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# Biochemical Studies on Benzoquinone Derivatives. V.[1] Structure-Activity Relationship between Benzoquinone Derivatives and Inhibition of Respiration of Rat Liver Intact Mitochondria[2]

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Alkyldihydroxy-*p*-benzoquinone and related compounds were newly prepared and effects of these compounds on a respiration of rat liver intact mitochondria were examined. Structure activity relationship between the benzoquinone derivatives and effects on the mitochondrial respiration was discussed.

1. 2-Methyl-5-octyl-3,6-dihydroxy-1,4-benzoquinone and ardisiaquinone B showed specific inhibition of State-3 respiration. But in high concentration they inhibited both State-3 and State-4 respiration.

2. 2-Octyl-3,5-dihydroxy-1,4-benzoquinone and ardisiaquinone A inhibited both State-3 and State-4 respiration.

3. Rapanone, maesaquinone, polygonaquinone and related compounds having longer alkyl chains did not show any effect. As the result carbon numbers of the side chain have been found to be an important factor to exhibit the activity.

In the course of studies on actions of naturally occurring benzoquinone derivatives on mitochondrial functions, it was observed that some benzoquinone derivatives showed significant effects on mitochondrial respiration.[4-8] Particularly, alkyldihydroxy-*p*-benzoquinones have marked inhibitory actions on rat liver mitochondrial respiration and oxidative phosphorylation.[7,8]

In order to obtain more potent agents as inhibitors on electron transport system and oxidative phosphorylation reactions and to make further confirmation on the structure-activity relationship between the chemical structures of benzoquinones and the inhibition of mitochondrial respiration, several kinds of mono- and di-alkyl-3,6-dihydroxy-*p*-benzoquinones and the related compounds were prepared and the effects on the respiration were examined.

## Materials

2-Octyl-(VIII), -methyl-5-octyl-(IX), 2-methyl-5-hexadecyl-(X), and 2,5-dioctyl-3,6-dihydroxy-*p*-benzoquinone (XIII) were prepared from *p*-dimethoxybenzene (1a), 2,5-dimethoxytoluene (1b), or 2,5-dimethoxyoctylbenzene (1d) according to the method of Robinson,[9,10] as shown in Chart 1.

1) Part IV: *Seikagaku*, **39**, 233 (1967).
2) This work was presented at the 23rd Annual Meeting of Pharmaceutical Society of Japan, October 1966, at Sendai.
3) Location: *a*) *Kitayobancho, Sendai.* *b*) *Tamagawayoga, Setagaya-ku, Tokyo.*
4) H. Ozawa, K. Momose, S. Natori, H. Ogawa, and K. Yamaguchi, *Biochem. Biophys. Acta*, **86**, 395 (1964).
5) H. Ozawa, S. Natori, and K. Momose, *Chem. Pharm. Bull.* (Tokyo), **13**, 1029 (1965).
6) H. Ozawa, S. Natori, and K. Momose, *Chem. Pharm. Bull.* (Tokyo), **15**, 1095 (1967).
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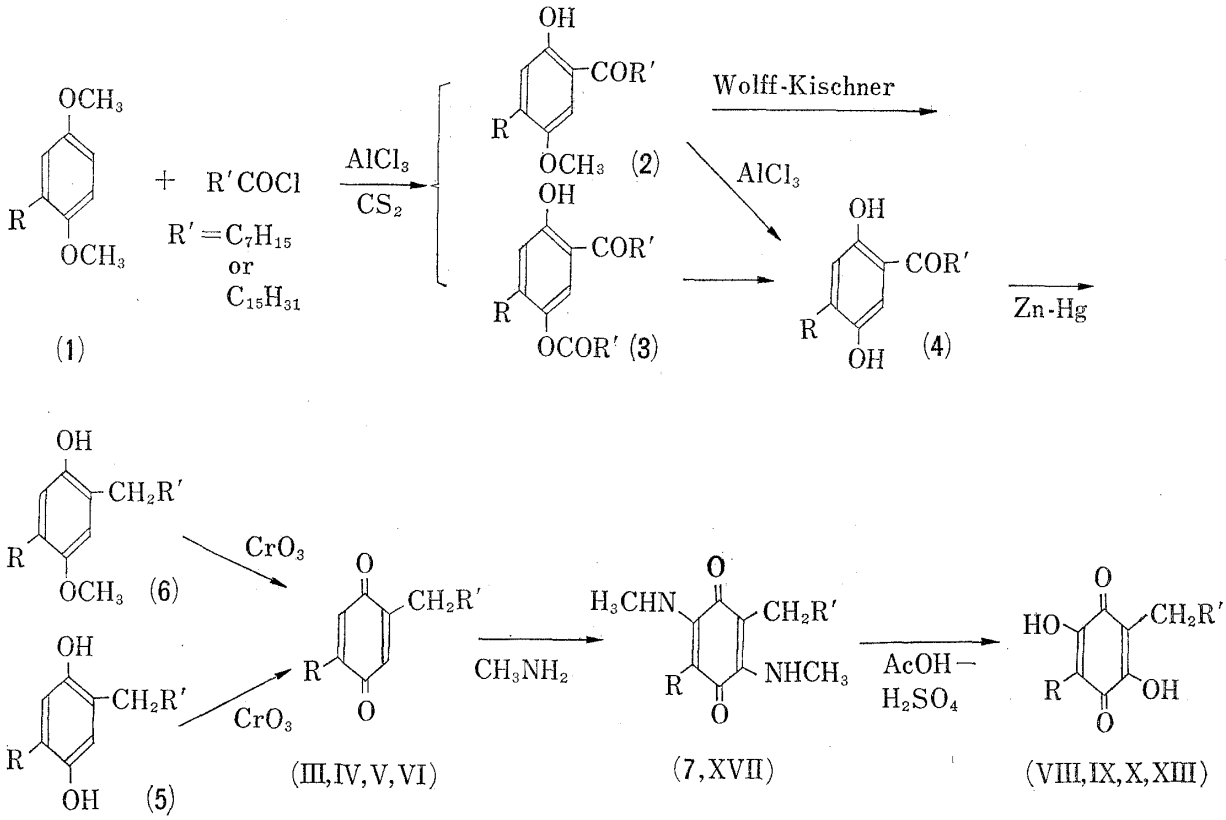
9) J.H. Cruickshank and R. Robinson, *J. Chem. Soc.*, **1938**, 2064; H. Hasan and E. Stedman, *ibid.*, **1931**, 2112; M. Asano and J. Hase, *Yakugaku Zasshi*, **60**, 650 (1940); K. Yamaguchi, *ibid.*, **70**, 24 (1950).
10) K. Yoshihira and S. Natori, *Chem. Pharm. Bull.* (Tokyo), **14**, 1052 (1966).




1a—7a, III, VIII, XVII : R=H, R'=$C_7H_{15}$ 1c—7c, V, X : R=$CH_3$, R'=$C_{15}H_{31}$
1b—7b, IV, IX : R=$CH_3$, R'=$C_7H_{15}$ 1d—7d, VI, XIII : R=$C_8H_{17}$, R'=$C_7H_{15}$

Chart 1

The position of the secondary introduced acyl groups in **2**, **3**, and **4** has been confirmed as follows: The IR spectra of alkyl aryl ketones (2b and 2d) show absorptions around 1640 $cm^{-1}$, which indicate the presence of hydrogen bond. On the other hand NMR spectrum of IV indicates allylic couplings of both of the methyl group and the allylic methylene with the respective adjacent ring protons (7.93$\tau$ (3H, d, $J$=1.5 cps) and 3.43 $\tau$ (1H, q, $J$=1.5 cps); 7.59 $\tau$ (2H, m) and 3.41 $\tau$ (1H, t, $J$=1.5 cps)). Thus the acyl group must be in 1,4-position of the alkyl group of 1b and 1d.

As for the preparation of bis(2,5-dihydroxy-3-alkylbenzoquinonyl)methane, homologues of vilangin,[11] the compounds having methyl and tridecyl side chains (XVIII and XIX) were synthesized by the method of Rao and Venkateswarlu.[12]

Ardisiaquinones A and B (XX and XXI), mp 149—153° and mp 109—112°, are novel bisbenzoquinonyl-olefine compounds from *Ardisia sieboldi* Miq. and the structural elucidation of the new natural products has been published.[13]

Other compounds used in this work are commercial products or have been prepared by the known methods: *p*-benzoquinone (I), mp 117°; 2-methyl-*p*-benzoquinone (II), mp 69°; 2-methyl-3,6-dihydroxy-*p*-benzoquinone (VII), mp 135—136°; polygonaquinone (XI),[10,14] mp 134°; 2,5-diphenyl-3,6-dihydroxy-*p*-benzoquinone (XII),[15] mp 306—307°; and 2-methyl-3,6-dibromo-*p*-benzoquinone (XIV),[16] mp 81—82°; 2,5-dihydroxy-*p*-benzoquinone (XV), mp 210—215° (decomp.); and 2,3,5,6-tetrahydroxy-*p*-benzoquinone (XVI), mp >300°.

**2-Hydroxy-5-methoxy-(2a) and 2-Hydroxy-5-octanoyloxy-octanophenone (3a)**——*p*-Dimethoxybenzene (1a) (12 g) in $CS_2$ was added to a mixture of octanoyl chloride (14 g), $AlCl_3$ (14 g), and $CS_2$ and the mixture was heated for 10 hr. The solvent was removed and the reaction mixture was treated with 2 N HCl and extracted with ether. Crystallization of the ethereal residue from EtOH gave yellow plates (2a) (10.5 g) of

11) Ch. B. Rao and V. Venkateswarlu, *J. Org. Chem.*, **26**, 4529 (1961).
12) Ch. B. Rao and V. Venkateswarlu, *Tetrahedron*, **18**, 361 (1962).
13) H. Ogawa and S. Natori, *Tetrahedron Letters*, **11**, 1387 (1968); *Chem. Pharm. Bull.* (Tokyo), **15**, 380 (1967).
14) H. Nakata, K. Sasaki, I. Morimoto, and Y. Hirata, *Tetrahedron*, **20**, 2319 (1964).
15) R. Pummerer and E. Prell, *Ber.*, **55**, 3095 (1922).
16) L.I. Smith and D.J. Byers, *J. Am. Chem. Soc.*, **63**, 616 (1941).

mp[17] 44—45°. IR[18] $cm^{-1}$: 1630 (bonded CO), 1240 ($CH_3O$). *Anal.* Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.18; H, 8.74.

The evaporation of the mother liquor of 2a and recrystallization from 80% EtOH afforded colorless needles of 3a (1.7 g), mp 58—59°. IR $cm^{-1}$: 1755 (ester CO), 1635 (bonded CO), 1170 (ester CO). *Anal.* Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 72.81; H, 9.13.

By the use of 2 moles of $AlCl_3$ and the acid chloride, 3a was obtained as the main product.

**2,5-Dihydroxyoctanophenone (4a)**——Hydrolysis of 3a (5 g) with EtOH–10% HCl, followed by the recrystallization from EtOH, gave yellow plates (4a) (3.8 g) of mp 86—87°. IR $cm^{-1}$: 3190 (OH), 1610 (bonded CO). *Anal.* Calcd. for $C_{14}H_{20}O_3$: C, 71.16; H, 8.53. Found: C, 70.91; H, 8.76.

**2,5-Dihydroxyoctylbenzene (5a)**——4a (2 g) in EtOH (200 ml) was treated with amalgamated zinc (20 g) and conc. HCl (100 ml) for 10 hr under heating. After filtration and evaporation, the separated crystals were recrystallized from EtOH to colorless plates (5a) (1.1 g), mp 96—97°. IR $cm^{-1}$: 3280 (OH). *Anal.* Calcd. for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 75.17; H, 9.51.

**2-Octylbenzoquinone (III)**——To the solution of 5a (0.5 g) in HOAc (40 ml) was added $CrO_3$ (0.2 g) in water and warmed on a water bath. After cooling and the addition of water, the residue was crystallized from EtOH to yellow needles (III) of mp 59—60° (0.38 g). IR $cm^{-1}$: 1658 (CO). *Anal.* Calcd. for $C_{14}H_{20}O_2$: C, 76.32; H, 9.15. Found: C, 76.22; H, 8.97.

**2,5-bis(Methylamino)-3-octylbenzoquinone (XVII)**———III (0.3 g) was treated with the solution of $CH_3NH_2$ in EtOH for 20 hr and the separated cyrstals were purified from EtOH to red-violet plates (0.12 g) of mp 162—163°. IR $cm^{-1}$: 3282 (NH), 1615 (CO). *Anal.* Calcd. for $C_{16}H_{26}O_2N_2$: C, 69.03; H, 9.41. Found: C, 68.85; H, 9.52.

**2,5-Dihydroxy-3-octylbenzoquinone (VIII)**——XVII (0.1 g) in HOAc (18 ml) was refluxed with 50% $H_2SO_4$ (10 ml) for 30 min. The deposit was crystallized from HOAc to yellow-brown needles (VIII) (0.05 g) of mp 154—155°. IR $cm^{-1}$: 3265 (OH), 1615 (CO). *Anal.* Calcd. for $C_{14}H_{20}O_4$: C, 66.64; H, 7.99. Found: C, 66.25; H, 7.75.

**2-Hydroxy-5-methoxy-4-methyloctanophenone (2b)**——The mixture of 2,5-dimethoxytoluene (1b) (2.5 g), octanoyl chloride (2.6 g), $AlCl_3$ (3 g) and $CS_2$ was heated as in the case of 2a. Working up usual and recrystallization from EtOH afforded pale yellow needles (2b) (4.1 g) of mp 57—59°. IR $cm^{-1}$: 1642 (bonded CO). *Anal.* Calcd. for $C_{16}H_{24}O_3$: C, 72.69; H, 9.15. Found: C, 72.60; H, 9.03.

**2,5-Dihydroxy-4-methyloctanophenone (4b)**——2b (4 g) was demethylated by boiling with $AlCl_3$ (2.5 g) in $CS_2$ (50 ml) for 34 hr. After decomposition with dil. HCl, the reaction mixture was extracted with ether and the residue was recrystallized from hexane to give yellow plates (4b) of mp 103—104° in an yield of 2.4 g. IR $cm^{-1}$: 3390 (OH), 1640 (bonded CO). *Anal.* Calcd. for $C_{15}H_{22}O_3$: C, 71.97; H, 8.86. Found: C, 72.19; H, 8.80.

**2,5-Dihydroxy-4-methyloctylbenzene (5b)**——4b (1 g) was reduced with amalgamated Zn by the same method as the preparation of 5a. 5b was obtained as colorless plates of mp 135—136° from benzene–hexane (1:9); yield, 0.8 g. IR $cm^{-1}$: 3260 (OH). *Anal.* Calcd. for $C_{15}H_{24}O_2$: C, 76.22; H, 10.24. Found: C, 76.34; H, 10.50.

**2-Methyl-5-octylbenzoquinone (IV)**——Chromic acid oxidation of 5b (0.2 g) afforded yellow plates (0.14 g) of IV, mp 65.5°. IR $cm^{-1}$: 1658 (CO). *Anal.* Calcd. for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.71; H, 9.21.

**3-Methyl-2,5-bis(methylamino)-6-octylbenzoquinone (7b)**——Treatment of IV (0.10 g) by the same method as before afforded violet-red plates (7b) (0.09 g) of mp 172—173° from EtOH. IR $cm^{-1}$: 3290 (NH), 1620 (CO). *Anal.* Calcd. for $C_{17}H_{28}O_2N_2$: C, 69.82; H, 9.65. Found: C, 69.86; H, 9.50.

**2,5-Dihydroxy-3-methyl-6-octylbenzoquinone (IX)**——Hydrolysis of 7b (0.05 g) by the same method as before afforded brown-red needles of mp 159.5°; yield, 0.03 g. IR $cm^{-1}$: 3320 (OH), 1612 (CO). *Anal.* Calcd. for $C_{15}H_{22}O_4$: C, 67.64; H, 8.33. Found: C, 68.03; H, 8.47.

**2,5-Dihydroxy-4-methylhexadecanophenone (4c)**——1b (5 g), hexadecanoyl chloride (18 g) and $AlCl_3$ (13 g) in $CS_2$ (70 ml) were reacted as in the case of the synthesis of 2a. Demethylation was accomapanied in the course of the reaction and yellow plates of 4c, mp 93.5° from hexane, was obtained in an yield of 12 g. IR $cm^{-1}$: 3360 (OH), 1640 (bonded CO). *Anal.* Calcd. for $C_{23}H_{38}O_3$: C, 76.19; H, 10.57. Found: C, 76.52; H, 10.38.

**2,5-Dihydroxy-4-methylhexadecylbenzene (5c)**——The Clemmensen reduction of 4c (3 g) was carried out as before and the reaction product was purified from benzene–hexane to colorless needles (1.83 g) of mp 107—108°. IR $cm^{-1}$: 3280 (OH). *Anal.* Calcd. for $C_{23}H_{40}O_2$: C, 79.25; H, 11.57. Found: C, 78.76; H, 11.26.

17) Melting points were determined by a Yanagimoto micro-melting point determination apparatus.
18) Infra-red spectra were determined by a Koken IRS recording spectrometer in Nujol mull unless otherwise specified.

**2-Hexadecyl-5-methylbenzoquionone (V)**——Oxidation of **5c** (0.7 g) with $CrO_3$ as before afforded V in yellow needles (0.5 g) of mp 81—82° from hexane. IR $cm^{-1}$: 1660(CO). *Anal.* Calcd. for $C_{23}H_{38}O_2$: C, 79.71; H, 11.05. Found: C, 79.64; H, 10.92.

**2,5-Dihydroxy-3-methyl-6-hexadecylbenzoquinone (X)**——V (0.5 g) was methylaminated by the same method as bofore and the bis(methylamino) derivative thus obtained was hydrolyzed. Recrystallization from EtOH gave brown needles (X) (0.4 g) of mp 123—124°. IR $cm^{-1}$: 3318 (OH), 1618 (CO). *Anal.* Calcd. for $C_{23}H_{38}O_4$: C, 72.97; H, 10.12. Found: C, 72.66; H, 10.04.

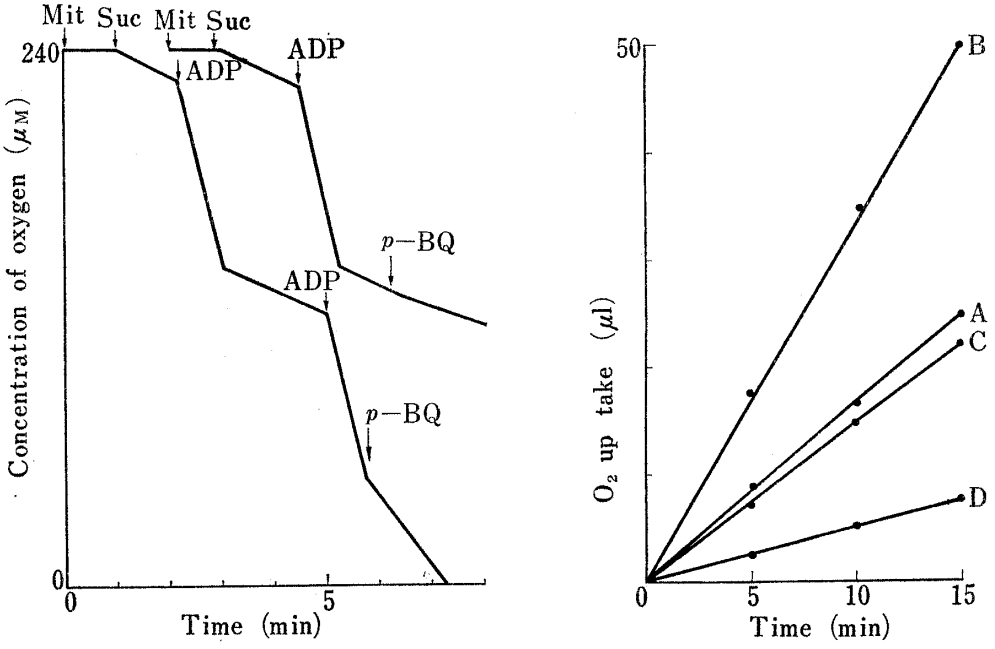
**2,5-Dimethoxyoctylbenzene (1d)**——The Clemmensen reduction of **2a** and/or **4a**, followed by methylation with dimethyl sulfate and NaOH, afforded the dimethyl ether, bp 144° (3 mmHg).

**2-Hydroxy-5-methoxy-4-octyloctanophenone (2d)**——**1d** (5 g) was acylated by the same method as before. Crystallization from hexane afforded yellow plates (2d) (4.9 g) of mp 53—54°. IR $cm^{-1}$: 1640 (bonded CO), 1250 ($CH_3O$). *Anal.* Calcd. for $C_{23}H_{38}O_3$: C, 76.19; H, 10.59. Found: C, 76.38; H, 10.58.

**2-Hydroxy-5-methoxy-1,4-dioctylbenzene (6d)**——2d (1.6 g) was dissolved in $(CH_2OH)_2$ (7 ml) and refluxed with $NH_2NH_2$ (80%, 0.5 g) for 1 hr. After the addition of KOH (2 g), the reaction mixture was heated to 200° and was mainatined at that temperature for 2.5 hr. After the addition of dil. HCl, the reaction mixture was extracted with ether and the residue was recrystallized from 80% EtOH to colorless needles (6d) (1.2 g) of mp 59°. IR $cm^{-1}$: 3320 (OH), 1210 ($CH_3O$). *Anal.* Calcd. for $C_{23}H_{40}O_2$: C, 79.25; H, 11.57. Found: C, 78.96; H, 11.44.

**2,5-Dioctylbenzoquinone (VI)**——6d (1 g) was oxidized by the same procedure as III. Recrystallization from EtOH afforded yellow needles (VI) (0.53 g) of mp 87—88°. IR $cm^{-1}$: 1655 (CO). *Anal.* Calcd. for $C_{22}H_{36}O_2$: C, 79.46; H, 10.92. Found: C, 79.16; H, 10.62.

**2,5-Dihydroxy-3,6-dioctylbenzoquinone (XIII)**——VI (0.4 g) was treated with $CH_3NH_2$ and then hydrolyzed with $HOAc-H_2SO_4$ as in the case of preparation of VIII from III. Recrystallization from EtOH




1A 1B

Fig. 1. Inhibition of Respiration by *p*-Benzoquinone

1A: Polarographic tracing. Incubation mixture: 0.3 M mannitol, 10 mM phosphate, 10 mM KCl, 2.5 mM $MgCl_2$, and 0.2 mM EDTA (pH 7.2). Total volume: 2.3 ml. Temperature: 25°. Additions: Mit=mitochondrial suspension (4 mg protein), 0.2 ml. Suc=0.025 ml of 0.5 M sodium succinate (final concentration=5 mM). ADP=10 μl of 0.025 M ADP sodium. *p*-BQ=25 μl of 10 mM *p*-benzoquinone (ethanolic solution. The final concentration is 0.1 mM). R.C. (respiratory control ratio): 5.

1B: Manometric results. Basic incubation mixture: 0.3 M mannitol, 10 mM KCl, 10 mM phosphate, 2.5 mM $MgCl_2$, 0.2 mM EDTA, 20 mM sodium succinate, 4 mM ATP and mitochondria (3.2 mg protein). Total volume: 3.0 ml, pH 7.2. 0.2 ml of 20% KOH was placed in a center well. Incubation was initiated by the addition of succinate from side arm at 30°.

Curve A: control. Curve B: 100 μmoles glucose and 150 units hexokinase were added as an inorganic phosphate (Pi) acceptor. Curve C: 100 μM of *p*-benzoquinone was added to the basic mixture. Curve D: same as cruve B except 100 μM *p*-benzoquinone was added.

afforded yellow plates of mp 144—145° in an yield of 0.1 g. IR $cm^{-1}$: 3350 (OH), 1612 (CO). *Anal.* Calcd. for $C_{22}H_{36}O_4$: C, 72.49; H, 9.96. Found: C, 72.53; H, 9.68.

**bis(2,5-Dihydroxy-3-methylbenzoquinonyl)methane and bis(2,5-Dihydroxy-3-tridecylbenzoquinonyl)methane (XVIII and XIX)**——To the solution of 3,6-dihydroxytoluquinone (VII) or rapanone (0.5 g) in AcOH (15 ml), was added 40% HCHO (2.5 ml) and the solution was warmed on a water bath for 10 min. After cooling and the addition of water, the deposit was collected and crystallized from dioxane. XVIII: Orange needles (0.37 g) of mp 281—283°. IR $cm^{-1}$: 3240 (OH), 1620 (CO). *Anal.* Calcd. for $C_{15}H_{12}O_8$: C, 56.25; H, 3.78. Found: C, 55.74; H, 3.55. XIX: Orange needles (0.41 g), mp 251°. IR $cm^{-1}$: 3235 (OH), 1615 (CO). *Anal.* Calcd. for $C_{39}H_{60}O_8$: C, 71.31; H, 9.21. Found: C, 71.45; H, 9.39.

## Methods

**Preparation of Rat Liver Mitochondria**——Liver mitochondria were isolated from Wister rats by the modified procedure of Schneider and Hogeboon[19] as previously reported by the present authors.[6-8]

**Determination of Oxygen Consumption**——Oxygen consumption was determined by both polarographic[20] and manometric methods as reported previously.[6-8] For polarographic method, oxygen electrode (Model PO-100, Yanagimto MFG. Co.) was used according to the procedure of Hagihara.[20] Benzoquinone derivatives

Table I. Effects of Various Benzoquinone Derivatives on Mitochondrial Respiration and Releasing by 2,4-Dinitrophenol

| Compounds ($10^{-4}$ M) | Respiration State-3 | Respiration State-4 | Ad. of DNP ($10^{-4}$ M) to inhibited state |
|---|---|---|---|
| (I) | ↓↓ | ↓ | - |
| (II) | ↓↓ | - | - |
| (III) | - | - | - |
| (IV) | - | - | - |
| (V) | - | - | - |
| (VI) | - | - | - |
| (VII) | - | - | - |
| (VIII) | ↓↓↓ | ↓↓↓ | slightly released |
| (IX) | ↓↓↓ | ↓↓ | slightly released |
| (X) | - | - | - |
| (XI) | - | - | - |
| (XII) | - | - | - |
| (XIII) | - | - | - |
| (XIV) | ↓↓↓ | ↓ | slightly released |
| (XV) | - | - | - |
| (XVI) | ↑ | ↑↑ | - |
| (XVII) | - | - | - |
| (XVIII) | - | - | - |
| (XIX) | - | - | - |
| (XX) | ↓↓↓ | ↓↓↓ | slightly released |
| (XXI) | ↓↓↓ | ↓↓ | slightly released |

↓ <30% ↓↓ 30—60% ↓↓↓ >60% ↑ 110—150% ↑↑ 150—200%

19) W.C. Schneider and G.H. Hogeboom, *J. Biol. Chem.*, **183**, 123 (1950).
20) B. Hagihara, *Biochem. Biophys. Acta*, **46**, 134 (1961).

were added to the incubation mixture as an ethanolic solution keeping the final concentration of ethanol not more than 2%. In this condition the influence of ethanol on the enzymyic activity was not observed. Detailed experimental conditions are shown in the legend of Fig. 1A and 1B. Methylamino-substituted benzoquinone derivatives were used as the solution in dioxane.

## Results

Effects of the benzoquinone derivatives on a respiration of rat liver mitochondria were determined by both polarographic and manometric methods. Fig. 1A is a polargraphic tracing showing the inhibition of respiration by *p*-benzoquinone. An addition of succinate slightly induced the respiration (State-4) and succesive addition of ADP gave rise to the remarkable increase of oxygen consumption (State-3). The respiratory control ratio was calculated at

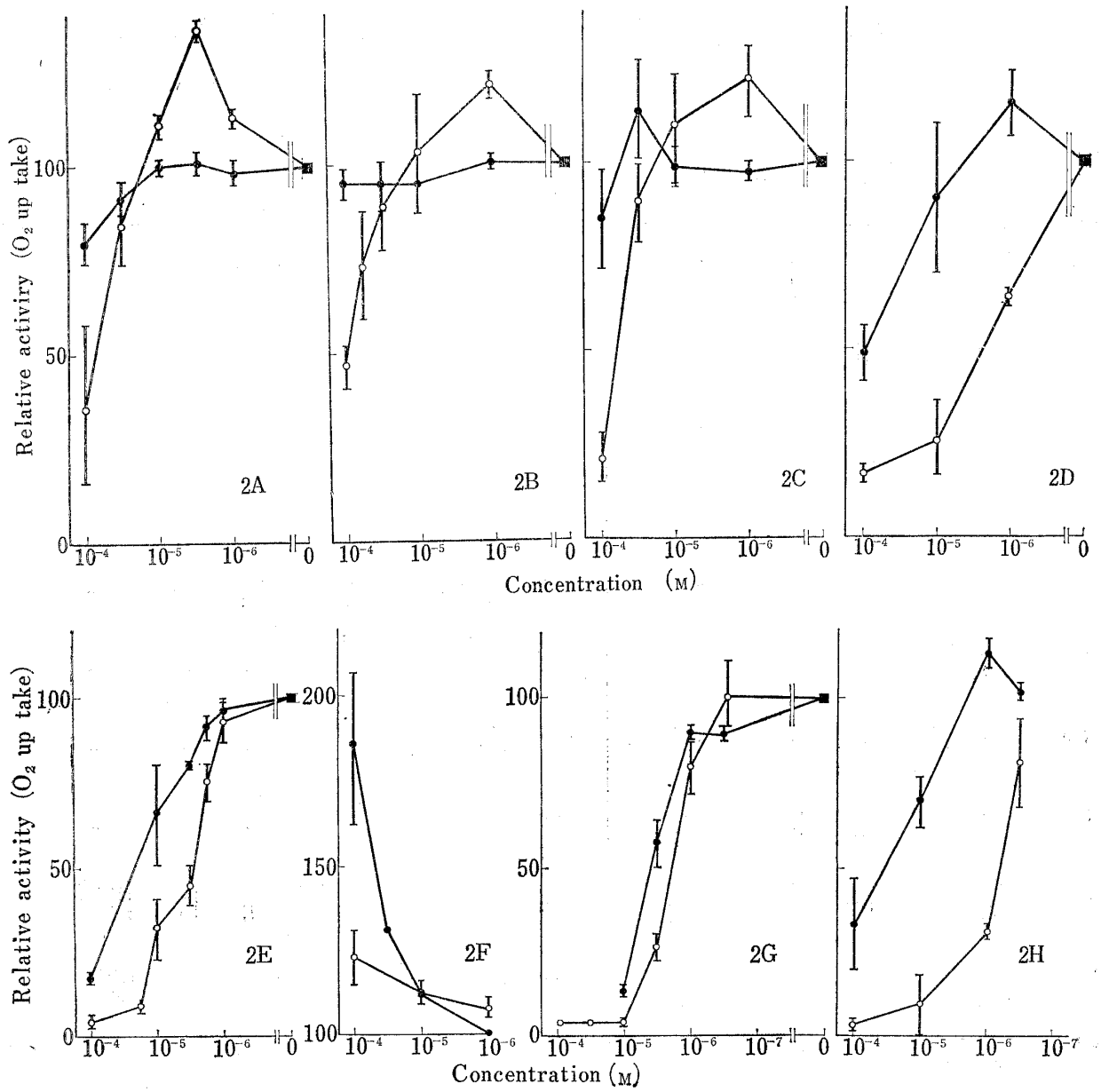



Fig. 2. Dose Responce Curves of Various Benzoquinone Derivatives on Inhibition of Respiration

2A: *p*-Benzoquinone, 2B: Toluquinone, 2C: Dibromotoluquinone, 2D: 2-Methyl-5-octyl-3,6-dihydroxy-*p*-benzoquionne, 2E: 2-Octyl-3,6-dihydroxy-*p*-benzoquinone, 2F: Tetrahydroxy-*p*-benzoquinone, 2G: Ardisiaquinone A, 2H: Ardisiaquinone B

5.0. An addition of 0.1 mM *p*-benzoquinone inhibited the both respiring state and inhibition of State-3 was stronger than that of State-4. Considering an interaction of quinone derivatives with platinum electrode of polarographic apparatus, manometric method was applied to confirm the inhibition of the respiration, and similar results were obtained as shown in Fig. 1B. By an addition of inorganic phosphate (Pi) acceptor (glucose and hexokinase) to the incubation mixture, the respiration was remarkably stimulated (curve B) and an inhibition of the stimulated respiration (curve D) was more extremely than the case of the absence of Pi acceptor (curve C).

Effects of the other benzoquinone derivatives newly synthesized on the respiration were also examined and results are listed in Table I. The final concentration the compounds added was at 0.1 mM. Effects of 2,4-dinitrophenol (DPN) at a concentration of 0.1 mM on the inhibited respiration are also described.

In Table I, it is seen that *p*-benzoquinone (I), toluquinone (II), 2-octyl-3,6-dihydroxy-*p*-benzoquinone (VIII), 2-methyl-5-octyl-3,6-dihydroxy-*p*-benzoquinone (MOQ, IX), dibromotoluquinone (XIV), ardisiaquinone A (XX) and ardisiaquinone B (XXI) were inhibitors for mitochondrial respiration. Tetrahydroxy-*p*-benzoquinone (XVI) was an uncoupler. Dose response curves of these compounds are shown in Fig. 2.

From these curves, the benzoquinone derivatives may be classified into three groups from the view point of the inhibition of the respiration:

1. *p*-Benzoquinone, toluquinone, and dibromotoluquinone stimulated the State-3 respiration at lower concentration, while the respiration was inhibited at higher concentration. On the other hand State-4 respiration was not remarkably influenced.

2. 2-Methyl-5-octyl-3,6-dihydroxy-*p*-benzoquinone and ardisiaquinone B showed specific inhibition of the State-3 respiration. In high concentration the compounds inhibited also the State-4 respiration, though a weak uncoupling action was observed at low concentration of them.

3. 2-Octyl-3,6-dihydroxy-*p*-benzoquinone and ardisiaquinone A showed potent inhibition of both State-3 and State-4 respiration, and the inhibition of the State-3 was slightly stronger than that of the State-4 respiration.

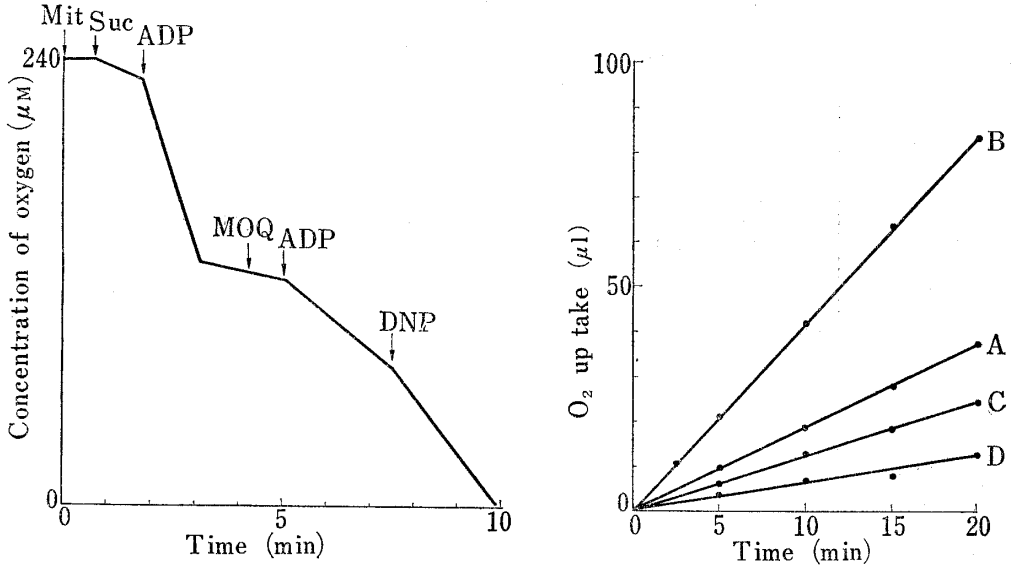


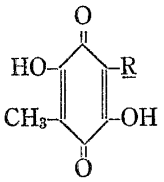
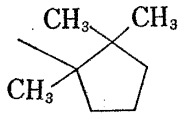
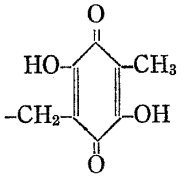
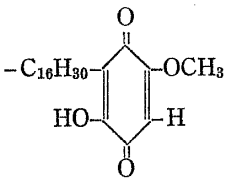
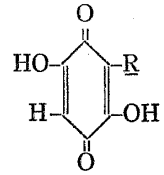
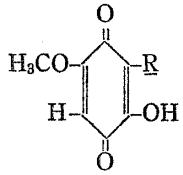
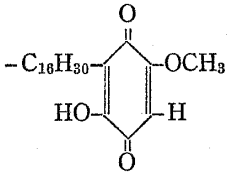

Fig. 3. Inhibition of Respiration by 2-Methyl-5-octyl-3,6-dihydroxy-1,4-benzoquinone (MOQ)

3A: Polarographic results. Detailed assay conditions were described in Fig. 1. The final concentration of the added MOQ was $5\times10^{-6}$ M.
3B: Manometric results. Experimental conditions were described in Fig. 1. Curve A: control. Curve B: Pi acceptor was addded. Curve C: MOQ was added to be a final concentration of $10^{-4}$ M. Curve D: both Pi acceptor and MOQ were added under the same conditions of cruve B and C.

The significant difference of the inhibitory pattern of the first group from the second group is whether low concentrations of those benzoquinone derivatives stimulate the State–3 respiration or not.

Fig. 3 shows an inhibition of respiration by 2–methyl–5–octyl–3,6–dihydroxy–*p*–benzoquinone (MOQ) which is one of the potent inhibitors on the State–3 respiration. Fig. 3A is a polarographic tracing which shows that sole addition of the MOQ to State–4 at a concentration of $5\times10^{-6}$ M did not influence on the respiration. But in the presence of the MOQ

Table II. Structure Activity Relationship between Alkyl–substitutes of Dihydroxy–*p*–benzoquinone and Effects on the Respiration of Mitochondria

| | R | Efiects on respiration |
|---|---|---|
|  | H | none |
| | $CH(CH_3)_2$ (dihydroxythymoquinone, DTQ) | inhibition (State–3>State–4)[3–5] |
| | $C_8H_{17}$ (MOQ) | inhibition (State–3>State–4) |
| | $C_{16}H_{33}$ | none |
| | $C_{19}H_{39}$ (dihydromaesaquinone) | none[3] |
| | $(CH_2)_{13}CH{:}CH(CH_2)_4H$ (maesaquinone) | none[3] |
| | $C_{21}H_{43}$ (polygonaquinone) | none |
| |  (helicobasidin) | potent uncoupling action[3] |
| |  | none |
| |  (ardisiaquinone B) | inhibition (State–3>State–4) |
|  | $C_8H_{17}$ | inhibition of both State–3 and State–4 |
| | $C_{13}H_{27}$ (rapanone) | none[3] |
|  |  (ardisiaquinone A) | inhibition of both State–3 and State–4 |

oxygen consumption of State–3 could not be observed by the successive addition of ADP. An addition of DNP could slightly release the inhibited respiration. Fig. 3B shows the manometric results. An addition of MOQ ($10^{-4}$ M) slightly inhibited the respiration when the Pi acceptor was absent (curve C). On the other hand, presence of Pi acceptor highly stimulated the respiration (curve B) and this was extremely reduced by MOQ at the same concentration of curve C (curve D). This fact may suggest that MOQ inhibits a certain part of phosphorylating reaction tightly coupled to respiration.

## Discussion

As mentioned above, the benzoquinone derivatives were classified into three groups according to their inhibitory action on the mitochondrial respiration. In each group, consistent similarities in their substitutes at benzene ring were observed as follows: 1) simple derivatives of *p*–benzoquinone, 2) *p*–benzoquinone substituted with two hydroxyl group at *para*–position, one methyl and one medium–sized alkyl chain, 3) compounds corresponding to the demethylated substances of the second group.

As previous reported by the same authors,[6–8] *p*–benzoquinone derivatives, particularly with two hydroxyl groups, were interested in their inhibitory actions on mitochondrial respiration and oxidative phosphorylation. It has been shown that the inhibition was in response to their substitutes at benzene ring. Table II indicates the relationship between the inhibition of respiration and various alkyl side chain of dihydroxy–*p*–benzoquinone, some of which have already reported in the inhibitory actions. It can be seen that dihydroxythymoquinone (2–methyl–5–isopropyl–3,6–dihydroxy–1,4–benzoquinone, DTQ), MOQ and ardisiaquinone B inhibit State–3 respiration stronger than that of the State–4. As previously reported by the present authors,[7,8] DTQ showed potent inhibitory actions on oxidative phosphorylation at low concentrations, and higher concentration of it showed an inhibition of electron transport system. Because of the similarities in the inhibitory pattern, MOQ and ardisiaquinone–B may inhibit oxidative phosphorylation and respiration in the same mechanism as DTQ. However, in the aspect of the length of alkyl substitutes ardisiaquinone B was an expectional case because of its large substitute whereas DTQ and MOQ had medium-sized alkyl substitute.

It is also interested that effects of ardisiaquinone A on the respiration is very similar to that of 2–octyl–3,6–dihydroxy–1,4–benzoquinone and both of them have a non–substituted position at benzene ring. The results that they inhibited respiration disregrading to the respiration states suggest that the both compounds are inhibitor of respiratory chain. It is reported that benzoquinone derivatives having non–substitute at benzene ring are reactive with sulfhydryl groups of the enzyme protein resulting an inhibition of respiration,[21] so the inhibition of those compounds might be due to the similar reaction.

Helicobasidin which has cyclopentyl group was a potent uncoupler and this compound could not be classified into any groups as mentioned above.

The other compounds having longer alkyl chain such as maesaquinone are not effective, although these quinone derivatives can restore the respiration of acetone treated mitochondria.[5,6]

It has been reported that alkylhydroxynaphthoquinone[22] and polyhydroxyanthraquinone[23] are inhibitor of mitochondrial respiration and oxidative phosphorylation, and that the inhibition of oxidative phosphorylation is stronger than the inhibition of the respiration. Considering these facts, some quinone compounds with alkyl and hydroxyl substitutes may

21) E.R. Redfearn and P.A. Whittaker, *Biochim. Biophys. Acta*, **56**, 440 (1962).
22) J.L. Howland, *Biochim. Biophys. Acta*, **105**, 205 (1965).
23) Y. Ueno, *Seikagaku*, **38**, 741 (1966).

possibly be inhibitors for mitochondrial functions. And it is also concluded that alkylated and hydroxylated *p*-benzoquinone derivatives structure of which should be belonged to a certain groups as mentioned inhibit both respiratory chain and oxidative phosphorylation, and an intensity of the inhibition of each reaction was variable according to the length of alkyl substitutes.

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