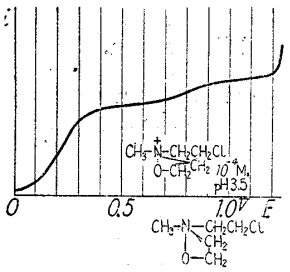
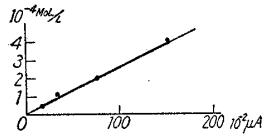


Fig. 2.



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.

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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.

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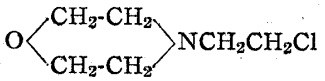
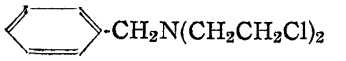
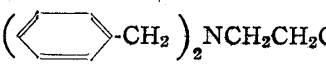
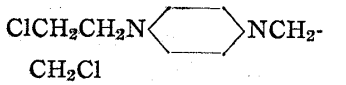
* M. Ishidate, Y. Sakurai: Studies on Cancerocidal Substances. V.

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

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

TABLE I

	Compounds*)	Min. concn. for**) inhibition		Compounds	Min. concn. for inhibition
1)	$(CH_3)_2NCH_2CH_2Cl$	$35\cdot10^{-4}$mol.	14)	Oxide of (13)	$4.2\cdot10^{-4}$mol.
2)	Oxide of (1)	30	15)	i-$C_3H_7N(CH_2CH_2Cl)_2$	23
3)	$(C_2H_5)_2NCH_2CH_2Cl$	14	16)	Oxide of (15)	10
4)	Oxide of (3)	53	17)	i-$C_5H_{11}N(CH_2CH_2Cl)_2$	4
5)		12	18)	Oxide of (17)	0.9
6)	Oxide of (5)	14	19)		1.8
7)		0.84	20)	Oxide of (19)	0.9
8)	Oxide of (7)	0.03	21)		17
9)	$CH_3N(CH_2CH_2Cl)_2$	5	22)	Dioxide of (21)	0.8
10)	Oxide of (9)	1.2	23)	$N(CH_2CH_2Cl)_3$	15
11)	$C_2H_5N(CH_2CH_2Cl)_2$	5	24)	Oxide of (23)	39
12)	Oxide of (11)	2.2	25)	$(ClCH_2CH_2)NCH_2CH_2N(CH_2CH_2Cl)_2$	2.6
13)	$C_3H_7N(CH_2CH_2Cl)_2$	23	26)	Dioxide of (25)	1.2
	TEM***	24		Aminopterin	1.7

* Compounds are all hydrochlorides.
** Minimum concentration for inhibition is observed after 24 hours' incubation. Composition of the media[2] used in these experiments was as follows: Glucose, 3.0 g.; $(NH_4)_2HPO_4\cdot2H_2O$, 5.0 g.; NaCl, 1.0 g.; $FeSO_4\cdot7H_2O$, 0.3 g.; $MgSO_4\cdot7H_2O$, 0.7 g.; K_2HPO_4, 5.0 g.; ad. Aq. 1,000 cc. pH 7.5.
*** TEM: Tris-ethyleneimino-s-triazine (Triethylenemelamine).

The results seem interesting at least in three aspects: *i.e.* 1) It is well known that the monofunctional nitrogen mustards or their N-oxides have none or only just one hundredth of the cancerocidal activity compared with that of the bi- or polyfunctional ones. This fact is likely to be true in their bacteriostatic activities *in vitro* against *E. coli*. 2) Despite the far more stagnant chemical reactivity of the chlorine atom of the N-oxides, their bacteriostatic activities *in vitro* are more potent than the corresponding tertiary amines. 3) The compounds having a benzyl group, such as Nos. 7, 8, 19, and 20, have exceptionally high activities of inhibition regardless of their functionalities of the molecules. It should be noted that these compounds have a marked lipophilic character.

The higher activity of the N-oxides against bacteria might be due to their stability in aqueous solution, as is indicated by following experiments. Ten mg. each of methyl-bis(β-chloroethyl)amine hydrochloride (HN_2) and its N-oxide hydrochloride (HN_2 N-oxide) was separately taken in two flasks and dissolved in 10 cc. of water. Both were kept at 25° and, with elapse of time, aliquots of the solution were tested as to their bacteriostatic activity against *E. coli by* dilution method. Results are shown in Table II.

TABLE II

Hours after being dissolved	Minimum concentration for inhibition *E. coli*: 24 hrs.' incubation, γ/cc.	
	HN_2	HN_2 N-oxide
0	100	50
3	125	50
6	500	50
9	—	50
14	—	50
30	—	50
53	—	50

When the solution of the free base of HN_2 N-oxide (60 mg. of the hydrochloride and 24 mg. of sodium bicarbonate in 6 cc. of water) was kept at 25°, the initial activity of

2) J. W. Bigger, G. C. Ware: Lancet, 259, 427(1950).

the solution against *E. coli* was maintained without lowering even after 96 hours. It had already been reported[3] that the N-oxides in this condition are invariably transformed into the ring-oxides, *e.g.* N,N-methyl-β-chloroethyldimethylene-1,2-oximinium chloride (in case of the compound No. 10), within several hours. Therefore the transformed compound should be as active against *E. coli* as the original N-oxide and the lowering of activity, caused by hydrolysis of the chlorine atoms, could only be first observed after 192 hours (Table III).

TABLE III

Hours after being dissolved in water with $NaHCO_3$	HN_2 N-Oxide Hydrochloride Minimum Concentration for Inhibition *E. coli*: 24 hrs.' incubation		
	100 γ/cc.	50 γ/cc.	25 γ/cc.
0	—	—	+
72	—	—	++
96	—	—	++
192	—	++	++

The minimum concentration for inhibition of the compound (No. 10) fell to $5 \cdot 10^{-3}$ mole in the medium containing casein hydrolyzate and similarly its activity fell almost to zero when sodium thiosulfate was added to the pure synthetic medium[2] in concentration of 0.5%.

Summary

Twenty-three derivatives of nitrogen mustards and their N-oxides were examined as to their bacteriostatic activities *in vitro* using *Escherichia coli*. It was found that, in general, the bi- or polyfunctional N-oxides were more active than the corresponding tertiary nitrogen mustards, but the monofunctional N-oxides were far less active, regardless of whether they were N-oxide derivatives or tertiary ones. The results agreed well with experiments with Yoshida sarcoma animals.

(Received September 4, 1953)

3) M. Ishidate, Y. Sakurai: J. Pharm. Soc. Japan, 72, 1297(1952).

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82. **Tadashi Sasaki:** Synthesis of Nitrofuran Sulfonic Acid Derivatives.*

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Nitrofuran derivatives with strong bactericidal activities, such as nitrofurfural semicarbazone or nitrofurylacrolein semicarbazone, are in general difficult to put into solution with ordinary organic solvents. This experiment was carried out in order to obtain nitrofuran derivatives having both characteristics of being easily soluble and also of being active in bacteriostasis. Several attempts[1] have been reported concerning this type of experiment. According to Hill and White 5-sulfofuran-2-carboxylic acid is nitrated by fuming nitric acid into nitrofuran-carboxylic acid and -sulfonic acid.[2]

Upon reëxamining this reaction, optimum conditions for preparation of nitrofuran

* Presented before the Annual Meeting of the Pharmaceutical Society of Japan, July, 1949.

** Yoshida-konoe-cho, Sakyo-ku, Kyoto (佐々木 正).

1) As a soluble furan, guanofuracin was prepared by Dr. Uota. Dr. Emoto later reported on some soluble furacin derivatives. Cf. M. Emoto: J. Soc. Chem. Ind. Japan, 55, 593(1952).

2) H. B. Hill, R. White: Am. Chem. J., 27, 196(1902).