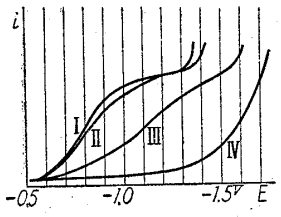
80. Isao Aiko: Polarographic Determination of Half-wave Potentials of Nitrogen Mustard N-Oxides*.

(*Yoshitomi Pharmaceutical Industries, Ltd.*)**

It had been reported in our preceeding papers[1~2] that the N-oxides of nitrogen mustards have a marked cancerolytic activity and were strongly oxidative. They readily submitted to reduction with mild reducing agents such as cysteine in neutral medium, even at an ordinary temperature.

The assumption that the N-oxide acts as a cancerolytic agent *in vivo* after being reduced to the corresponding tertiary amine seems not to be improbable, because the chemical properties of these N-oxides are fairly stable in themselves compared to their biological activities which we reported already in our experiments with the Yoshida sarcoma animals.

We, therefore, considered it interesting to compare the tendency of the compounds to be reduced and carried out polarographic determination of the half-wave potentials of the compounds.



I: pH 3.5, II: pH 4.0, III: pH 5.0, IV: pH 7.0

Fig. 1.

The samples were dissolved in Clark Lubs' or Kolthoff's buffer in concentration of about 10^{-4} mol. and tested at 25° in all cases. They gave characteristic reduction wave of a shallow slope in strongly acid medium and the inclination of the slope became more diminished with the decrease in acidity of the buffer, and at last disappeared in an alkaline range (Fig. 1). In the case of higher concentrations of more than 10^{-3} mol., a maximum of the wave appeared, which, however, could be inhibited by mere dilution or addition of gelatine or tylose.

The reduction potentials of the compounds thus obtained are shown in Table I.

TABLE I

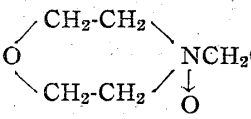
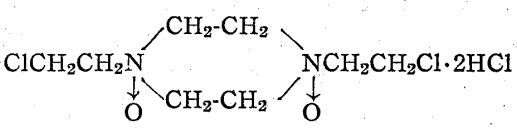
No.		$E_{\frac{1}{2}}$ *vs.* N.C.E.: pH 3.5	
		Original compounds	Transformed ring-oxide compounds†
I:	$CH_3N(CH_2CH_2Cl)_2\cdot HCl$ ↓ O	−0.78 V	−0.20 V
II:	$C_2H_5N(CH_2CH_2Cl)_2\cdot HCl$ ↓ O	−0.81	−0.13
III:	$C_4H_9N(CH_2CH_2Cl)_2\cdot HCl$ ↓ O	−0.68	−0.15
IV:	*iso*-$C_5H_{11}N(CH_2CH_2Cl)_2\cdot HCl$ ↓ O	−0.60	−0.13
V:	$C_6H_5\text{-}CH_2N(CH_2CH_2Cl)_2\cdot HCl$ ↓ O	−0.63	−0.11
VI:	$(CH_3)_2NCH_2CH_2Cl\cdot HCl$ ↓ O	——	−0.46

* M. Ishidate, Y. Sakurai: Studies on Cancerocidal Substances. VI.

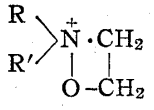
** Yoshitomi-machi, Fukuoka-ken (愛甲軍雄).

1) I. Aiko, S. Owari, M. Torigoe; J. Pharm. Soc. Japan, **72**, 1297(1952).

2) Y. Sakurai, M. Izumi: This Bulletin, **1**, 297(1953).

VII:	$(C_2H_5)_2NCH_2CH_2Cl\cdot HCl$ (O)	−1.04	−0.37
VIII:	$(C_6H_5\text{-}CH_2)_2NCH_2CH_2Cl\cdot HCl$ (O)	−0.74	−0.13
IX:	$N(CH_2CH_2Cl)_3\cdot HCl$ (O)	−0.60	−0.08
X:		−0.78	−0.25
XI:		−0.79	−0.13
XII:	$(ClCH_2CH_2)_2NCH_2CH_2N(CH_2CH_2Cl)_2\cdot 2HCl$ (O) (O)	−0.80	+0.02

† Refer to the literature 1) : this transformation occurs in general in cases of oxides, general formula of which is as follows:



R,R′=alkyl, aralkyl or β-chloroalkyl.

The elements of the wave of the compound (I) in the table, which was graphed at pH 3.5, almost satisfied the relation induced from the equation of Heyrovsky and Ilkovic, i.e.

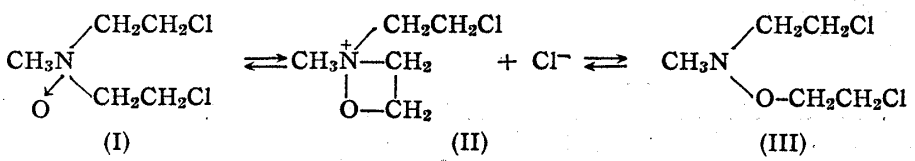
$$E=E_{\frac{1}{2}}-\frac{0.059}{n}\log\frac{i}{i_d-i}\quad(25°)$$

But the electrode reaction should be irreversible because the value of n was 0.48. $i_d/C=1.845\cdot n$ was calculated from the equation: $i_d/C=605\cdot n\cdot D^{1/2}\,m^{2/3}\,t^{1/6}$, in which m=0.832 mg./sec., t=3.50 sec. (at $E_{1/2}$ in pH 3.5 buffer, 25°, respectively), D is a diffusion coefficient, which was unknown for the compound (I) but was calculated from the equation:

$D=\frac{RT\lambda^0_i}{ZF^2}$, in which Z is the valency of an atom, R, the gas constant per mole, T, the absolute temperature, F, the Faraday constant, and λ^0_i, the equivalent conductivity of the ion in an infinite dilution (29.8 $ohm^{-1}\,cm^2\,equiv.^{-1}$), which was analogized from that of a quaternary ammonium ion. As the experimental value of i_d/C was 3.87, the equation $1.845\cdot n=3.87$ was induced and, therefore, n is approximately 2.

Zdevék Vodrážka[3] had reported that the hydroxylamine submits to two-electron reduction under the cleavage of nitrogen-oxygen linkage by mercury dropping electrode. It seems probable that the reduction wave of the present series of compounds is due to their reduction to the corresponding tertiary amines. It was also confirmed that the nitrogen mustards themselves, not their N-oxides, never gave such reduction waves.

It had been reported that the compound (I) undergoes the following transformation reaction in a neutral aqueous medium.[1]



(I) (II) (III)

3) Zdevék Vodrážka: Chem. Listy, 45, 293(1951) (C. A., 46, 1392(1952)).

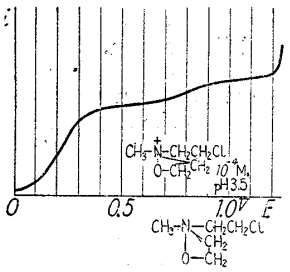
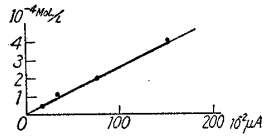


Fig. 2.



pH 3.5, m=0.382 mg., t=3.50 sec.

Fig. 3.

Out of the above three, (II) showed a characteristic reduction wave in acid, neutral, and alkaline media and a half-wave potential at pH 3.5 was 0.20V (*vs.* N.C.E.) (Fig. 2), while (III) showed no such wave. In fact, the compound (II) can be more readily reduced than (I) by ordinary reducing agents.

Furthermore, it was found that, in the case of (I), the diffusion current was in exact proportion to the concentration within the range of 5.10^{-5} to 4.10^{-4} mol. at pH 3.5 (Fig. 3). The method is, therefore, applicable to the quantitative determination of (I).

Whether the difference in the potentials of the compounds has a decisive meaning upon their toxicity and efficacy or not cannot be said as yet in the present stage of experiments because the condition of the reduction by polarographic method is very different from that of *in vivo*. Concerning the data here obtained, however, no reasonable relation between $E_{1/2}$ and their biological activities was found.

This study was carried out at the Iatrochemical Institute of the Pharmacological Research Foundation, Tokyo, and the author is very grateful to Prof. Morizo Ishidate and Dr. Takashi Isshiki of the Tokyo University and to Dr. Yoshio Sakurai of the Iatrochemical Institute for their interests and advices in this experiment.

Summary

The half-wave potentials of twelve N-oxides of the nitrogen mustards were determined by polarographic method and it was found that the methyl-bis(β-chloroethyl)amine N-oxide can be quantitatively determined by this method. Concerning the relation between the potentials and their biological activities, little was found in the present experiment.

(Received September 4, 1953)

---

81. **Yoshio Sakurai and Hanako Komai:** Bacteriostatic Activity of the Nitrogen Mustard N-Oxides against *Escherichia coli.**

(*Iatrochemical Institute of the Pharmacological Research Foundation***)

It was reported in our previous paper[1] that nitrogen mustard N-oxides in general show more favorable chemotherapeutic effects in amimal experiments with the Yoshida sarcoma than the corresponding tertiary nitrogen mustards. Some nitrogen mustards and their N-oxides were examined as to their bacteriostatic activities in the present study.

On account of the chemical reactivity of nitrogen mustards against amino acids, polypeptides, and proteins, *Escherichia coli* was employed in this experiment, as this bacterium can be readily cultivated in pure synthetic medium without amino acids or pepton.

Results are summarized in Table I.

---

* M. Ishidate, Y. Sakurai: Studies on Cancerocidal Substances. V.

** Nishigahara, Kita-ku, Tokyo (桜井欽夫, 駒井華子).

1) Y. Sakurai, M. Izumi: This Bulletin, 1, 297(1953).