was allowed to stand at room temperature for 2 hrs. The deep red solution gradually became colorless. The mixture was filtered to remove the insoluble matter, and a sufficient amount of H_2O was added to the filtrate to produce a precipitate (0.11 g.). After recrystallization from 50% EtOH using activated carbon, it melted at 269~273.5°, undepressed on admixture with an authentic sample of biphenol.

8) **Benzoquinone Monoguanylhydrazone**—To 3.5 g. (0.032 mole) of benzoquinone dissolved in 13 cc. of hot EtOH, was added 4.0 g. (0.029 mole) of aminoguanidine nitrate (dissolved in 12 cc. of H_2O containing 3 drops of HNO_3 (sp. gr. 1.38)). After refluxing for 1 hr., a crystalline precipitate began to deposit which was collected by filtration after chilling. Benzoquinone monoguanylhydrazone was obtained as brown red needles after recrystallization from EtOH. Yield: 2.3 g., 32%, m.p. 186°(decomp.). It oxidized KI, dissolved in dil. H_2SO_4, within 10 mins. at 100°.

9) **Disodium Biphenol Biphosphate**—To a cooled mixture of 6 cc. (0.066 mole) of $POCl_3$ and 10 cc. of pyridine was added a solution of 3.9 g. (0.021 mole) of biphenol in 20 cc. of pyridine. After standing for 4 hrs. at room temperature, an excess of $POCl_3$ was decomposed with 70 cc. of H_2O. Addition of a sufficient amount of 5*N* HCl produced a thick gummy substance, which was dissolved in H_2O and made slightly alkaline to phenolphthaleine with 1*N* NaOH. It was evaporated to dryness under a reduced pressure. Yield: 4.6 g. (56%). For analysis, 0.002 mole of the above crude product was dissolved in H_2O (8 cc.) and an aqueous solution of *p*-toluidine-HCl (0.008 mole) was added. The precipitated *p*-toluidine salt was collected on a glass filter and washed first with H_2O, then with EtOH, and finally with ether. *Anal.* Calcd. for $C_{26}H_{30}O_8N_2P_2$: N, 5.00. Found: N, 4.69.

10) **Determination of Cysteine Uncombined with Benzoquinone**—A mixture of 25.0 cc. of the sample solution (cf. Table II), 5 cc. of 12*N* H_2SO_4, and 0.3 g. of Zn dust[13] was shaken gently for 30 mins. Excess of Zn was removed through a glass filter and washed repeatedly with distd. water. To the filtrate, 2 cc. of 10% aq. KI solution and crushed ice were added and titrated with *M*/300 KIO_3, which was preliminary standardized against pure cystine by a blank test by the same procedure. The titration had to be carried out at a temperature below 3°[14] and the end point of the titration was easily recognized by the yellowish coloring of the solution. No starch indicator was used. Neither hydroquinone nor cystine consumed *M*/300 KIO_3 under the conditions mentioned above.

Summary

Certain quinones and related compounds were synthesized and their antimitotic effect on the Yoshida sarcoma and bacteriostatic activity against *E. coli* were examined. None of these compouds were found to show a significant inhibition on the Yoshida sarcoma. A ratio of cysteine up-take of benzoquinone in an aqueous medium was analytically investigated. The results suggested that benzoquinone acts monofunctionally against sulfhydryl groups of the compounds *in vivo*.

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67. Sakahiko Owari: Studies on Cancerocidal Substances. XIV.* Preparation of some New Bis(β-chloroethyl)amines and their N-oxides.

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A number of acyl derivatives of bis(β-chloroethyl)-β-hydroxyethylamine and their N-oxides were prepared in order to discuss their biological activity against the Yoshida sarcoma. The compounds prepared are shown in Tables I, II, and III.

The hydroxyethyl compounds were synthesized generally by acylating triethanolamine with one mole of the corresponding acid chlorides. In order to isolate the pure mono-

13) Y. Okuda: J. Biochem. (Japan), **5**, 217 (1925).
14) C. Yoshimura, M. Matsuoka: J. Chem. Soc. Japan, **73**, 786 (1952).

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Table I. Hydroxyethyl Compounds $\left(R\text{-}O\cdot C_2H_4N<\begin{matrix}C_2H_4OH\\C_2H_4OH\end{matrix}\right)$

	R	Free base, m.p. °C	Picrate, m.p. °C	Picrylsulfonate, m.p. °C
I	Benzoyl	Oil	122~123(Needles)	—
II	*p*-Nitrobenzoyl	59~60(Plates)	169~170(Plates)	178~179(Needles)
III	Diphenylacetyl	Oil	—	142~143(Plates)

Table II. Chloroethyl Compounds $\left(R\text{-}O\cdot C_2H_4N<\begin{matrix}C_2H_4Cl\\C_2H_4Cl\end{matrix}\right)$

	R	Free base m.p. °C	Hydrochloride m.p. °C	Picrate m.p. °C	Picrylsulfonate m.p. °C
IV	Benzoyl	Oil	114 (Needles)	110 (Prisms)	—
V	*p*-Nitrobenzoyl	Oil	138~139 (Needles)	157~158 (Prisms)	—
VI	Diphenylacetyl	Oil	147~148 (Prisms)	101~102 (Needles)	—
VII	*p*-Aminobenzoyl	—	—	—	238(decomp.) (Prisms)
VIII	*p*-(4-Dimethylaminophenyl-azo-1)benzoyl	86~86.5 (Plates)	165 (Prisms)	—	—
IX	*p*-(Naphthol-2-azo-1)benzoyl	103~104 (Plates)	207~208 (Prisms)	—	—
X	*p*-(Naphol-2-disulfonic acid-6,8-azo-1)benzoyl	—	205 (Needles)	—	—
XI	H	—	—	104~105 (Needles)	131~132[a] (Plates)

a) Bergmann[2] reported m.p. 127~129° for this compound.

Table III. N-Oxide Compounds $\left(R\text{-}O\cdot C_2H_4N(\rightarrow O)<\begin{matrix}C_2H_4Cl\\C_2H_4Cl\end{matrix}\right)$

	R	Hydrochloride, m.p. °C	Picrate m.p. °C	Picrolonate, m.p. °C
XII	Benzoyl	138~139(Needles)	—	—
XIII	*p*-Nitrobenzoyl	120~122(Plates)	—	—
XIV	Diphenylacetyl	—	—	132~133(Prisms)
XV	H	—	110.5~111.5(Prisms)	—
XVI	Cyclic transformation product[b] of (XV).	—	150~160(decomp.) (Prisms)	—

b) $HO\cdot C_2H_4N<\begin{matrix}C_2H_4Cl\\ \diagup CH_2\\ O \diagdown CH_2\end{matrix}$

esters, however, it was sometimes necessary to carry out a repeated recrystallization or a fractional precipitation of their free bases from the strong acid solution by neutralization. The chlorinated compounds were obtained by treating the corresponding hydroxyethyl compounds with thionyl chloride by the usual process.

The compound No. VII, which had an unsubstituted amino group, was obtained by catalytic reduction of the chlorinated nitro compound in hydrochloric acid, while the azo compounds, VIII, IX, and X, were prepared by previous diazotizing of VII with sodium nitrite followed by coupling with dimethylaniline, β-naphthol, and G-acid, respectively.

Oxidation of the tertiary amines to the N-oxides was performed by the process previously reported.[1] However, some of the compounds, having unsubstituted amino group or azo group, could not be oxidized without decomposition. XV was once described by Bergmann[2] but it was already pointed out by the author[3] from the analytical data that Bergmann's product was not an N-oxide but a secondarily transformed product, and the true N-oxide is newly reported in this paper.

1) I. Aiko, S. Owari, M. Torigoe: J. Pharm. Soc. Japan, **72**, 1297.
2) M. A. Stahmann, M. Bergmann: J. Org. Chem., **11**, 586 (1946).
3) S. Owari: This Bulletin, **1**, 353(1953).

Owing to the existence of aromatic residue in the molecules, these compounds had in general a lipophilic character, even in the case of their N-oxides. Among these compounds, β-(*p*-aminobenzoyloxy)ethyl-bis(β-chloroethyl)amine (Ⅶ) and the azo derivatives of Ⅶ or β-(diphenylacetyloxy)ethyl-bis(β-chloroethyl)amine (Ⅵ) had a constitutional resemblance to procaine or to synthetic antispasmodics, viz. β-diethylaminoethyl diphenylacetate.

The aim of this study was to discuss whether such a change of physical property of nitrogen mustard or an addition of a characteristic group or residue to the molecule could give profitable influence upon their chemotherapeutic index in animal experiments employing the Yoshida sarcoma.

The detail of the animal experiments will be published in Gann (The Japanese Journal of Cancer Research) this year. The author is indebted to Mr. D. Ohata for microanalyses in this study.

Experimental

β-Benzoyloxyethyl-bis(β-hydroxyethyl)amine (I)—Into 30% acetone solution of triethanolamine (90 g.) was added dropwise 28 g. of benzoyl chloride dissolved in 2 volumes of acetone, under vigorous stirring. After being cooled, the crystallized precipitate was filtered off and dry HCl was passed through the filtrate. This was continued until a precipitation of the crystalline hydrochloride of triethanolamine ended, which was removed by filtration. Excess of HCl should be avoided lest the separation of a syrupy hydrochloride of the monoacylated amine should follow. The filtrate was evaporated and the residue was treated with 3% HCl. This acid solution was then neutralized with alkali and extracted with $CHCl_3$. It was not obtained as crystals and decomposed by distillation even *in vacuo*. It gave a yellow picrate, m.p. 122~123°. Yield, 30 g. *Anal.* Calcd. for $C_{12}H_{19}O_4N \cdot C_6H_3O_7N_3$: C, 47.30; H, 4.56; N, 11.62. Found: C, 47.41; H, 4.54; N, 11.50.

β-(*p*-Nitrobenzoyloxy)ethyl-bis(β-hydroxyethyl)amine (II)—It was obtained by treating 90 g. of triethanolamine with 37 g. of *p*-nitrobenzoyl chloride by the same process as for (I). It was soluble in 1% HCl and the free base was recrystallized from benzene. Yield, 10 g. *Anal.* Calcd. for $C_{13}H_{18}O_6N_2$: C, 52.17; H, 6.35; N, 9.37. Found: C, 52.04; H, 6.25; N, 9.40.

β-Diphenylacetyloxyethyl-bis(β-hydroxyethyl)amine(III)—It was obtained from diphenylacetic chloride (46 g.) and triethanolamine (120 g.). Its free base, hydrochloride, or picrate was not crystallizable, but its picryl sulfonate alone was obtained as crystals and purified by recrystallization from EtOH. Yield, 45 g. (as a free base). *Anal.* Calcd. for $C_{20}H_{25}O_4N \cdot C_6H_3O_9N_3S$: C, 49.06; H, 4.40; N, 8.81. Found: C, 49.04; H, 4.39; N, 8.91.

Chlorination of (I), (II), and (III)—The hydroxyethyl derivatives (30 g.) were chlorinated by refluxing with a mixture of $CHCl_3$ (100 cc.) and $SOCl_2$ (60 g.) on water bath for 1 hr. After the reaction ended, an excess of $SOCl_2$ and $CHCl_3$ were distilled off *in vacuo*. The residue solidified to a crystalline mass and was recrystallized from acetone.

The hydrochloride of (Ⅳ) was found difficult to be obtained in a pure state by the above-mentioned process and its pure specimen was prepared by the catalytic reduction of its N-oxide (Ⅻ) and recrystallized from an acetone-ether mixture. *Anal.* Calcd. for $C_{13}H_{18}O_2NCl_3$: C, 47.78; H, 5.51; N, 4.29. Found: C, 47.79; H, 5.36; N, 4.40.

Crystalline hydrochloride of (Ⅴ) was obtained directly from a reaction mixture. Yield, quantitative. *Anal.* Calcd. for $C_{13}H_{17}O_4N_2Cl_3$: C, 41.99; H, 4.58; N, 7.51. Found: C, 41.96; H, 4.60; N, 7.47.

The yield of hydrochloride of (Ⅵ) was 29 g. starting from 30 g. of the free base (Ⅲ). *Anal.* Calcd. for $C_{20}H_{24}O_2NCl_3$: C, 57.62; H, 5.76; N, 3.36. Found: C, 57.62; H, 5.82; N, 3.40.

Oxidation of (Ⅳ), (Ⅴ), and (Ⅵ)—Oxidation was accomplished by the usual process previously reported,[1] using Ac_2O and H_2O_2 in ether solution.

The crystalline hydrochlorides of (Ⅳ) and (Ⅴ) were obtained by extracting the reaction mixture with conc. HCl. The former hydrochloride was easily purified by recrystallization from acetone and the latter was obtained from a readily soluble portion of an acetone extract of the crude chloride.

Hydrochlorides of the N-oxides were sparingly soluble in water and not hygroscopic, and easily oxidized KI solution.

Hydrochloride of (Ⅻ) was recrystallized from acetone to needles. Yield, 8.5 g. (from 16 g. of hydrochloride of (Ⅳ)). *Anal.* Calcd. for $C_{13}H_{18}O_3NCl_3$: C, 45.55; H, 5.26; N, 4.32. Found: C, 45.41; H, 5.27; N, 4.54.

Hydrochloride of (XIII) was recrystallized from an acetone-ether mixture to plates. Yield, 1 g. (from 8 g. of the hydrochloride of (V)). *Anal.* Calcd. for $C_{13}H_{17}O_5N_2Cl_3$: C, 40.25; H, 4.39; N, 7.22. Found: C, 40.32; H, 4.29; N, 7.17.

(XIV) was not obtained as a pure hydrochloride but its picrolonate alone became crystalline, which was recrystallized from EtOH to prisms. Yield, 8 g. as syrupy hydrochloride (from 10 g. of hydrochloride of (VI)). *Anal.* Calcd. for $C_{20}H_{23}O_3NCl_3 \cdot C_{10}H_8O_5N_4$: C, 54.55; H, 4.70; N, 10.61. Found: C, 54.51; H, 4.79; N, 10.67.

β-(*p*-Aminobenzoyloxy)ethyl-bis(β-chloroethyl)amine (VII)—Five g. of finely powdered (V) was suspended in 90 cc. of 3% HCl and shaken with H_2 over Pd-C catalyst (10 cc. of 0.5% $PdCl_2$ and 1 g. of activated carbon). Absorption of H_2 stopped nearly at 500 cc.

The primary amine was so unstable in air that it could not be isolated from the reaction mixture except as a picryl sulfonate. Prisms, m.p. 238°(decomp.) (from dil. HCl). *Anal.* Calcd. for $C_{13}H_{18}O_2N_2Cl_2 \cdot 2C_6H_3O_9N_3S$: C, 33.67; H, 2.69; N, 12.56. Found: C, 33.74; H, 2.77; N, 12.70.

β-[*p*-(4-Dimethylaminophenylazo-1)benzoyloxy]ethyl-bis(β-chloroethyl)amine (VIII)—A solution of (VII) in 3% HCl (500 cc.), which was prepared by the catalytic reduction from 5 g. of (V), was immediately diazotized with $NaNO_2$ (0.9 g.) and then poured into an ice-cooled mixture of dimethylaniline (2 g.) and water under stirring. After 30 min., the deep red solution was neutralized with Na_2CO_3, and a crystalline precipitate was gathered, which was recrystallized from EtOH. m.p. 86~86.5°. Yield, 6 g. *Anal.* Calcd. for $C_{21}H_{26}O_2N_4Cl_2$: C, 57.66; H, 5.95; N, 12.81. Found: C, 57.53; H, 5.86; N, 12.97.

β-[*p*-(Naphthol-2-azo-1)benzoyloxy]ethyl-bis(β-chloroethyl)amine (IX)—Five g. of (V) was reduced and diazotized without isolation of the primary amine. This was added into an ice-cooled mixture of β-naphthol (2 g.), NaOH (0.6 g.), Na_2CO_3 (20 g.), and water (250 cc.). A dark red precipitate immediately formed, which was recrystallized from acetone or EtOH. m.p. 103~104°. Yield, 3.5 g. *Anal.* Calcd. for $C_{23}H_{23}O_3N_3Cl_2$: C, 60.00; H, 5.04; N, 9.13. Found: C, 59.92; H, 5.01; N, 9.31.

Its hydrochloride was recrystallized from acetone or EtOH containing a small amount of HCl to prisms, m.p. 207~208°. *Anal.* Calcd. for $C_{23}H_{24}O_3N_3Cl_3$: C, 56.39; H, 4.83; N, 8.46. Found: C, 56.24; H, 4.82; N, 8.44.

β-[*p*-(Naphthol-2-disulfonic acid-6,8-azo-1)benzoyloxy]ethyl-bis(β-chloroethyl)amine (X)—Five g. of (V) was reduced and immediately diazotized without isolating the amine. This was added to an aqueous solution of potassium salt of G-acid (3.5 g.). It was then made weakly alkaline with addition of dil. NaOH solution. A yellow precipitate, which was produced at the beginning of the reaction, disappeared again by the addition of NaOH and the solution turned gradually to deep red. Then the solution was acidified with HCl and an orange precipitate was filtered off and recrystallized from 50% EtOH containing a small amount of HCl. Dark red or black needles when dried. Yield, 2.5 g. Its analytical data agreed well with the hydrochloride. *Anal.* Calcd. for $C_{23}H_{24}O_9N_3Cl_3S_2$: C, 44.19; H, 3.84; N, 6.73. Found: C, 44.08; H, 3.97; N, 6.74.

Hydrolysed Product of β-Acyloxyethyl-bis(β-chloroethyl)amine—β-Acyloxyethyl derivative was heated with conc. HCl on a water bath for 1 hr. After being cooled, the filtrate was evaporated to dryness *in vacuo* and added with an aqueous picric acid solution. The picrate was recrystallized from EtOH or acetone. It melted at 104~105°, and was identical with the picrate of β-hydroxyethyl-bis(β-chloroethyl)amine. *Anal.* Calcd. for $C_6H_{13}ONCl_2 \cdot C_6H_3O_7N_3$: C, 34.73; H, 3.89; N, 13.50. Found: C, 34.72; H, 3.95; N, 13.23.

Hydrolysed Product of β-Acyloxyethyl-bis(β-chloroethyl)amine N-oxide—By a similar hydrolyzing procedure as described above, the N-oxide gave a picrate of the cyclic transformation product of N-bis(β-chloroethyl)-β-hydroxyethylamine N-oxide, m.p. 150~160°(decomp.), which was confirmed by analysis. *Anal.* Calcd. for $C_6H_{13}O_2NCl \cdot C_6H_3O_7N_3$: C, 36.50; H, 4.05; N, 14.19. Found: C, 36.28; H, 3.91; N, 14.30.

β-Hydroxyethyl-bis(β-chloroethyl)amine N-oxide (XVI)—(XI) (4.2 g.) was oxidized with EtOH solution of H_2O_2 and Ac_2O and then the reaction mixture was shaken with a small amount of water. The N-oxide was obtained from the water layer as a picrate, from which its hydrochloride was regained by treating with HCl as a syrupy product. It oxidized a solution of KI. Yield, 1 g. *Anal.* Calcd. for $C_6H_{13}O_2NCl_2 \cdot C_6H_3O_7N_3$: C, 33.43; H, 3.74; N, 12.99. Found: C, 33.56; H, 3.70; N, 12.82.

Summary

A number of new derivatives of β-acyloxyethyl-bis(β-chloroethyl)amines and their N-oxides were prepared for the purpose of screening their anticancer effectiveness using the Yoshida sarcoma.

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