Chem. Pharm. Bull.
15(8)1112~1119(1967)

UDC 615.771.7 : 547.831.04 : 543.253

138. **Misako Tachibana, Shohei Sawaki,**[*1] **and Yutaka Kawazoe :**

Studies on Chemical Carcinogens. III.[*2] Polarographic Reduction Potentials of Nitroquinoline Derivatives.

(*National Cancer Center Research Institute*[*3])

The polarographic reduction potentials ($NO_2 \rightarrow NHOH$) were measured of 35 kinds of nitroquinoline derivatives. Discussions were made on the $E_{1/2}$ values in connection with their chemical structures.

(Received May 10, 1966)

Since Nakahara and his co-workers demonstrated strong carcinogenicity of 4-nitroquinoline 1-oxide (4NQO)[1] and some of its derivatives, many efforts have been made for elucidation of the chemical structure-carcinogenicity relation in this class of chemical carcinogens.[1~23] Among many mono- and di-nitroquinoline and their 1-oxides, 4NQO is the only one derivative that has been demonstrated to be carcinogenic on the skin of animals so far. It is of interest, further, that not all the substituted 4NQO derivatives induced malignant tumor, some induced but some did not. Another important finding[12~15] was reported in 1963 in connection with the carcinogenic mechanism of these carcinogens that 4-hydroxyaminoquinoline 1-oxide (4HAQO), one of the reduction metabolites of 4NQO, also induced fibrosarcoma and carcinoma at the site of its injection, whereas no cancerization has been proved with other possible metabolites such as 4-nitroquinoline, 4-aminoquinoline and its 1-oxide, 4,4′-azoxyquinoline 1,1′-dioxide, etc. The finding[20,22,23] that 4NQO is converted to carcinogenic 4HAQO *in vivo* strongly suggests that 4NQO and 4HAQO induce cell cancerization through a common mechanism, one of which being perhaps the more essential

---

[*1] Present address : Department of Pharmacy, Tokyo College of Science. Ichigaya, Shinjuku-ku, Tokyo.
[*2] Part II. This Bulletin, **15**, 1 (1967).
[*3] Tsukiji, Chuo-ku, Tokyo (橘美佐子, 沢木正平, 川添　豊).
1) W. Nakahara, F. Fukuoka, T. Sugimura : Gann, **48**, 129 (1957).
2) T. Okabayashi : Yakugaku Zasshi, **73**, 964 (1953).
3) F. Fukuoka, T. Sugimura, S. Suzuki : Gann, **48**, 263 (1957).
4) W. Nakahara, F. Fukuoka, S. Sakai : *Ibid.*, **49**, 33 (1958).
5) H. Endo : *Ibid.*, **49**, 151 (1958).
6) W. Nakahara, F. Fukuoka : *Ibid.*, **50**, 1 (1959).
7) W. Nakahara : Progr. Experiment. Tumor Res., **2**, 158 (1961).
8) T. Okabayashi : This Bulletin, **10**, 1127 (1962).
9) T. Okabayashi, A. Yoshimoto : *Ibid.*, **10**, 1221 (1962).
10) Al-Kassab, B. Boyland, K. Williams : Biochem. J., **87**, 5 (1963).
11) T. Okamoto, M. Itoh : This Bulletin, **11**, 785 (1963).
12) Y. Shirasu, A. Ohta : Gann, **54**, 221 (1963).
13) H. Endo, F. Kume : *Ibid.*, **54**, 443 (1963).
14) Y. Shirasu : *Ibid.*, **54**, 487 (1963).
15) H. Endo, F. Kume : Naturwiss., **50**, 525 (1963).
16) C. Nagata, A. Imamura, K. Fukui, H. Saitoh : Gann, **54**, 401 (1963).
17) W. Nakahara : Arzneim. Forsch., **14**, 842 (1964).
18) H. Endo, F. Kume : Gann, **56**, 261 (1965).
19) T. Kawachi, Y. Hirata, T. Sugimura : *Ibid.*, **56**, 415 (1965).
20) T. Sugimura, K. Okabe, H. Endo : *Ibid.*, **56**, 489 (1965).
21) C. Nagata, M. Kodama, Y. Tagashira, A. Imamura : Biopolymers, **4**, 409 (1966).
22) H. Hoshino, F. Fukuoka, Okabe, T. Sugimura : Gann, **57**, 71 (1966).
23) T. Sugimura, K. Okabe, M. Nagao : Cancer Res., **26**, 1717 (1966).

carcinogen. In this laboratory extensive chemical and biological studies on quinoline 1-oxide derivatives in this connection have been undertaken to supply basic data for the consideration of carcinogenic mechanism from the point of view of organic chemical reactions and quantum biology. As one of our serial studies along this line, this paper describes half-wave reduction potentials of nitro groups to the corresponding hydroxyamino groups of thirty-five kinds of nitroquinoline derivatives which were synthesized in this laboratory for bioassay for their carcinogenicity.[24] Although the purpose originally intended in this study is to correlate the carcinogenic activity with the reduction potential of the compounds examined, this paper includes only the basic data of the reduction potentials and some discussions in relation to their chemical structures. Further results and detailed discussion will be reserved for forthcoming papers.

Experimental

Materials——The compounds examined, which are listed in Table I and II, were synthesized by authentic preparative methods described in the literatures referred to in the tables.

Polarography——The polarogram of each compound was obtained from their $5\sim7\times10^{-5}$ molar aqueous solutions by an Yanagimoto PA-102 polarograph with an ordinary dropping mercurry electrode at 25°. The solutions were adjusted to the pH's of 3.78, 6.98, and 9.85 with the acetic acid-acetate ($1/10M$), the K-Na-phosphates ($1/15M$), and the glycine-NaOH ($1/10M$) buffered solutions, respectively. Each half-wave reduction potential ($E_{1/2}$) was calibrated against the saturated calomel electrode. The dissolved oxygen was removed by bubbling nitrogen gas through the solution for about 15 minutes.

Results

No systematic studies on the polarographic reduction of nitroquinolines and their 1-oxides has been reported previously.[25,26] A single, well-defined wave was observed around -0.1 to -0.5V for the reduction of nitro to hydroxyamino for each compound examined. Other two waves, which are associated with a further reduction to amino group and with deoxygenation of 1-oxide group, are far removed from the one concerned. Fig. 1 shows the polarograms of 4NQO and its reduction products, 4HAQO and 4-aminoquinoline 1-oxide, in pH 3.78 solutions at 25°, comparison of these polarograms making it possible to assign the reduction waves to the correct reduction process indicated in the figure. In Table I and Fig. 2 and 3 are reported the half-wave reduction potentials of mononitroquinolines, their 1-oxides and some reference nitro compounds in three kinds of buffered solutions. Those of substituted 4NQO derivatives in neutral solutions are reported in Table II and Fig. 4.

Discussion

In general, the lower the energy level of the lowest vacant molecular orbital is, the more easily the addition of electrons should occur at the dropping mercury electrode. Let us consider the molecular orbitals of nitro group perturbed by electronic resonance interaction with an aromatic nucleus. It can be qualitatively assumed that more electron-withdrawing contribution of aromatic nucleus brings about a more profound stabilization of the lowest vacant orbital energy level of nitro group, resulting in positive shift of the reduction potential of the nitro group. Thus, a nitro group which is bonded to a more electron-deficient aromatic nucleus must be

---

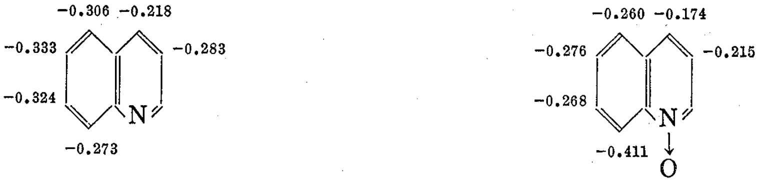
[24] Y. Kawazoe, M. Tachibana: This Bulletin, **15**, 1 (1967).
[25] E. Ochiai: Yakugaku Zasshi, **69**, 1 (1949).
[26] *Idem*: Proc. Imp. Acad. (Tokyo), **19**, 309, 575 (1943).

Table I. Half-Wave Reduction Potentials of Nitroquinolines and Their 1-Oxides ($E_{1/2}$ volt *vs.* S.C.E.)

Compounds	pH			Ref. for synthesis
	3.78	6.98	9.85	
3-Nitroquinoline	−0.170	−0.283	−0.423	27
4-Nitroquinoline	−0.070	−0.218	−0.299	28
5-Nitroquinoline	−0.161	−0.306	−0.442	29
6-Nitroquinoline	−0.198	−0.333	−0.458	30
7-Nitroquinoline	−0.184	−0.324	−0.430	31
8-Nitroquinoline	−0.148	−0.273	−0.373	32
3-Nitroquinoline 1-oxide	−0.097	−0.245	−0.357	33
4-Nitroquinoline 1-oxide	−0.081	−0.174	−0.272	34
5-Nitroquinoline 1-oxide	−0.134	−0.260	−0.390	35
6-Nitroquinoline 1-oxide	−0.173	−0.276	−0.419	36
7-Nitroquinoline 1-oxide	−0.163	−0.268	−0.376	37
8-Nitroquinoline 1-oxide	−0.185	−0.411	−0.582	35
4-Nitropyridine 1-oxide	−0.145	−0.241	−0.340	45
Nitrobenzene	−0.278	−0.460	−0.595	
α-Nitronaphtalene	−0.196	−0.367	−0.508	
5-Nitrotropolone		−0.421		46

more easily reduced than that bonded to a more electron-rich nucleus. For instance, the $E_{1/2}$ value of nitropyridine is more positive than that of nitrobenzene. This may be true in comparison of the potentials between the position isomers of one sort of aromatic homologues. For example, 4-nitropyridine should have a more positive $E_{1/2}$ value than 3-nitropyridine because electron-withdrawing resonance effect of the ring nitrogen influences the nitro group at position-4 more than that at position-3. In other words, a nitro group bonded to a more electrophilic aromatic carbon, which is apt to suffer nucleophilic attacks, must more easily accept electrons at the negative electrode.

The polarographic reduction of nitro group is, in itself, strongly dependent on the pH of the solution. In addition to this, in basic nitro compounds, protonation of the



$|E_{1/2}|$: 4<8<3<5<7<6 4<3<5<7<6<8

Chart 1. $E_{1/2}$ Values of Mononitroquinolines and Their 1-Oxides in pH 6.98 Solutions

---

27) Chemisches Zentralblatt. : II, 962 (1921).
28) M. Hamana : Yakugaku Zasshi, **75**, 135 (1955).
29) Dofton : J. Chem. Soc., **61**, 782 (1892).
30) L. Haskelberg : J. Org. Chem., **12**, 434 (1947).
31) K.S. Chofield, J.C.E. Simpson : J. Chem. Soc., **1945**, 512.
32) F. Misani, M.T. Bogert : J. Org. Chem., **10**, 347 (1945).
33) E. Ochiai, C. Kaneko : This Bulletin, **5**, 56 (1957).
34) E. Ochiai, M. Ishikawa, Z. Sai : Yakugaku Zasshi, **63**, 280 (1943).
35) E. Ochiai, T. Okamato : *Ibid.*, **70**, 384 (1950).
36) E. Ochiai, M. Ishikawa : *Ibid.*, **64**, 72 (1944).
37) J.K. Landquist : J. Chem. Soc., 1885 (1956).
38) M. Ishikawa : Yakugaku Zasshi, **65B**, 99 (1945).
39) T. Okamoto : *Ibid.*, **71**, 727 (1951).

Table II. Half-Wave Reduction Potentials of 4-Nitroquinoline 1-Oxide Derivatives

Compounds	$E_{1/2}$ (*vs.* S. C. E.)	Ref. for synthesis
2-Methyl-4-nitroquinoline 1-oxide	−0.197	38
3-Methyl-4-nitroquinoline 1-oxide	−0.258	24
5-Methyl-4-nitroquinoline 1-oxide	−0.192	24
6-Methyl-4-nitroquinoline 1-oxide	−0.176	39
7-Methyl-4-nitroquinoline 1-oxide	−0.187	24
8-Methyl-4-nitroquinoline 1-oxide	−0.187	24
9-Nitro-tetrahydroacridine 10-oxide	−0.337	
2-Chloro-4-nitroquinoline 1-oxide	−0.140	[a]
3-Fluoro-4-nitroquinoline 1-oxide	−0.199	[b]
3-Chloro-4-nitroquinoline 1-oxide	−0.177	24
3-Bromo-4-nitroquinoline 1-oxide	−0.181	40
5-Chloro-4-nitroquinoline 1-oxide	−0.181	24
6-Chloro-4-nitroquinoline 1-oxide	−0.157	39
6-Fluoro-4-nitroquinoline 1-oxide	−0.157	[b]
7-Chloro-4-nitroquinoline 1-oxide	−0.156	41
6,7-Dichloro-4-nitroquinoline 1-oxide	−0.144	24
3-Methoxy-4-nitroquinoline 1-oxide	−0.270	24
3-Diethylmaronyl-4-nitroquinoline 1-oxide	−0.227	42
6-Carboxy-4-nitroquinoline 1-oxide	−0.193	43
4,5-Dinitroquinoline 1-oxide	(−0.201)[c] (−0.201)[c]	44
4,6-Dinitroquinoline 1-oxide	−0.154 (−0.323)	44
4,7-Dinitroquinoline 1-oxide	−0.147 (−0.267)	
4,8-Dinitroquinoline 1-oxide	−0.086 (−0.350)	44

a) Our sincere thanks are indebted to Professor Masatomo Hamana for a gift of this compound which was newly prepared by his group.

b) 3- and 6-monofluoro derivatives were kindly supplied by Mr. Mineo Saneyoshi of this institute for our present study. He is preparing to publish the papers on the syntheses of fluoroquinoline derivatives.

c) Two waves were partly overlapped with each other on the polarogram, so that the $E_{1/2}$ values in parentheses may include certain amounts of error.

basic centers of the molecule affects the reduction potential of nitro group seriously so that the pH dependency in our compounds must be rather complex, the data being reported in Table II and Fig. 2. Now, pending consideration of the pH dependency of the $E_{1/2}$ values, comparisons were made of $E_{1/2}$ values of mononitroquinolines and their 1-oxides in neutral solutions, as illustrated in Chart 1. The fact that 4-nitro group of the 1-oxide is most easily reduced, followed by 4-nitro of the free quinoline is in agreement with that fact that the position-4 of the 1-oxide is the most reactive toward nucleophilic reagents such as -SH, -Cl, followed by the position-4 of the free base.[11,47] Comparison of these $E_{1/2}$ values reveals another feature that each position isomer of the 1-oxide has always considerably smaller reduction potential than the corresponding position isomer of the free quinoline except for 8-nitro derivatives. This feature indicates the stronger electron-withdrawing effect of N^+-O^- structure than that of the free amine nitrogen on the pi-electrons of the aromatic nuclei. As

---

40) E. Okamoto : Yakugaku Zasshi, **70**, 376 (1950).

41) S. Yoshida : *Ibid.*, **66B**, 158 (1946).

42) H. J. Richter, N. E. Rustad : J. Org. Chem., **29**, 3381 (1964).

43) E. Ochiai, S. Suzuki, Y. Utsunomiya, T. Oomoto, N. Nakagome, M. Itoh : Yakugaku Zasshi, **80**, 339 (1960).

44) M. Ishikawa : Proc. Imp. Acad. (Tokyo), **20**, 599 (1944).

45) E. Ochiai, M. Ishikawa, K. Arima : Yakugaku Zasshi, **63**, 79 (1943).

46) T. Nozoe, *et al.* : Ann. Rep. Non-Aqueous Res. Inst. Univ. Tohoku, **7**, 13 (1957).

47) T. Okamoto, H. Hayatsu, Y. Baba : This Bulletin, **8**, 892 (1960).

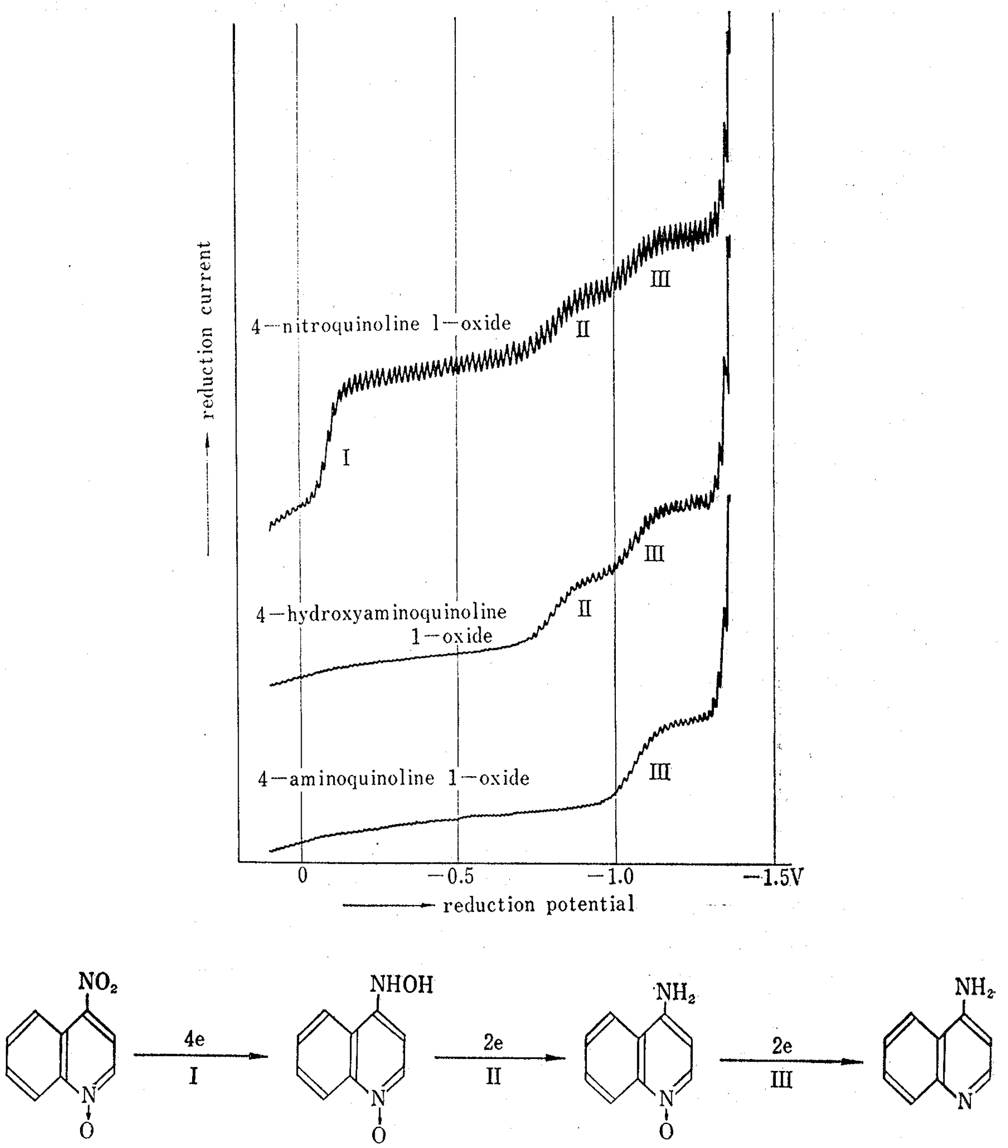


Fig. 1. Polarograms of Three Compounds denoted in the Figure in Phosphate-buffered Solution pH=3.78.

The reduction potential was referred to the saturated calmoel electrode.

for 8-nitroquinoline 1-oxide, where the nitro group should be twisted out of the plane of the aromatic nucleus because of the serious electric repulsion with an electronegative 1-oxide group, the molecular orbital perturbation of the nitro group by aromatic pi-orbitals must be much less than in cases of other nitro derivatives, because of interruption of the resonance interaction between nitro group and aromatic pi-electrons. The reduction potential should, therefore, be much larger than that which is expected. Acidification of the solution is expected to result in increase of the resonance interaction between nitro group and the rest of the molecule. Therefore, the reduction potential of this 1-oxide must be decreased by acidification through this mechanism in addition to the ordinary decrease by pH dependency of the reduction potentials of nitro groups. The polarographic data of 8-nitroquinoline 1-oxide confirmed this assumption as shown in Fig. 3. It is worth noting that, since the nitro group of carcinogenic 4NQO is the most easily reducible of the mononitroquinolines

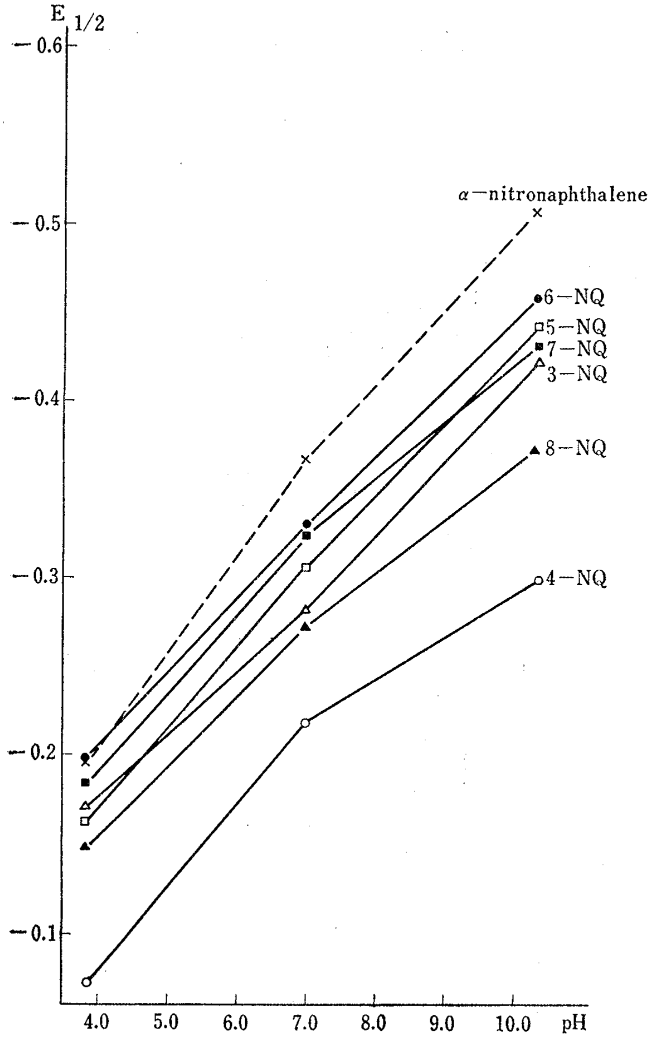


Fig. 2. Half-wave Reduction Potentials (*vs.* S. C. E.) of Mononitroquinolines in Buffered Solutions pH= 3.78, 6.98, and 9.85 at 25°

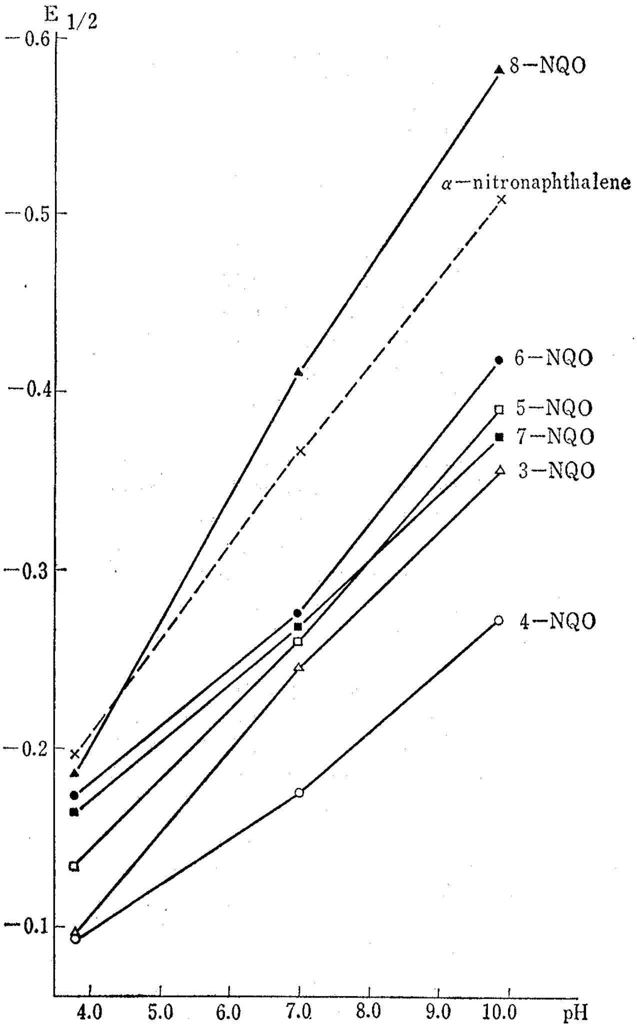


Fig. 3. Half-wave Reduction Potentials (*vs.* S. C. E.) of Mononitroquinoline 1-Oxides in Buffered Solutions pH=3.78, 6.98, and 9.85 at 25°

examined, 2-nitroquinoline 1-oxide,[*4] which must be expected to have a further smaller reduction potential, is of great interest to be assayed for their carcinogenic activity.

Now, the polarographic data of substituted 4NQO derivatives are summarized in Table II and Fig. 4. The substituent effects were clearly observed on the $E_{1/2}$ values, depending on the kind and position of substituents. Among them, 3-methyl, 3-methoxyl, and 3-diethylmalonyl derivatives showed most remarkable deviations from the $E_{1/2}$ value of the parent 4NQO. This may be due to mesomeric interaction and to steric hindrance with the substituents, both of which bring about a negative shift of $E_{1/2}$ through the molecular orbital perturbation mechanism as illustrated in this paper. It is probable for 3-halogeno 4NQO's to be dehalogenated prior to reduction of nitro group. With regard to 3-substituted 4NQO's, however, it has been still unsuccessful to synthesize the corresponding hydroxyamino derivatives, so that the definite assignment of the reduction waves must be left for future investigation. Introduction of another nitro group at position-8 to 4NQO caused a remarkable

---

[*4] The synthesis of 2-nitroquinoline 1-oxide is still now under investigation in our and some other laboratories.

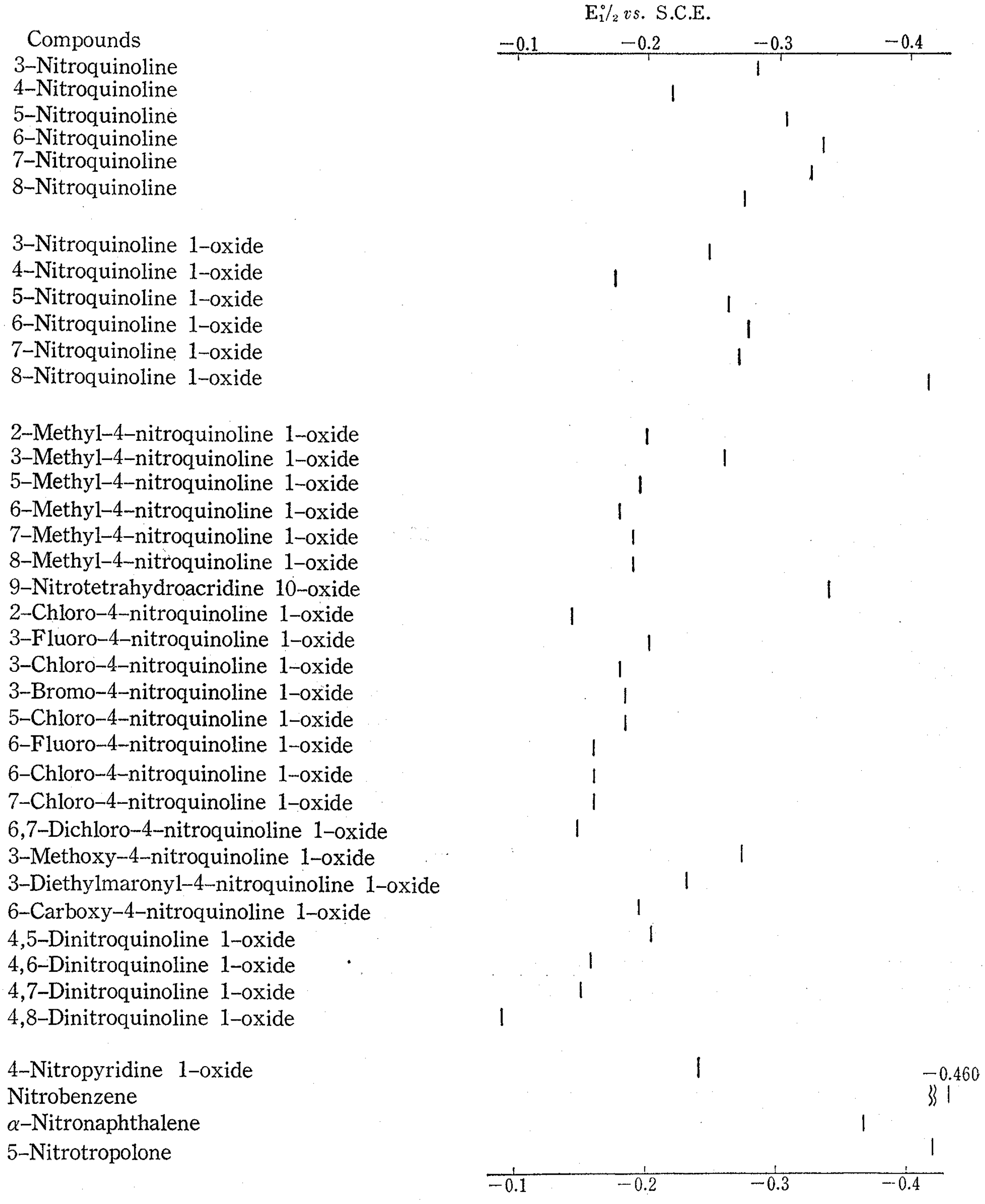


Fig. 4. Half-wave Reduction Potentials ($E_{1/2}$ vs. S. C. E.) of Nitroquinoline Derivatives in Buffer Solution pH=6.98 at 25°

positive shift of $E_{1/2}$ value of 4-nitro group. This is an anomalously large deviation from cases of other di-nitro derivatives but no reasonable explanation is available at present.

Further discussions will be made in forthcoming papers on correlation of these polarographic potentials with the Hammett's parameters and UV absorption maxima of these compounds and also on the reduction potentials from hydroxyamino to amino group, which might be important in connection with the stability of the intermediate reduction metabolite, 4HAQO's, *in vivo*. Their theoretical treatment from MO calculation are being undertaken in relation to the reduction potential at one electron step using the non-aqueous polarographic technique.

The authors are greatly indebted to Dr. Waro Nakahara, Director of National Cancer Center Research Institute, for his continuing encouragement and valuable discussion throughout this work. Thanks are also due to Professor Keizo Tada, Kyoritsu Women's College of Pharmacy, and Professor Chikara Kaneko, Tokyo Medical and Dental University, for their useful discussions. They also thank Professors Kazuhiko Kubota, Tadashi Nakai, and Shunji Ishikura, Tokyo College of Science, for making the polarography instrument available to us. One of us (S. S.) express his grateful acknowledgment to Professor Bunsuke Umezawa, Tokyo College of Science, for his kind encouragement. The financial support from the Ministry of Education for a part of this work is gratefully acknowledged.