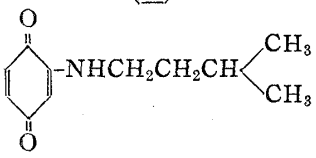
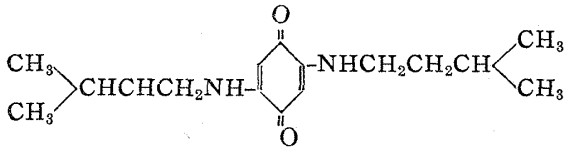
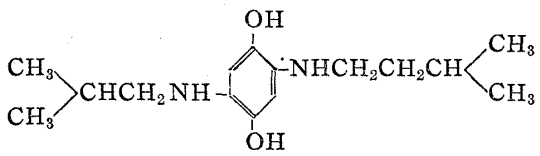
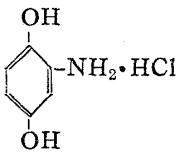
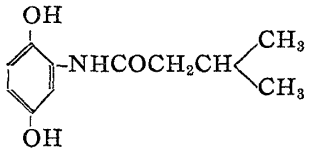
66. **Masahiro Torigoe**: Studies on Cancerocidal Substances. XIII.* Effect of some Quinones and Related Compounds upon the Yoshida Sarcoma.

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

Results of many experiments have been published on the antimitotic activity of quinone and related compounds, employing chick fibroblast cells, tubifex eggs, root tips of plants, experimental tumors of animals, and also human carcinoma. Hayashi[1] and Naito[2] reported bacteriostatic action of the quinones and Takizawa[3] found a carcinogenic activity of benzoquinone in mice. Such biological activity of quinone and its related compounds has aroused interest in the field of cancer chemotherapy.

The following is a report on a part of a series of studies[4] on cancerocidal substances and describes the synthesis of some quinones and compounds with quinoid structures, their mitotic effect upon the Yoshida sarcoma cell, and also their bacteriostatic activity *in vitro* against *Escherichia coli*. Compounds studied herein are listed in Table I.

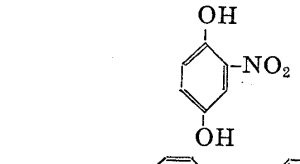
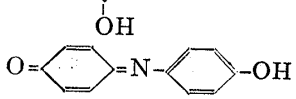
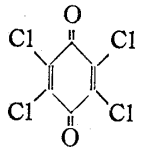
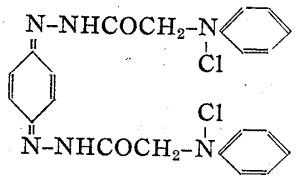
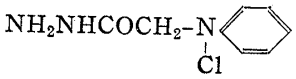
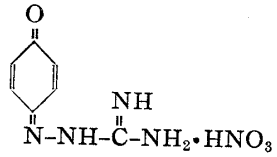
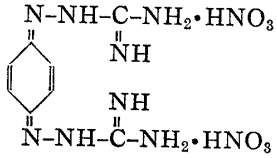
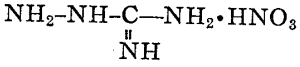
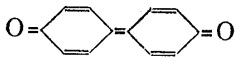
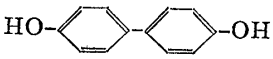
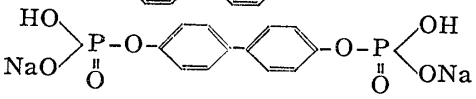
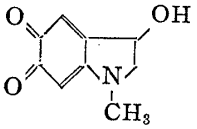
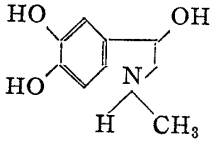
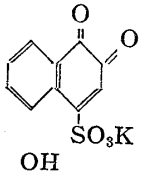
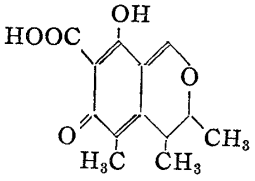
TABLE I.

Compounds	Min. concn.[a] for inhib. *E. coli* (γ/cc.)	LD_{50} in rats (intrap.) (mg./kg.)	Tumor cell response[b] (Yoshida sarcoma)
	1.25	30	(±)
	25		
	>20		
	>100		
	>100		
	5	30	(−)
	10		

* This is a part of a series entitled "Studies on Cancerocidal Substances" by M. Ishidate and Y. Sakurai. Pat XII: see Footnote 4)

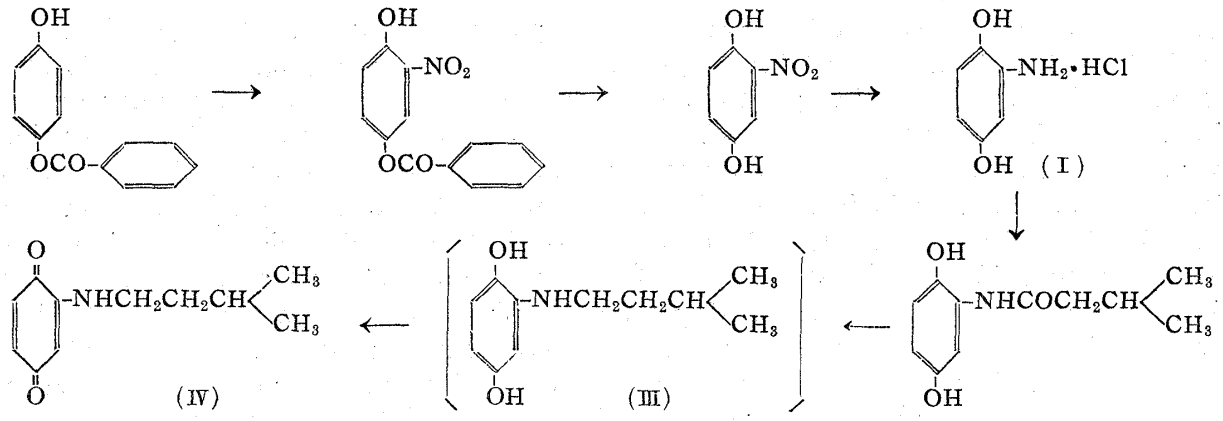
** Nishigahra-machi, Kita-ku, Tokyo (鳥越政宏).

1) S. Hayashi: Yakugaku (Science of Drug), **4**, 60(1950).
2) M. Naito, *et al.*: J. Pharm, Soc. Japan, **72**, 1047(1951).
3) N. Takizawa: Gann, **39**, 56(1948).
4) M. Ishidate, Y. Sakurai: This Bulletin, **3**, 88(1955)

	25		
	25	30	(−)
	10	500	(±)
	50		
	>1000		
	50	750	(−)
	250		
	>1000		
	>20	300	(±)
	100	750	(−)
	>1000	750	(−)
		>100	(−)
	100		
	100	200	(−)
		50	(−)

a) The synthetic medium used in this experiment was reported by J. W. Bigger and G. C. Ware (Lancet, **259**, 427(1950)).
b) The maximum tolerable dose of the compounds is given in each experiment. (±) indicates the cacodylate-like action on the Yoshida sarcoma cells.[8]

Among these compounds, indophenol, Girard-P reagent, chloranil, aminoguanidine nitrate, biphenoquinone,[5] biphenol, and adrenochrome[6] were prepared according to the methods described in the literatures. 2,5-Bis(isoamylamino)benzoquinone and 2-isoamylaminobenzoquinone, however, have not been prepared previously. The former was obtained as red crystals by treating benzoquinone with isoamylamine in ethanol and the latter by the following process.



The acylation of (I) with isovaleroyl anhydride in water in the presence of sodium sulfite gave (II), which was reduced to (III) with lithium aluminum hydride in ether. (III) was not obtained in a pure state because of its instability and was subjected without further purification to oxidation with ferric chloride to obtain (IV).

Treatment of biphenol in pyridine with phosphoryl chloride gave its diphosphoric ester (V), identification of which was confirmed by its crystalline *p*-toluidine salt. The condensation product of benzoquinone with Girard-P reagent was isolated by mixing both components in ethanol, containing hydrochloric acid, but biphenoquinone was only reduced by Girard-P and gave biphenol in quantitative yield.

Biphenoquinone was more easily oxidized than benzoquinone, and their oxidation-reduction potentials according to Rao and Peterson[7] are ca. 950 mV. and ca. 700 mV., respectively. In a neutral aqueous solution, biphenoquinone oxidized potassium iodide to iodine, while benzoquinone did not. Benzoquinone monoguanylhydrazone was less oxidizable than the original quinone by ferrous ion.

The inhibiting activity of the compounds against *E. coli* and their action on rats bearing the Yoshida sarcoma are summarized in Table I.*

From Table I, it can be concluded that none of these quinones were effective against the tumor, although some of them exhibited weak damaging effect upon the nuclei of the tumor cells, causing scattering of lacerated chromosomes and caryolysis. Such effect upon the nuelei has already been observed when tumor rats were treated with sodium cacodylate[8] and it had been found that the potency was not sufficient to prolong the life span of the tumor animals.

5) R. Willstätter, L. Kalb: Ber., **38**, 1235(1905).
6) J. H. Mason: J. Chem. Soc., **1950**, 1276.
7) K. V. Rao, W. H. Peterson: J. Am. Chem. Soc., **76**, 1340(1954).
8) T. Asano: Tohoku J. Exptl. Med., **57**, 227(1953).
* Details of animal experiments were reported at the Annual Meeting of the Japanese Cancer Association held in Kyoto on April 4, 1955.

Yoshida[9] has described that hydrogen peroxide inhibited mitosis of Yoshida sarcoma. The author investigated the antimitotic activity of perbenzoic acid, as it was considered possible that the oxidizing action of quinones may have some relation to their biological activity. The peracid, however, was found to show no significant inhibition on mitosis of the Yoshida sarcoma.

The bacteriostaic activity of benzoquinone was remarkably lowered by substitution of its hydrogen atoms, and the fact that monosubstituted benzoquinone remained highly active in comparison with disubstituted benzoquinone suggests the significance of chemical reactivity in the biological activity of quinone.

In 1944, Kuhn and Beinert[10] suggested that inactivation of carboxylase by benzoquinone was due to its reaction upon thiol groups of the enzyme. They studied the chemical reaction between cysteine ethyl ester and benzoquinone, succeeded in isolating some of the condensation products from the reaction mixture and further determined that quinone could be regarded as a kind of alkylating agent against the thiol group. The molecular ratio of cysteine up-take of benzoquinone in dilute aqueous solution was studied analytically by the author under the same conditions as in the determination of the reaction ratio between β-chloroethylamines[11] and cysteine. A neutral or an acid aqueous solution of the components was kept at 28~29°. After standing for 6, 24, and 48 hours, aliquots were pipetted out and treated with zinc dust to reduce cystine to cysteine, the former having been produced by benzoquinone or air oxygen during treatment. The filtrate was then titrated with potassium iodate in 2*N* sulfuric acid in the presence of potassium iodide. The data obtained are summarized in Table II.

Table II. Cysteine Up-take of Benzoquinone in Aqueous Solution
(Concentration: Benzoquinone, $10^{-3}M$/L., cysteine, $4\times10^{-3}M$/L., $NaHCO_3$, $12\times10^{-3}M$/L., at 28~29°)

Condition of reaction	Cysteine up-take (mol. equiv.)			
	10 min.	6 hrs.	24 hrs.	48 hrs.
Neutral medium[a] (pH: 6.6~7.1) anaerobic	0.92		1.0~1.1	0.91
Neutral medium (pH: 6.6~7.1) aerobic	0.92	1.13	1.7~1.9	
Acid medium[b] (pH: 2.6) aerobic	0.87~0.91			0.91~0.97

a) Water, used in dissolving the compounds, was redistilled and made oxygen-free by the bubbling through of hydrogen. The sample solution was sealed in ampules, in which the atmosphere was displaced by CO_2.
b) $NaHCO_3$ was not added to the sample solution.

The conclusion is that, either in neutral or in acid aqueous medium, benzoquinone instantly consumed about one molar equivalent of cysteine and the characteristic yellow color of benzoquinone disappeared. No further cysteine was consumed even after 48 hours under anaerobic conditions, but the reaction mixture again darkened intensively in the presence of air oxygen, and further consumption of cysteine occurred.

It was found that the reaction velocity of benzoquinone with cysteine under such a condition was high even when compared with nitrogen mustards, but benzoquinone is supposed to act monofunctionally against thiol or amino groups *in vivo*.

Biphenoquinone was expected to react bifunctionally with cysteine in an anaerobic environment, but its cysteine up-take in an aqueous solution could not be determined due to its extremely poor solubility or its strong oxidizing activity against cysteine. Biphenoquinone was also found to be no more significant than benzoquinone in its bio-

9) T. Yoshida: "Yoshida Nikushu" (The Yoshida Sarcoma) 2nd Ed., Nara-Shobo, Tokyo, 186 (1952).
10) R. Kuhn, H. Beinert: Ber., **76**, 904(1943); **77**, 606(1944).
11) M. Torigoe: This Bulletin, **1**, 349(1953).

logical effects upon the Yoshida sarcoma rats or *E. coli*.

The author is indebted to Mr. H. Sato and Mr. H. Imamura for the animal experiments and to Miss H. Komai for the determination of bacteriostatic activity of the compounds. The microanalyses presented in this paper were carried out by Mr. D. Ohata to whom the author wishes to express his gratitude.

Experimental

1) **2-Nitrohydroquinone**—2-Nitro-4-benzoyloxyphenol (7.6 g., 0.029 mole) was treated with 98 cc. of 10% aq. KOH (0.17 mole) at 12~13°, and after 30 mins. the solid that remained insoluble was removed by filtration. Orange red crystals precipitated when the filtrate was acidified. Yield: 2.8 g.(62%). The crude product was recrystallized from dil. EtOH (10%).

2) **2-Isovaleroylaminohydroquinone**—2-Aminohydroquinone (I)-HCl was obtained from 2-nitrohydroquinone by reduction with Sn and conc. HCl.[12] *Anal.* Calcd. for $C_6H_8O_2NCl$: N, 8.67. Found: N, 8.59.

To a mixture of (I)-HCl (2.6 g., 0.016 mole), Na_2SO_3 (2.4 g., 0.019 mole), and H_2O (38 cc.) was added isovaleroyl anhydride (3.6 g., 0.019 mole) and kept at a room temperature for 8 hrs. The precipitated colorless crystals were recrystallized from AcOEt-benzene mixture to colorless needles, m.p. 190~192°. *Anal.* Calcd. for $C_{11}H_{15}O_3N$: C, 63.15; H, 7.23; N, 6.70. Found: C, 63.27; H, 7.22; N, 6.46.

3) **2-Isoamylaminobenzoquinone-1,4**—In a two-necked flask provided with a sealed stirrer and a Soxhlet extractor surmounted by a reflux condenser, $LiAlH_4$ (0.8 g.) and ether (70 cc.) were placed and 1.75 g.(0.0084 mole) of isovaleroylaminohydroquinone was placed in the extractor thimble. During a period of refluxing and stirring for 40 hrs., $LiAlH_4$ was added four times with ether (0.32, 0.32, 0.16, and 0.16 g. of $LiAlH_4$ and 20 cc. of ether in every addition). Powdered Na_2SO_3 (1.75 g.) was then added and an excess of the hydride was decomposed by a portionwise addition of 2*N* HCl (125 cc. in total) under strong cooling with brine. After the ether layer was removed, the water layer was extracted repeatedly with AcOEt. The AcOEt extracts were combined with the ether layer removed earlier and evaporated to dryness. A solid residue (0.36 g.) was recrystallized from AcOEt to colorless needles of 2-isovaleroylaminohydroquinone, m.p. 191~192°. The aqueous layer was first added with 4 cc. $FeCl_3$ solution (10 g. of $FeCl_3 \cdot 6H_2O$ dissolved in 50 cc. H_2O) and then extracted with benzene (10 cc.). This benzene extract contained some unknown substance which was not examined closely. The water layer was again oxidized with 1 cc. $FeCl_3$ solution and extracted twice with 10 cc. each of benzene. The benzene extract was concentrated to a small volume under reduced pressure. Upon cooling, 0.18 g.(11%) of red crystals deposited. It was recrystallized four times from a benzene-petroleum ether mixture. *Anal.* Calcd. for $C_{11}H_{15}O_2N$: C, 68.38; H, 7.83; N, 7.25. Found: C, 68.70, 68.77; H, 7.64, 7.60; N, 7.32. λ_{max} 304~307, 480~485 mμ (in benzene).

4) **2,5-Bis(isoamylamino)benzoquinone-1,4**—To a solution of 5.4 g.(0.05 mole) of benzoquinone in 190 cc. EtOH was added EtOH solution of isoamylamine (isoamylamine-HCl 3.1 g., metallic Na 0.58 g., and EtOH 50 cc.). After standing overnight at room temperature, EtOH was removed *in vacuo* at 30~40°. When placed in a refrigerator, red crystals of 2,5-bis (isoamylamino)-benzoquinone separated. Yield: 1.0 g. (29%). It was dissolved in acetone and purified by alumina chromatography. The eluate was evaporated and recrystallized from acetone, m.p. 170~171°. *Anal.* Calcd. for $C_{16}H_{26}O_2N$: C, 69.05; N, 10.07. Found: C, 69.19; H, 9.42; N, 10.28.

5) **2,5-Bis(isoamylamino)hydroquinone-1,4**—A mixture of 2,5-bis(isoamylamino)benzoquinone (0.28 g.), 1*N* HCl (6 cc.), and EtOH (100 cc.) was shaken with hydrogen over Pd-C catalyst (prepared from 0.04 g. of carbon and 0.4 cc. of 0.5% aq. $PdCl_2$). After removal of the catalyst and evaporation of the solvent, a colorless solid remained which was recrystallized from EtOH-ether mixture in H_2 atmosphere, m.p. ca. 210°(decomp.). *Anal.* Calcd. for $C_{16}H_{30}O_2N_2Cl_2$: C, 54.39; H, 8.56; N, 7.93. Found: C, 54.38; H, 8.53; N, 7.96.

6) **Condensation Product of Benzoquinone with Girard-P Reagent**—To a solution of 1.1 g. (0.01 mole) of beozoquinone in 25 cc. EtOH was added 0.027 mole of Girard-P reagent (Girard-P reagent 5.0 g., 5*N* HCl 5 cc., and H_2O 2.5 cc.). When the mixture was chilled in a refrigerator, yellow crystals of the addition product separated out slowly. Yield, 0.3 g.(6%). It was recrystallized four times from dil. EtOH. *Anal.* Calcd. for $C_{20}H_{20}O_2N_6Cl_2$: C, 53.70; H, 4.56; N, 18.79. Found: C, 53.54; H, 5.15; N, 19.03. Calcd. for $C_{20}H_{20}O_2N_6Cl_2 \cdot 2H_2O$: C, 49.70; H, 5.01; N, 17.39; Cl, 14.67; H_2O, 7.46. Found: C, 49.25; H, 5.46; N, 17.81; Cl, 14.48; H_2O, 7.33, 7.68.

7) **Reaction between Biphenoquinone and Girard-P Reagent**—A mixture of 0.11 g.(0.0006 mole) of biphenoquinone, 0.3 g.(0.0016 mole) of Girad-P reagent, 0.3 cc. of 5*N* HCl, and 6 cc. of EtOH

12) F. Henrich: Ber., **54**, 2506(1921).

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).

* This paper constitutes a part of series entitled "Studies on Cancerocidal Substances" by M. Ishidate and Y. Sakurai. Part XIII: This Bulletin, **3**, 337 (1955).

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