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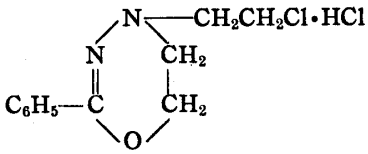
1,1-Bis(2-chloroethyl)hydrazine and its Antitumor Activity

In the study on the preparation of the masked derivatives of nitrogen mustard, the compounds, which might liberate 1,1-bis(2-chloroethyl)hydrazine hydrochloride (I) as an active agent in the body of a tumor animal, have been synthesized by the present writers and tested as to its anticancer effect during 1958.

To this end, the active form of the compound (I) was first prepared by chlorination of 1-benzoyl-2,2-bis(2-hydroxyethyl)hydrazine (II) with thionyl chloride.

Recently, a short communication of Preussmann[1] and others was found describing the preparation of the same compound. The present writers wish to describe here their own results obtained so far, although the experiment is yet continuing.

The compound (II) was prepared by treating benzoylhydrazine with ethylene oxide in *N* AcOH in 71~72% yield as white prisms, m.p. 87~88°(from AcOEt). (II) was chlorinated with $SOCl_2$ in $CHCl_3$ at room temperature for 36 hr. and the two reaction products were isolated after evaporating $SOCl_2$ *in vacuo*. The one came as colorless needles, m.p. 102~103° (from petr. ether), which was easily soluble in EtOH and identified as 1-benzoyl-2,2-bis (2-chloroethyl)hydrazine (III) (*Anal.* Calcd. for $C_{11}H_{14}ON_2Cl_2$: C, 50.59; H, 5.40; N, 10.73. Found : C, 50.42; H, 5.04; N, 10.73.) The other came as colorless plates, m.p. 144~145°(from acetone), which was sparingly soluble in EtOH and water. From the data of its analysis (*Anal.* Calcd. for $C_{11}H_{14}ON_2Cl_2$: C, 50.59; H, 5.40; N, 10.73; Cl, 27.16; Cl^-, 13.58. Found : C, 50.62; H, 5.23; N, 10.52; Cl, 27.59; Cl^-, 13.51) and the fact that one of its two chlorine atoms ionized in solution, this compound (IV) was presumed to have the following formula.



The yield of (III) was about 40% and that of (IV), 30%.

After heating a solution of (III) in conc. HCl on a boiling water bath for 10~17 hr., the reaction mixture was diluted with water to 5% HCl, and extracted with ether to remove both unchanged starting material and liberated benzoic acid. After evaporating the aqueous layer to dryness, (I) was obtained as colorless scales, m.p. 132~133°(uncorr.) (from EtOH-ether) (*Anal.* Calcd. for $C_4H_{11}N_2Cl_3$: C, 24.83; H, 5.73; N, 14.48. Found: C, 24.86; H, 5.61; N, 14.38), while Preussmann reported m.p. 133~135° (from EtOH-petr. ether) for his sample. Yield, 46%. About 45% of the starting material was recovered unchanged from the ether extract of the reaction mixture.

On refluxing (I) with AcCl in dry benzene for 2 hr., it yielded 1-acetyl-2,2-bis(2-chloroethyl)hydrazine (V) as colorless prisms, m.p. 76~77°(from hexane) (*Anal.* Calcd. for $C_6H_{12}ON_2Cl_2$: C, 36.20; H, 6.08; N, 14.07. Found : C, 36.13; H, 5.82; N, 14.08).

1-Isopropylidene-2,2-bis(2-chloroethyl)hydrazine hydrochloride (VI) was also obtained by heating (I) with acetone for 30 min. Colorless plates, m.p. 150~151° (from acetone) (*Anal.* Calcd. for $C_7H_{15}N_2Cl_3$; C, 36.00; H, 6.47; N, 12.00. Found : C, 35.79; H, 6.23; N, 12.07).

Of these compounds, only (I) and (VI) reduced instantly Fehling's solution and (I), (II), and (VI) were easily soluble in water. Dragendorff's color reaction was strongly

1) R. Preussmann, *et al.* : Angew. Chem., **70**, 743(1958).

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positive in the case of (IV) and (VI), but only faintly positive in the case of (I) and (III). Rate of Cl^- liberation in a carbonate buffer solution at 37° is in Table I.

Table I.

	Liberated Cl^- in mol. equiv.					
	10 min.	30 min.	1 hr.	2 hr.	5 hr.	24 hr.
(I)	0.01	0.25	0.28	0.38	1.25	1.63
(III)	0.00	0.00	0.00	0.00	0.13	0.49
(IV)	0.01	0.01	0.08	0.35	1.35	1.79

From these results, it seemed likely that the chemical reactivity of (I) was markedly depressed by acylation as seen in the case of (III) but not by substitution with alkylidene group. The biological effects of these compounds, tested so far using the Yoshida sarcoma rat and *in vitro*-cultured cell of the same tumor, are shown in Tables II and III.

Table II.*

	LD_{50} on Rat mg./kg.(i. p.)	M. E. D. mg./kg.	M. T. D. mg./kg.
(I)	7.5	0.5	5
(III)	175	50	100
(V)	175	50	100
(VI)	50	5	50

M. E. D. : Minimum effective dose.
M. T. D. : Maximum tolerable dose.

* The test on the Yoshida sarcoma rat was carried out by Dr. H. Satoh of this Institute. The test method was published by M. Ishidate, T. Yoshida, *et al.* (Gann, **44**, 342(1953)).

Table III.*

	M. E. C. mg./L.	Response of tumor cells		M. E. C. mg./L.	Response of tumor cells
(I)	5×10^{-3}	+	(V)	2.5×10^{-2}	+
(III)	2.5×10^{-2}	+	(VI)	2.5×10^{-3}	+
(IV)		−			

M. E. C. : Minimum effective concentration which induced the characteristic response in tumor cell.

* The method of the cell-culture test will be published in due course. These results were obtained by keeping the tumor cells (5×10^4/cc.) in contact with the test compound serially diluted in the medium through 72-hr. incubation.

The action of the acyl derivatives on the tumor animal was profoundly retarded and they hardly exhibited any effect on the *in vitro*-cultured Yoshida sarcoma cells, if the time of contact with the test solution was cut down to 30 min., which was sufficient length of time for other derivatives of nitrogen mustard to manifest their activity. In these experiments, concentration of the cells varied from 10^5/cc. to 10^8/cc. through 30 min.' incubation with the test solution, but there was found no damage on the figures of the cells through the succeeding culture for 72 hr. in a fresh medium.

The various derivatives, especially those having sugar or α-amino acid residue are now being investigated.

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