting this solution to pH 7 with 10% NaOH sol., this

7) E. Ochiai, M. Ishikawa, Z. Sai : Yakugaku Zasshi, **63**, 280 (1943).
8) M. Hamana : *Ibid.*, **75**, 135 (1955).
9) S. Yoshida : *Ibid.*, **66B**, 158 (1946).
10) M. Ishikawa : Proc. Imp. Acad. (Tokyo), **XX**, 599.
11) *Idem* : Yakugaku Zasshi, **65B**, 99 (1945).
12) E. Ochiai, M. Ishikawa, K. Arima : *Ibid.*, **63**, 79 (1943).
13) T. Okabayashi : *Ibid.*, **74**, 936 (1953).

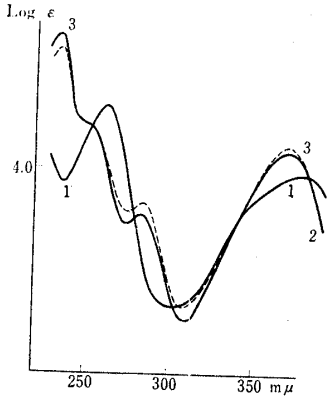




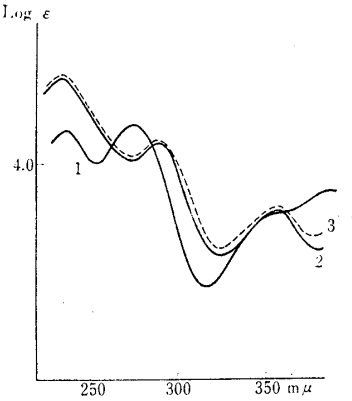
1 : 4-nitroquinoline 1-oxide
2 : 4-quinolinethioacetic acid 1-oxide
3 : reaction sol.



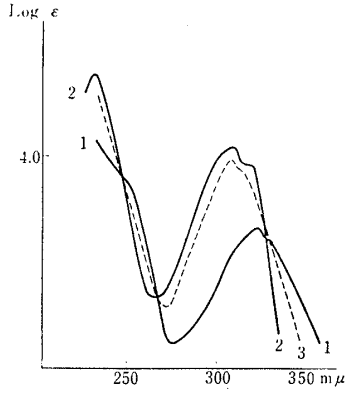
1 : 4-nitro-7-chloroquinoline 1-oxide
2 : 7-chloro-4-quinolinethioacetic acid 1-oxide
3 : reaction sol.



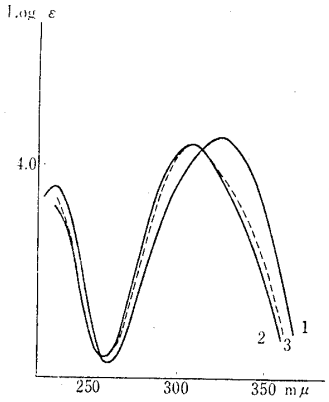
1 : 4,8-dinitroquinoline 1-oxide
2 : 8-nitro-4-quinolinethioacetic acid 1-oxide
3 : reaction sol.



1 : 4,6-dinitroquinoline 1-oxide
2 : 6-nitro-4-quinolinethioacetic acid 1-oxide
3 : reaction sol.



1 : 4-nitroquinoline
2 : 4-quinolinethioacetic acid
3 : reaction sol.



1 : 4-nitropyridine 1-oxide
2 : 4-pyridinethioacetic acid 1-oxide
3 : reaction sol.

Fig. 1. Ultraviolet Spectra of Reactants, Products and Reaction Sol.

solution was heated at 70° for 5 hr. to afford crude product 0.392 g. by similar procedures (yield. 65%). By recrystallization from MeOH containing H_2O, colorless needles was obtained, m.p. 223~225°. *Anal.* Calcd. for $C_{10}H_9O_2NS$: C, 60.27; H, 4.10; N, 6.39. Found : C, 59.72; H, 3.78; N, 6.33.

Ethyl 4-Pyridinethioacetate 1-Oxide (VII)——Into the solution of 0.76 g. 4-nitropyridine 1-oxide in 200 ml. of 50% EtOH, 1 g. of thioglycolic acid was added. After adjusting this solution to pH 7 with 10% NaOH sol., this was heated for 2 days at 50°. Then, the solution was evaporated to dryness *in vacuo*, and the residue was esterified by absolute EtOH and dry HCl gas. The residue was purified by the alumina-chromatography(benzene), and recrystallized from benzene containing Me_2CO to afford coloriess needles 0.68 g., yield. 59%, m.p. 89~90°. *Anal.* Calcd. for $C_9H_{11}NO_3S$: C, 50.70; H, 5.16; N, 6.57. Found : C, 50.76; H, 4.99; N, 6.62.

Reaction Solvent——The reaction solvent systems were obtained by mixing the solvents A and B, and the solvents A and B had following compositions.

Solvent A			Solvent B	Dioxane/H_2O of reaction sol.
Dioxane (ml.)	H_2O (ml.)	Phosphate buffer (ml.)	Dioxane (ml.)	(%)
5	5	2	2	50 (v/v)
6	4	2	2	57.1
7	3	2	2	64.3

UV-Spectra of Reactants and Products——1 ml. of the reaction solution by which rate measurement was made, (to be mentioned in next section.) was pippeted into 10 ml. of 95% EtOH, and UV spectrum of this solution was determined, as shown in Fig. 1. These spectra were compared with those of the previously mentioned products in the same solvent systems.

Form the spectra, no by-product was detected, and UV spectra of the reactants in 95% EtOH are also shown in Fig. 1.

Rate Measurement by The Amperometic Titration Technique[14]——4-Nitroquinoline 1-oxide was dissolved in the solvent A at the concentration of 1×10^{-3}~$10^{-4}M$ (solution A). In the solvent B thioglycolic acid was dissolved at the concentration of 1×10^{-2}~$10^{-3}M$ (solution B).

Flask A (containing solution A) and Flask B (sol. B) were immersed into the thermostat adjusted to the definite temperature (25°, 30°, 35°, 40° and 45°±0.1°). When sol. A and B are returned to the temperature of thermostat, the reaction between 4-nitro compound and thioglycolic acid was initiated by mixing 2 ml. of solution B into the solution A, and this time was recorded as zero time. 1 ml. of the reaction sol. was pippetted into the amperometric titration solution (containing 10 ml. of ammonia-buffer, 0.2 ml. of 0.01*N* EDTA sol. and 1 ml. sample of the reaction solution.) in which a platinium electrode was rotating. Then SH concentration in the sample was determined by the titration with standard silver nitrate solution($10^{-3}M$). Subsequently, each 1 ml. sample of the reaction solution were removed and titrated at definite intervals, all times being recorded.

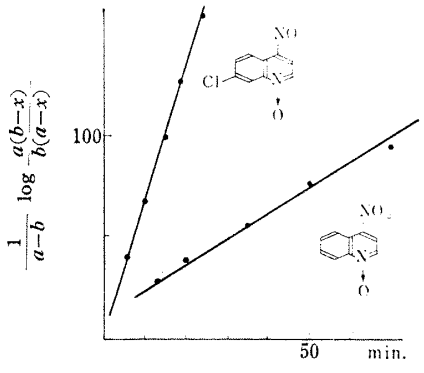
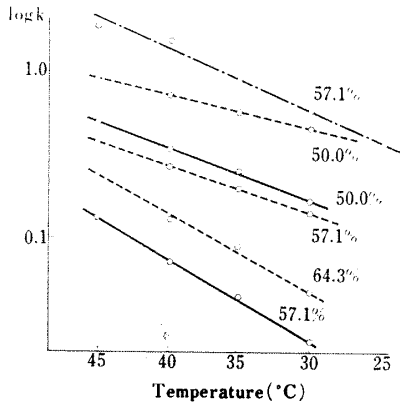


Fig. 2. Example of Plots of $\frac{1}{b-a}\log\frac{a(b-x)}{b(a-x)}$ *vs.* Time



-·-·-·: 4,8-dinitroquinoline 1-oxide
- - - - : 4-nitro-7-chloroquinoline 1-oxide
——— : 4-nitroquinoline 1-oxide

Fig. 3. Example of Plots of log *k* *vs.* $1/T$

14) I. M. Kolthoff : Ind. End. Chem., Anal. Ed., 18, 161 (1946).

If it was assumed that the reaction between 4-nitro compound and thioglycolic acid followed second order kinetics,[15] rate constants were calculated from a following equation; $k=\frac{1}{t(b-a)}\ln\frac{a(b-x)}{b(a-x)}$ where a and b are the initial concentration of nitro compound and thioglycolic acid, respectively, and x is the concentration of the product at time t. Fig. 2 shows the plots of $\frac{1}{b-a}\log\frac{a(b-x)}{b(a-x)}$ *vs.* t, and the plots were linear. Furthermore, ks were independent on a and b.

Thus, the reaction between these nitro compounds and thioglycolic acid was proved to follow the second order kinetics. Second order rate constants, ks were determined from the slopes of the lines which were calculated by the method of least squares. Fig. 3 shows the plots of $\log k$ *vs.* $1/T$, the plots are linear. Energies of activation were calculated from the slopes of this lines (by the method of least squares) by Arrhenius relationship : $\log k=\log A-\frac{\Delta E^{\ddagger}}{2.303\times RT}$

Entropies of activation $\Delta S^{\ddagger}$ at 313.0°K (40°) were obtained by the following equation :

$$\Delta S^{\ddagger}=2.303R\left(\log k-\log\frac{kT}{h}+\frac{\Delta E^{\ddagger}}{2.303RT}\right)$$

Results and Discussion

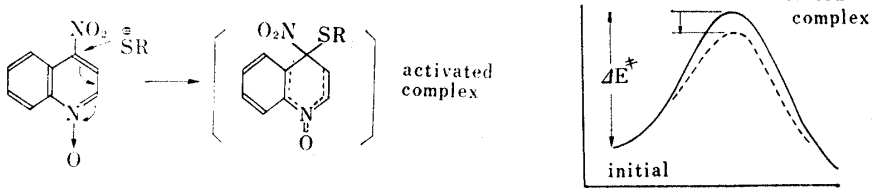
Rate constants at 40°C, energies of activation $\Delta E^{\ddagger}$ and entropies of activation $\Delta S^{\ddagger}$ are summarized in Table I.

As shown in the Table, 4-nitroquinoline 1-oxide reacts 5~30 times faster than 4-nitroquinoline. Further, the substituted 4-nitroquinoline 1-oxide which have electro-attractive group in benzene ring react more faster than 4-nitroquinoline 1-oxide itself. The 1-oxide group is considered as the strong accelerating group for this nucleophilic substitution reaction. As the solvent become more polar (the ratio water/dioxane increases,[16]) the rate constant of 4-nitroquinoline 1-oxide greatly increases, but that of 4-nitroquinoline increases only slightly. This fact shows that the effect of 1-oxide group to the 4-position are largely influenced by the condition of reaction solution. And this fact is referred to the variation of $\Delta S^{\ddagger}$, which will be discussed further.

The polarity of a solvent greatly affects the reaction rate, and the order of rate constants of a given compound are parallel to that of the polarity of a solvent. In the 4-nitroquinoline 1-oxide, the variation of K, $\Delta E^{\ddagger}$ and $\Delta S^{\ddagger}$ by the polarity of the solvent are shown as follow :

H_2O/dioxane	small	⟶	large
$\Delta E^{\ddagger}$	large	⟶	small
$\Delta S^{\ddagger}$	large	⟶	small (large negative)
K	small	⟶	large

On the contrary, in 4-nitroquinoline, these variation are unexpectedly small, even if the polarity of a reaction solvent are increased. Consequently, it may be considered that the interaction between 1-oxide group and solvent molecules is characteristic in this nucleophilic substitution reaction. Thus, assuming the activated complex as indi-



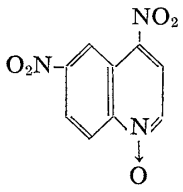
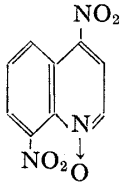
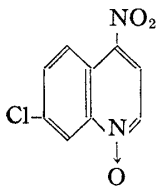
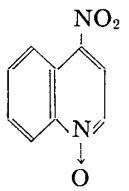
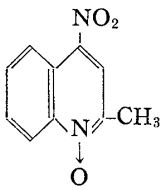
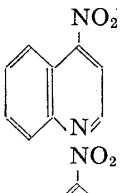
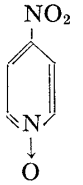
15) J.F. Bunnett, R.E. Zahler : Chem. Revs., **49**, 382 (1951). J.F. Bunnett : Quart. Revs. (London), XII, 1(1958).

16) S. Brownstein : Can. J. Chem., **38**, 1590 (1960).

cated in a next formula, the formula may be stabilized by the increasing of solvent polarity, and consequently $\Delta E^{\neq}$ seems to be decreased.[17]

It is known that the rate of reaction involving the formation of a stabilized activated complex is dependent on a solvent, and $\Delta E^{\neq}$ are decreased by increasing solvent polarity.[18] On the other hand, as indicated in Table, the entropies of activation are greatly dependent on the solvent system and decreased (large negative value) with a increasing of solvent polarity.

Table I.

Dioxane; H_2O / Nitro comp.	50.0%			57.1%			64.3%		
	$K\times10^{-3}$ [a]	$\Delta E^{\neq}$ [b]	$\Delta S^{\neq}$ [c]	$K\times10^{-3}$ [a]	$\Delta E^{\neq}$ [b]	$\Delta S^{\neq}$ [c]	$K\times10^{-3}$ [a]	$\Delta E^{\neq}$ [b]	$\Delta S^{\neq}$ [c]
				3588					
				1512	15.6	− 7.9	286	12.2	−22.8
	690	8.7	−31.5	252	15.2	−12.3	125	21.7	− 6.6
	342	14.4	−14.7	70	17.1	− 9.5	27.7	19.3	− 4.1
	149	13.8	−22.7	17.0	14.6	−24.3			
	11.2	12.5	−27.5	8.3	12.8	−27.1	6.6	15.5	−21.7
				6.4	18.7	− 8.9			

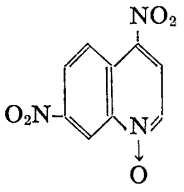
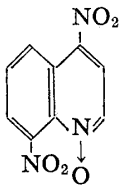
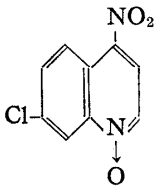
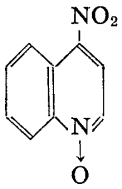
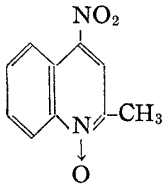
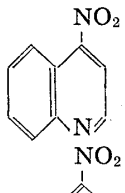
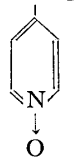
a) K : 1/mol. sec. *b*) $\Delta E^{\neq}$: kcal./mol. *c*) $\Delta S^{\neq}$: cal./deg. mol. (e. u.).
Errors : 5% for K, 1 kcal. for $\Delta E^{\neq}$, 3 e. u. for $\Delta S^{\neq}$.

17) H. Eyring : The Theory of Rate Process., 185, 416 (1941).
18) *Idem* : *Ibid.*, 185, 418 (1941).

These phenomena are quite different with the observation in the nucleophlic substitution reaction between 4-haloqinoline 1-oxide and piperidine, in which the $\Delta E^{\ddagger}$ increases slightly and $\Delta S^{\ddagger}$ greatly increases, as the solvent becomes more polar.[3] But, this fact seems to be referred to the difference between solvation of initial state and that of activated complex.

In 4,8-dinitroquinoline 1-oxide, k and $\Delta S^{\ddagger}$ largely increase, as the solvent becomes more polar. This increasing of k is due to the increasing of $\Delta S^{\ddagger}$. From this fact, it

Table II.

Nitro comp.	$K \times 10^{-3}$	A	B	C
	3588			−
	1512			−
	252	(3.361)[a]	(0.189)[a]	+
	70	3.113	0.186	+
	17.0	1.913	0.178	+
	8.3	1.579	0.176	−
	6.4	1.092	0.149	−

A : Approximate super delocalizability. K. Fukui, C. Nagata : Gann, **51**, 119 (1960).
B : Frontier electron density at 4-possition of quinoline
C : Skin cancer producing activity. W. Nakahara, *et al.* : Gann, **48**, 129 (1957). *Ibid.*, **49**, 33 (1959). *Ibid.*, **50**, 1 (1960).
a) for 4-nitro-6-chloroquinoline 1-oxide.

may be considered that the interaction between 1-oxide group and polar solvent molecules in initial state is influenced with 8-nitro group. And this fact is explained from the assumption that the entropy of solvation in initial state is larger than that in its activated complex, and as the results, the rate constant greatly increases.

On the carcinogenic aspects of these 4-nitro compounds, the obtained activity values of 4-nitro groups show a good agreement with the theoretical results by Fukui's calculation[19] and with Endo's observation.[6] Furthermore, comparing these quantitative results and Fukui's estimation with the carcinogenic activities, one would notice a definite relation between these chemical constants and carcinogenicity. There seems to be a certain "upperthreshold" as well as "the lower threshold"[19] for the occurrence of carcinogenic activity. But, as previouly mentioned, ΔE^{*} and ΔS^{*} are influenced with the composition of the reaction solution.

From this results, if one assume 4-nitroquinoline 1-oxide to react with a certain "nucleophilic site" of the biochemical system for the occurrence of the carcinogenic action,[20] it seems to be difficult to estimate immediatly the carcinogenic activity by only the chemical activity of these compounds.

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Summary

4-Nitroquinoline 1-oxide and related compounds react easily with SH-group of thioglycolic acid affording the corresponding sulfides of these compounds. With these nitro compounds, the present authors studied the nucleophilic substitution reaction between 4-nitro group and thioglycolic SH-group, and determined the reaction rate constants, energies of activation and entropies of activation in several kinds of solvent system.

On the other hand, 4-nitroquinoline 1-oxide and some related compounds have been known to be skin cancer producing agents. Then, the chemical activity of 4-nitro groups which were obtained were discussed on comparison with the carcinogenic activity.

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19) K. Fukui, C. Nagata : Gann (Tokyo), **51**, 119 (1960).
20) W. Nakahara, F. Fukuoka : *Ibid*, **50**, 1 (1959).