CHEMICAL & PHARMACEUTICAL BULLETIN

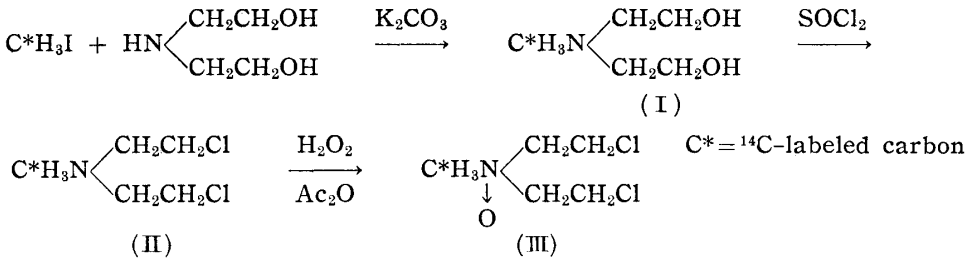
Vol. 8 No. 2 **February 1960**

UDC 615.771.7[547.233'222]-034-092.259

15. Morizo Ishidate and Shigeru Tsukagoshi : The Synthesis of N-Methyl[^{14}C]-bis(2-chloroethyl)amine N-Oxide and its Excretion in the Urine of Dog.

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[*1])

The synthesis of N-methyl[^{14}C]-bis(2-chloroethyl)amine N-oxide was carried out using methyl[^{14}C] iodide for the labeled carbon. The process was as shown in the following formulae :



Since the product of the first reaction, viz. N-methyl[^{14}C]-bis(2-hydroxyethyl)amine, was always mixed with a fair amount of the quaternary amine, it was acetylated with acetic anhydride without purification. The crude acetylated product was then purified by distillation *in vacuo* and successively hydrolyzed by heating with hydrochloric acid.

(I) afforded ^{14}C-labeled nitrogen mustard (II) by chlorination with thionyl chloride and (II) was oxidized to the N-oxide (III) with hydrogen peroxide and acetic anhydride. The properties of the N-oxide thus prepared were quite identical with those of the authentic preparation of N-methyl-bis(2-chloroethyl)amine N-oxide except in radioactivity. The specific activity of (III) was determined as 6,500 counts/min./μmole by Q-gas flow counter. The overall yield from methyl[^{14}C] iodide to the N-oxide was about 30%.

On a descending paper chromatogram, (II) and (III) were located respectively at Rf 0.5 and 0.7 (BuOH:EtOH:H_2O=8:1:1). The Rf values, determined by the location of radioactivity, were quite identical with those developed with Dragendorff reagent or potassium iodide-starch solution.

Excretion of N-methyl[^{14}C]-bis(2-chloroethyl)amine N-oxide (III) in urine was quantitatively determined by radioactivity measurement, after it was given intravenously to a normal dog.

Experimental

(1) Synthesis of N-Methyl[^{14}C]-bis(2-chloroethyl) amine N-Oxide : N-Methyl[^{14}C]-bis(2-hydroxyethyl)amine (I)—70 mg. of $^{14}CH_3I$ (1 mc.) was diluted about 100 times with non-labeled CH_3I. The diluted $^{14}CH_3I$ (7.1 g.), diethanolamine (15.8 g.), and anhyd. K_2CO_3 (13.8 g.) were mixed in MeOH (250 cc.),

*1 Hongo, Tokyo (石館守三, 塚越 茂).

refluxed for 1.5 hr., and filtered. After the filtrate was evaporated to dryness, Ac_2O (80 cc.) was added dropwise to the residue under stirring and ice-cooling. The mixture was then refluxed for 1 hr. and Ac_2O was distilled off. The residue was distilled again *in vacuo* and N-methyl[^{14}C]-bis(2-acetoxyethyl)-amine was obtained as a viscous fluid at b.p_3 105°. For purification, it was redistilled and a distillate of b.$p_{3.5}$ 110° was collected. Yield, 60%. The diacetate was dissolved in conc. HCl under ice-cooling and then refluxed for 1 hr. in an oil bath at 140°, evaporated under water suction, and (I) was obtained as an oily residue. It was used as such without purification for the next step of reaction.

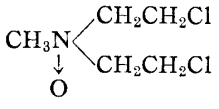
N-Methyl[^{14}C]-bis(2-chloroethyl)amine (II)—The crude (I) was well dried and added with $SOCl_2$ (3 times of (I) in volume). The mixture was heated at below 80° for about 15 min. and $SOCl_2$ was removed under reduced pressure. A solid residue was recrystallized from MeOH. The hydrochloride of (II) was obtained as white prisms, m.p. 108~110°. Yield, 58%. Specific activity, 6,500 counts/min./μmole.

N-Methyl[^{14}C]-bis(2-chloroethyl)amine N-Oxide (III)—The oxidation of (II) with H_2O_2 and Ac_2O in ether solution was carried out similarly as reported by Aiko, *et al.*[1] for preparation of N-methyl-bis(2-chloroethyl)amine N-oxide. The hydrochloride was obtained as colorless prisms, m.p. 108~110° (from acetone). Yield, 84%. *Anal.* Calcd. for $C_5H_{11}ONCl_2 \cdot HCl$: C, 28.77; H, 5.75; N, 6.7. Found : C, 29.09; H, 5.83; N, 6.91. Specific activity, 6,500 counts/min./μmole by Q-gas flow counter.

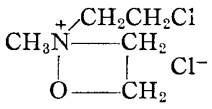
(2) Excretion of N-Methyl[^{14}C]-bis(2-chloroethyl)amine N-Oxide (III) : Materials—A single dose (5 mg./kg.) of (III) was injected during 5 sec. into the vein of a forefoot of dog (15 kg. body weight), which had been anesthetized by thiopenthal sodium. The fractions of urine were taken out from the opened bladder at intervals through 4 hr. of the experiment. The urine was acidified at once with dil. HCl and kept in an ice-box.

Radioactivity Measurement—Each urine sample was diluted 100 times with distilled water. One cc. of the diluted solution was taken on the counting dish and a small quantity of acetone was dropped on it in order to make the sample flat. After drying the sample, Q-gas flow counter was used for measurement. 3,100 c.p.m. represented approximately 1 mg. of (III).

Paper Chromatography of the Sample—The diluted urine was developed on the HCl-treated filter paper strip (Toyo Roshi No. 51-A) with a solvent mixture (BuOH:EtOH:H_2O=8:1:1) for about 7 hr. The spots were detected by Dragendorff or KI-starch reagent. There were found one or two spots on the paper, the one (Rf 0.7) for (III) and the other (Rf 0.2) for (IV).



(III)



(IV)

Results

The excretion curves and the paper chromatogram of the samples are demonstrated in Fig. 1.

The concentration of the excreted (III), calculated from the data of radioactivity measurement, was found higher than that of bioassay which was reported by Satoh, *et al.*,[2] because the curve in Fig. 1 must correspond to the sum of concentration of all radioactive substances which consisted of the original (III) and its transformed compound, e. g. 2-methyl-2-(2-chloroethyl)-1,2-oxazetidinium

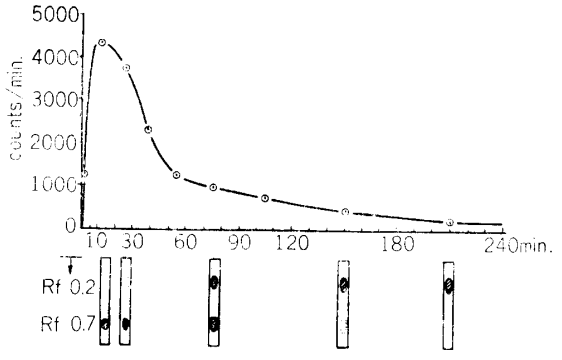


Fig. 1.

1) I. Aiko, S. Owari, M. Torigoe : Yakugaku Zasshi, **72**, 1297(1952).
2) J. Tokuoka, H. Satoh : Japan. J. Cancer Clinics, **5**(1959), in press.

chloride (IV). It was also demonstrated by the results of paper chromatography; the appearance of (IV) in urine could be detected 20 min. after the injection began and its quantity ratio to (III) increased gradually with lapse of time.

The authors are indebted to Drs. Y. Sakurai and H. Satoh of Iatrochemical Institute of Pharmacological Research Foundation for many valuable advices and the bioassay in this experiment, and also express their gratitude to Mr. S. Kawai for preliminary experiment.

Summary

N-Methyl[^{14}C]-bis(2-chloroethyl)amine N-oxide (^{14}C-labeled nitrogen mustard N-oxide) was synthesized for pharmacological and biochemical experiments using methyl-[^{14}C] iodide. Its specific activity was determined as 6,500 counts/min./μmole by Q-gas flow counter.

The labeled compound was given to a dog intravenously and the urine was taken out from the opened bladder. Concentration of the compound in the urine was quantitatively determined by radioactivity measurement.

(Received July 3, 1959)

UDC 615.771.7[547.233'222]-06 : 616-006.446-085

16. Morizo Ishidate, Yoshio Sakurai, and Eiichi Matsui : Studies on Carcinostatic Substances. XXIII.*1 Correlation between Anti-tumor and Leucopenia-inducing Effects of Alkylating Agents.

(*Iatrochemical Institute of Pharmacological Research Foundation**2)

Recently, there have been found a few derivatives of nitrogen mustard, which have been recognized as available anti-tumor agents, at least against such human tumors as Hodgkin's disease, reticulosarcoma, seminoma, and some kinds of lymphosarcoma. During the course of the treatment, however, hematopoietic tissues of the host were affected and, very frequently, a serious leucopenia was induced.

It has been a matter of particular interest to know whether or not the two actions of nitrogen mustard, the one against tumor and the other against bone marrow, could not be inclined to the former by modifying chemical structure of the molecule.

At the stage of animal experiment, a ratio of LD_{50} to MED (minimum effective dose) was usually noticed as a chemotherapeutic index, but, in most clinical treatment, the unfavorable side-effect of the alkylating agents, requiring discontinuation of the medication, was not the acute toxicity which led rats to death in animal experiment, but the unavoidably accompanying leucopenia of the patient. It was deemed important, therefore, to evaluate the clinical effectiveness of the agents by comparing the rate of one action to the other in animal experiment.

This paper deals with the experiment, in which 10 derivatives of nitrogen mustard, including some N-oxides having characteristic properties or structures, were given to rats bearing Yoshida sarcoma, and their percentage survival diagrams for 30 days and the decrease rate of leucocyte number were checked.

---

*1 Part XXII : This Bulletin, 7, 873(1959).

*2 26 Nishigahara 1-chome, Kita-ku, Tokyo (石館守三, 桜井欽夫, 松井英一).