Tetrahedron Vol. 46, No. 9, pp. 3199-3210, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

SYNTHESIS AND INHIBITORY ACTIVITY OF BROMOQUINONE DERIVATIVES*

Lianquan Gu[1], Cuihua Liu [1], Jingxing Xu [2] and Tsoo E. King
Institute For Structural and Functional Studies, University City Science Center and
Department of Biochemistry and Biophysics
University of Pennsylvania
Philadelphia, PA 19104 USA

(*Received in Japan* 7 *November* 1989)

2,5 Dibromo-3-methyl-6-isopropyl-1, 4-benzoquinone has been reported inhibitory to b_6-f and b-c_1 complexes [Malkin, R. (1977) Biochim. Biophys. Acta., 501, 552-554]. In this paper, four classes of the analogues are synthesized. They were 5-bromo-3-methyl, 3-bromo-2-methoxy-5-methyl, 2,5-dibromo-3-methyl, and 3, 5-dibromo-2-methyl derivatives with the side chain $-(CH_2)_nH$ of n=5,7,9,11 and 13 at position 6. Dibromo compounds are usually more inhibitory in the mitochondrial ubiquinol cytochrome *c* reductase; the maximal inhibition was usually with 9 methylene in a functional group.

2, 5-Dibromo-3-methyl-6-isopropyl-1, 4-benzoquinone (DBMIB) was first synthesized and used as an inhibitor for electron pathway in chloroplasts by Trebst *et al.* (1). Lately, it was found also inhibitory to electron transfer in mitochondria (2). It is well known that the inhibitory site of DBMIB in chloroplasts is located at the cytochrome b_6-f complex and in mitochondria is at cytochrome b-c_1 complex regions (3,4,5). There are few bromoquinones were studied for structure and function relationship especially the inhibition of bromoquinones in b_6-f or b-c_1 systems.

Recently, we have synthesized four groups of total 20 new bromoquinones with a 1, 4-benzoquinoid ring, but different positional arrangement of functional groups and side chains in an attempt to understand their inhibitory properties and mechanisms .

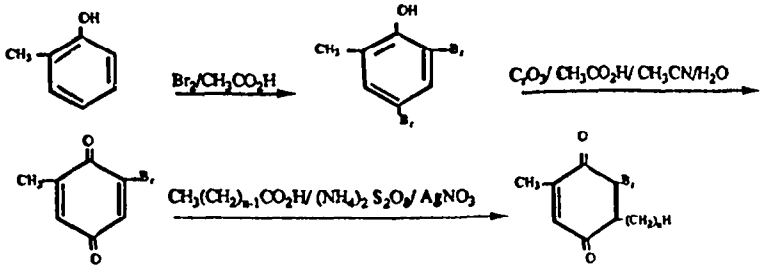
In this paper, we present the synthetic procedures to 5-bromo-3-methyl-6-alkyl-1, 4-benzoquinone (I_{a-e}), 2, 5-dibromo-3-methyl-6-alkyl-1, 4-benzoquinone (II_{a-e}), 3, 5-dibromo-2-methyl-6-alkyl-1, 4-benzoquinone (III_{a-e}) and 3-bromo-2-methoxy-5-methyl-6-alkyl-1, 4-

* This paper is dedicated to Professor Wang Yu, an innovative chemist, creative poet, my most beneficial teacher and an intimate friend. Although we have been separated by a wide ocean for so many decades but he has remained as a best friend. Our communications always exist. -- TEK

[1]Permanent Address: Department of Chemistry, Zhongshan University, Guangzhou, China

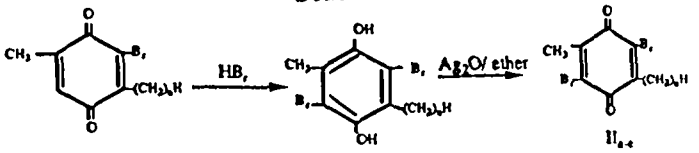
[2]Permanent Address: Institute of Biophysics, Academia Sinica, Beijing, China

Scheme I



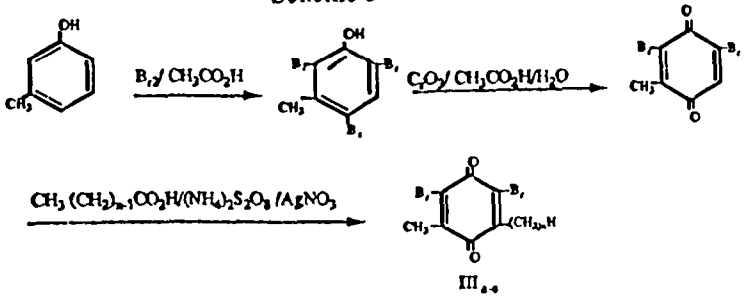
n=5 (a), 7 (b), 9 (c), 11 (d), 13 (e)

Scheme 2



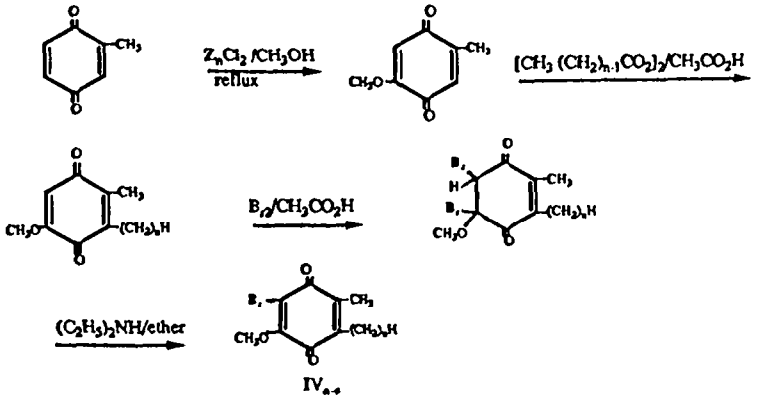
n=5 (a), 7(b), 9 (c), 11 (d), 13 (e)

Scheme 3



n=5 (a), 7 (b), 9 (c), 11 (d), 13 (e)

Scheme 4

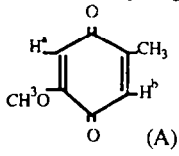


n=5 (a), 7(b), 9 (c), 11 (d), 13 (e)

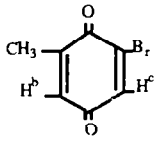
benzoquinone (IV_{a-e});* and more important, to show the inhibitory characteristic of the new synthetic bromoquinones in mitochondria. The synthetic pathway is listed in scheme 1-4.

Bromization of *o*-cresol and *m*-cresol with B_rZ-CH_3 CO_2 H in a 1:2 or 1:3 ratio of cresol to bromine gave desired products with high yields (6). With a modified oxidation method (7), treatment of bromophenols with a equal-molar C_rO_3 in a solvent mixture of CH_3 CO_2H-CH_3CN-H_2O , gave better yields and easier handling.

Radical alkylation (8,9) for both 3-bromo-5-methyl-1, 4-benzoquinone and 2-methoxy-5-methyl-1, 4-benzoquinone have good selective substitution. Considerations of reactivity are similar to the aromatic substrates (10), that is, we need to know which position on the quinone ring will be attached to give the products. Although the mechanism is still unknown, however, the position of attack seems to be apparent. We found that the electronic environments of vinylic hydrogens on the quinone ring is evidently important -- the greater the electron density around a vinylic hydrogen, the more difficult for the hydrogen to be abstracted.



(A)



(B)

δH (ppm): H^a = 5.91, H^b = 6.54 in (A), 6.62 in (B), H^c =7.20

From ^{1}HNMR data of 2-methoxy-5-methyl-1, 4-benzoquinone (11) and 3-bromo-5-methyl-1, 4-benzoquinone (12), the chemical shift order of various vinylic hydrogens is found to be H^c>H^b>H^a. In case of the position next to the less electron donative group is active for attack of alkyl radical substituted. Under our experimental condition, 2-methoxy-5-methyl-6-alkyl-1, 4-benzoquinone and 5-bromo-3-methyl-6-alkyl-1, 4-benzoquinone are almost only mono-substituted products, respectively. In other words, in (A) and (B) the hydrogen at H^b and H^c are much more dominated to be abstracted than H^a and H^b respectively.

Addition of bromoquinone derivatives 10 even 100 times of cytochrome c_1 to succinate cytochrome *c* reductase with succinate as the substrate (electron donor) and Q_2* plus 2,6-dichlorophenol- indophenol as acceptor , no inhibition was observed. On the other hand, when cytochrome *c* was used as an acceptor, inhibition was clearly demonstrated. These results were in

* For convenience, all alkyl side chain is assigned at 6-position of quinone ring with subscript a, b, c, d, e for the number of CH_2 group of 5, 7, 9, 11 and 13 respectively.

* Q: ubiquinone; QH_2: ubiquinol (fully reduced form of ubiquinone); Q_2: ubiquinone-2 (or 2,3-dimethoxy-5-methyl-6-geranyl-1,4-benzoquinone).

agreement with the observation of the lack of inhibition of succinate to Q activity by directly using purified succinate ubiquinone reductase. Likewise inhibition took place when purified ubiquinol cytochrome *c* reductase was used with QH_2 and cytochrome *c* as electron donor and acceptor respectively. These results unequivocally indicate that bromoquinone derivatives acts between ubiquinol and cytochrome *c*, i.e. inhibits the ubiquinol cytochrome *c* reductase and does not inhibit succinate to Q at all. Actually it is expected since the bromoquinone derivatives are ubiquinone like compounds and are expected to be competitively by binding at Q site in the reductase (3). Experimentally the inhibition was found to be partially reversed by exogenous Q_2. Thus in experimental system for convenience succinate was used as substrate and cytochrome *c* as the electron acceptor. The activity was found to be in mol of the inhibitor to mol of enzyme.

Fig. 1 shows the comparison of inhibitory activity of bromoquinones with different number and arrangement of bromine, on the ubiquinol cytochrome *c* reductase.

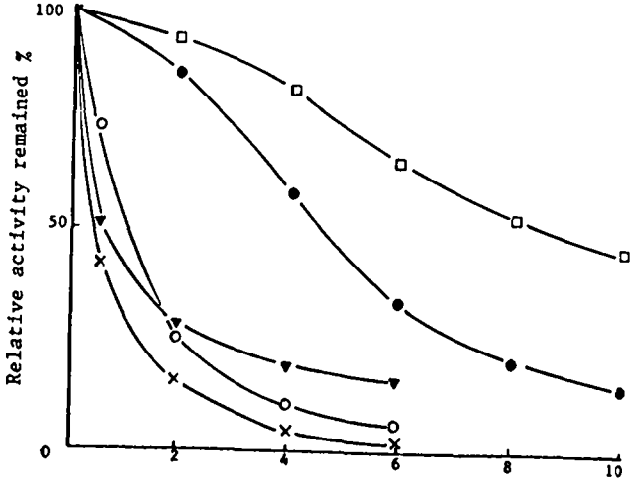


Fig. 1. Inhibitory effect of bromoquinone derivatives on ubiquinol cytochrome *c* reductase activity. (●) I_C, (x) II_C, (○) IIIc, (□) IV_C and (▼) DBMIB. The amount of inhibitor to cytochrome c_1 ratio in the enzyme as indicated was added to a 10 µl of succinate cytochome *c* reductase (1.5 nmol of cytochrome c_1, 1 mg protein) in phosphate buffer, pH 7.4. Ubiquinol cytochrome *c* reductase activity was assayed after the the mixture was incubated for 10 min at O° C. The basal mixture for assay contains 0.3 mM EDTA, 20 mM succinate and 0.1 mM cytochrome *c* in 0.1 M phosphate buffer, pH 7.4, total volume = 1 ml. 100% activity was 9 µmol cytochrome *c reduced*/min/mg.

The inhibition potency in decreasing order was found to be 2, 5-dibromo-3-methyl-6-nonyl-1, 4-benzoquinone DBMNB > DBMIB> 3, 5-dibromo-2-methyl-6-nonyl-1, 4-benzoquinone> 5-bromo-3-methyl-6-nonyl-1, 4-benzoquinone> 3-bromo-2-methoxy-5-methyl-6-nonyl-1, 4-benzoquinone. These results may suggest that the molecules of bromoquinone are oriented when they bind to the proteins.

The potency of inhibitors was dependent on the alkyl side chain length of bromoquinones (Fig. 2). Maximum inhibition fell to the region of the chain length from 9-11 carbons. Further increase of the length of chain decreased the inhibition.

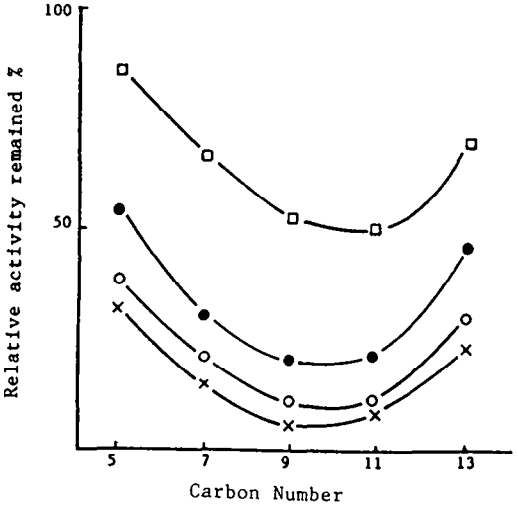


Fig. 2. Effect of the length of alkyl side chain of bromoquinone derivatives on ubiquinol cytochrome *c* reductase activity. (●) I, (x) II, (○) III and (□) IV. The mola ratio of bromoquinones to cytochrome c_1 (succinate cytochrome *c* reductase) was set at 6:1. The experimental conditions and other symbols are the same as the legend of Fig. 1.

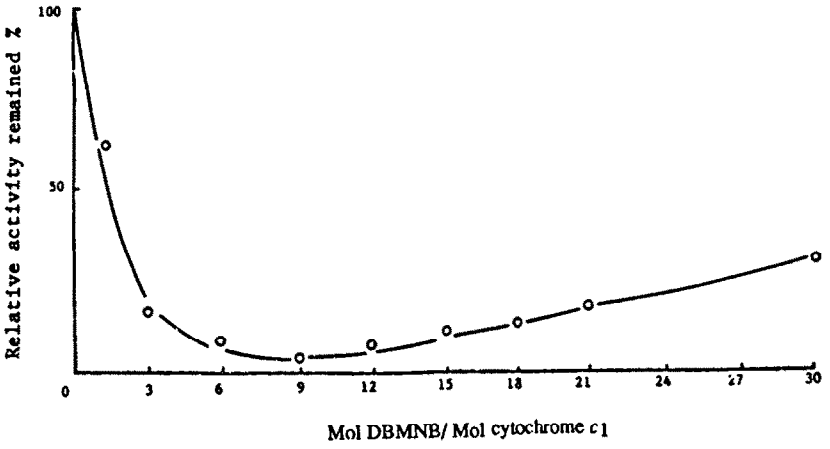


Fig. 3. Inhibitory effect of molar ratio of DBMNB (IIc) to cytochrome c_1 content in the enzyme. The experimental conditions are the same as the legend of Fig. 1.

All bromoquinones appeared an unusual concentration dependent on inhibitory efficiency. Fig. 3 shows effect of concentration of 2, 5-dibromo-3-methyl-6-nonyl-1, 4-benzoquinone (DBMNB) on inhibition of ubiquinol cytochrome *c* reductase.

The maximal inhibition of DBMNB was found to be at 6 - 12 mol of the inhibitor to 1 mol of reductase in terms of cytochrome c_1. When concentration of DBMNB was beyond this range, the inhibitory activity was slightly decreased with the increase of DBMNB concentration . The oxidized form of DBMNB did not apparently inhibit the first turn over of cytochrome *b* and c_1, but oxidize the reduced cytochrome *b* (Fig. 4), and reduced form ($DBMNBH_2$) did cause a reduction of cytochrome c_1 directly in succinate cytochrome *c* reductase. This observation is in agreement with Lenaz (3) that the real inhibitory form of DBMNB is semi-DBMNB ($DBMNB^-$ or DBMNBH) which is formed by the oxidation of reduced cytochrome *b*. The semi-DBMNB can bind with proteins to stop normal electron transfer from cytochrome *b* to endogenous Q to cytochrome c_1. In the case of high concentration of DBMNB, the extra free semi-DBMNB dismutates to DBMNB and $DBMNBH_2$, that causes both increase of cytochrome *b* oxidized and cytochrome c_1 reduced, which behaves as if a redox reaction of the system itself.

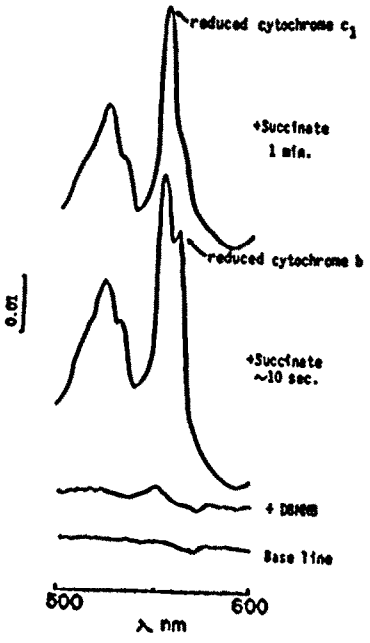


Fig. 4. Effect of DBMNB on cytochrome *b* and c_1 redox behavior. They were first reduced by endogenous QH_2, but reduced cytochrome *b* was oxidized. 1 ml of succinate cytochrome c reductase (1.37 mg/ml) in 0.1 M phosphate buffer, contained 0.5% sodium cholate, pH 7.4. 2 μl of DBMNB (10 mM) was added to the reductase. After baseline was scanned, 10 μl of succinate (5 mM) was added to the system and immediately scanned again. Finally approximately 1 min, it was scanned again.

It is generally agreed that all mitochondrial enzymes, perhaps other than cytochrome *c*, is partially or completely imbeded in bilayer phospholipid. Plate 1 shows the comparison of CPK models of the inhibitor (DBMNB) with phospholipid and ubiquinones Q_2 and Q_{10}. Q_{10} naturally occurs in mammalian tissues perhaps with exception of rat. Whereas Q_2 does not exist in nature but is more soluble and thus used widely, if not universally in *all invitro* experiments. A notable feature occurs in the Plate, the length of the side chain of the inhibitor approximates that of Q_2. Then question will be asked what is the role of the long poly-isoprenoid tail of Q_{10}. We do not have slightest intention to suggest the non functionality of the 10 isoprenoid units but it seems to be the benzoquinoid head at least plays some role physiologically.

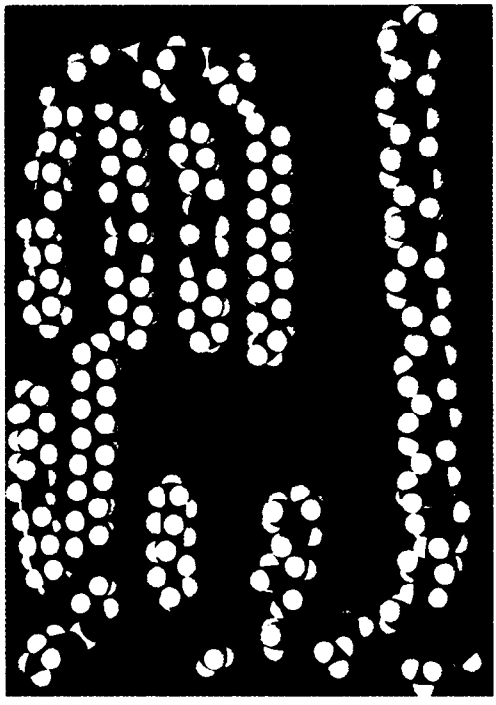


Plate 1. Comparison of the inhibitor with phospholipids
upper: diphosphoditylglycerol (cardiolipin), fatty acids from left to right are: 18:1, 18:2, 18:2, 18:0
down: phosphatidylethanolamine, fatty acids from left to right: 18:1, 18:0
2,5-dibromo-3-methyl-6-nonyl-1,4-benzoquinone (DBMNB)
ubiquinone - 2
ubiquinone - 10

EXPERIMENTAL

All chemicals were purchased from commercial sources in the most purified form available. Succinate cytochrome *c* reductase was prepared and assayed according to the reported methods (13, 14).

The UV spectra were measured in Shinadzu UV-3000 or UV-250. NMR spectra were measured in Varian XL-200. IR spectra were obtained on a Perkin-Elmer 983 spectrometer. Mass spectra were obtained on a Hitach M-80A spectrometer. Elemental analyses were performed on Perkin-Elmer 240-B.

Synthesis of 5-bromo-3-methyl-6-alkyl-1, 4-benzoquinone ($I_{a\text{-}e}$): (**1**) 2, 4-dibromo-6-methylphenol----Bromine (16g, 0.10 mol) was dissolved in 50 ml acetic acid. *o*-Cresol (5.4g,

0.05 mol, in 10ml of CH_3CO_2H) were added dropwise to above solution in 10 min with stirring at room temperature and continued to stir for 20 min. The solution was diluted with 80 ml of water. The white crystals of product was precipitated and collected, which was recrystallized from chloroform to give a yield of 95%, m.p. 56~57°C (ref. 15, 58°C). (2) 3-bromo-5-methyl-1, 4-benzoquinone----2, 4-dibromo-6-methylphenol (5.3g, 0.02 mol) was dissolved in 200ml of CH_3CO_2H-CH_3CN-H_2O (5:1:1). The solution was then heated at 60° C. Chromic acid (CrO_3, 2g, 0.02 mol) was added in 30 min with stirring and continued to stir for one hour at 60-65°C. The solution was then cooled and extracted with chloroform. The chloroform layer was washed with a 1M $NaHCO_3$ and water, then dried over Na_2SO_4. After removing solvent, the residue was applied to a silica gel column which eluted by a hexane-ether (2:1). A yellow eluate was collected to give a yield of 75%. The product is a yellow crystals, m.p. 93-94° C (ref. 16, 95°C). (3) 5-bromo-3-methyl-6-alkyl-1, 4-benzoquinone----3-bromo-5-methyl-1, 4-benzoquinone (0.4g, 2 mmol) was placed in 30 ml of CH_3CN-H_2O (1:1). The fatty acid (of different chain length) (4 mmol) and $AgNO_3$ (0.34g, 2 mmol) were added to the solution, which was then heated to 60-70° C until completely dissolved. $(NH_4)_2S_2O_8$ (0.91g, 4 mmol, in 10 ml H_2O) were dropwise in 30 minutes with stirring and continued to stir for 30 min at 60° C. The solution was then cooled and extracted with either. The ether layer was washed with diluted $NaHCO_3$ solution and water, then dried over Na_2SO_4. After removing ether, the residue was applied to the silica gel plates. The plates were developed with a hexane-ether-CCl_4 (16:1:1). A yellow band with R_f of 0.5~0.7 (dependent upon side chain length) was collected and eluted with ether to give a yield about 40% of compounds $I_{a\text{-}e}$.

I_a: UV λ_{max} (EtOH): 265 nm. IR (KB_r): 1652 (quinone), 1590 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t, CH_2 $(CH_2)_3$ *CH_3*), 1.20-1.65 (6H, m, CH_2 (*CH_2*)$_3CH_3$), 2.10 (3H, s ,*CH_3*), 2.65 (2H,t, *CH_2* $(CH_2)_3CH_3$), 6.60 (1H, s, 2-*H*) ppm. Mass (^{m}/z): 270 (M^+), 272 (M^++2). Calc for $C_{12}H_{15}BrO_2$: C, 53.14; H, 5.54. Found: C, 53.70; H, 5.16.

I_b: UV λ_{max} (EtOH): 265 nm. IR (KB_r): 1660 (quinone), 1592 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.20-1.65 (10H, m), 2.10 (3H, s), 2.65 (2H, t), 6.60 (1H, s) ppm. Mass (^{m}/z): 298 (M^+), 300 (M^++2). Calc for $C_{14}H_{19}BrO_2$: C, 56.19; H, 6.35. Found: C, 55.91; H,6.82.

I_c: UV λ_{max} (EtOH): 265 nm. IR (KB_r): 1659 (quinone), 1580 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.20-1.65 (14 H, m), 2.10 (3H, s), 2.64 (2H, t), 6.61 (1H, s) ppm. Mass (^{m}/z): 326 (M^+), 328 (M^++2). Calc for $C_{16}H_{23}BrO_2$: C, 58.71; H, 7.03. Found: C, 58.15; H, 6.72.

I_d: UV λ_{max} (EtOH): 265 nm. IR (KB_r): 1650 (quinone), 1590 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H,t), 1.20-1.65 (18 H, m), 2.10 (3 H, s), 2.64 (2H, t), 6.61 (1H, s) ppm. Mass (^{m}/z): 354 (M^+), 356 (M^++2). Calc for $C_{18}H_{27}BrO_2$: C, 60.85; H, 7.61. Found : C, 60.22; H, 7.18.

I_e: UV λ_{max} (EtOH): 265 nm. IR (KB_r): 1653 (quinone), 1590 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.20-1.65 (22H, m), 2.10 (3H,s), 2.65 (2H, t), 6.62 (1H, s) ppm. Mass (^{m}/z): 382 (M^+), 384 (M^++2). Calc for $C_{20}H_{31}BrO_2$: C, 62.66; H, 8.09. Found: C, 62.57; H, 7.90.

Synthesis of 2, 5-dibromo-3-methyl-6-alkyl-1, 4-benzoquinone ($II_{a\text{-}e}$): 5-bromo-3-methyl-6-alkyl-1, 4-benzoquinone (4 mmol) was added to a concentrated HB_r (46%, 5 ml). The solution was stirred at room temperature for 1h, and then diluted with 20 ml of water, which following extracted with chloroform. The organic extracts were dried over Na_2SO_4. Ag_2O (0.8g) was added to the solution and stirred for 1.5h. After removing solid phase, the organic solvent was removed in *vacuo*. The concentrate was purified by silica gel plates, which developed by a hexane-ether-CCl_4 (15:1:1). A yellow band was collected to give a yield over 50% of $II_{a\text{-}e}$.

II_a: UV λ_{max} (EtOH): 290 nm. IR (KB_r): 1660 (quinone), 1590 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t, CH_2 $(CH_2)_3$ CH_3), 1.25-1.70 (6H,m,CH_2 $(CH_2)_3$ CH_3), 2.28 (3H,s, CH_3), 2.74 (2H, t, CH_2 $(CH_2)_3$ CH_3) ppm. Mass (^{m}/z): 348 (M^+), 350 (M^++2), 352 (M^++4). Calc for C_{12} H_{14} $B_{r2}O_2$: C, 41.14; H, 4.00. Found: C, 41.50; H,4.32.

II_b: UV λ_{max} (EtOH): 290 nm. IR (KB_r): 1661 (quinone), 1590 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.25-1.70 (10 H, m), 2.28 (3H, s), 2.74 (2H, t) ppm. Mass (^{m}/z): 376 (M^+), 378 (M^++2), 380 (M^++4). Calc for C_{14} H_{18} $B_{r2}O_2$: C, 44.44; H, 4.76. Found: C, 44.01; H.5.22.

II_c: UV λ_{max} (EtOH): 290 nm. IR(KB_r): 1660 (quinone), 1592 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.25-1.70 (14H, m), 2.28 (3H,t), 2.74 (2H, t) ppm. Mass (^{m}/z): 404 (M^+), 406 (M^++2), 408 (M^++4). Calc for C_{16} H_{22} $B_{r2}O_2$: C, 47.29; H, 5.42. Found: C, 46.87; H, 5.02.

II_d: UV λ_{max} (EtOH): 290 nm. IR (KB_r): 1661 (quinone), 1592 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.25-1.70 (18H,m), 2.28 (3H, s), 2.74 (2H, t) ppm. Mass (^{m}/z): 432 (M^+), 434 (M^++2), 436 (M^++4). Calc for C_{18} H_{26} $B_{r2}O_2$: C, 49.77; H, 5.99. Found: C, 49.20; H, 6.32.

II_e:UV λ_{max} (EtOH): 290 nm. IR (KB_r): 1665 (quinone), 1590 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.25-1.70 (22H, m), 2.28 (3H, s), 2.74 (2H,t) ppm. Mass (^{m}/z): 460 (M^+), 462 (M^++2), 464 (M^++4). Calc for C_{20} H_{30} $B_{r2}O_2$: C, 51.95; H, 6.49. Found: C,51.74; H, 6.01.

Synthesis of 3, 5-dibromo-2-methyl-6-alkyl-1, 4-benzoquinone (III_{a-e}):(**1**) 2,4,6-tribromo-3-methylphenol----*m*-Cresol (5.4g, 0.05 mol) was treated with a bromine (24g, 0.15 mol) by a similar procedures with 2, 4-dibromo-6-methylphenol synthesized. The crude product was purified by recrystallization from hexane to give a yield 98%, m.p. 82-83°C (ref. 15, 84°C). (**2**) 3, 5-dibromo-2-methyl-1, 4-benzoquinone----2,4,6-tribromo-3-methylphenol (13.8g, 0.04 mol) was dissolved in 500 ml of CH_3CO_2H-H_2O (7:3), which was then heated to 70°C. C_rO_3 (4g. 0.04 mol) was added in 10 min with stirring and continued to stir for 30 min. The mixture was diluted with water (1500 ml). The yellow solid was precipitated and collected , which was recrystallized from C_2H_5OH-H_2O to give a yield of 75%,m.p. 114-115°C (ref. 15, 117°C). (**3**) 3, 5-dibromo-2-methyl-6-alkyl-1, 4-benzoquinone----3, 5-dibromo-2-methyl-1, 4-benzoquinone (0.56g, 2 mmol) was placed in 30 ml of CH_3CN-H_2O (1:1). The fatty acid (4 mmol) and $AgNO_3$ (0.34g, 2 mmol) were added to the solution, which then heated to 70-80° C and stirred. $(NH_4)_2S_2O_8$ (0.91g, 4 mmol, in 10 ml H_2O) were added dropwise with stirring and continued to stir for 1h at 70-80° C, which then cooled and extracted with ether. The ether layer was washed with diluted $NaHCO_3$ solution and water, then dried over Na_2SO_4 and concentrated in *vacuo*. The concentrate was purified by silica gel plates, which was developed with a hexane-ether-CCl_4 (8:1:1). A yellow band with a R_f 0.5-0.6 was collected and eluted with ether to give a yield around 50% of III_{a-e}.

III_a: UV λ_{max} (EtOH): 288 nm. IR (KB_r): 1650 (quinone), 1587 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t, CH_2 $(CH_2)_3$ CH_3), 1.25-1.70 (6H, m, $CH_2(CH_2)_3$ CH_3), 2.30 (3H,s, CH_3), 2.70 (2H, t, CH_2 $(CH_2)_3CH_3$) ppm. Mass (^{m}/z): 348 (M^+), 350 (M^++2), 352 (M^++4). Calc for C_{12} H_{14} $B_{r2}O_2$: C, 41.14; H, 4.00. Found: C,40.67; H, 4.20.

III_b: UV λ_{max} (EtOH): 288 nm. IR(KB_r): 1649 (quinone), 1587 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.25-1.70 (10H, m), 2.30 (3H,s), 2.70 (2H, t) ppm. Mass (^{m}/z): 376 (M^+), 378 (M^++2), 380 (M^++4). Calc for C_{14} H_{18} $B_{r2}O_2$: C, 44.44; H, 4.76. Found: C, 44.22; H, 5.18.

III_c: UV λ_{max} (EtOH): 288 nm. IR (KB_r): 1649 (quinone), 1588 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.25-1.70 (14H, m), 2.30 (3H, s), 2.70 (2H, t) ppm. Mass ($^m/_z$): 404 (M^+), 406 (M^++2), 408 (M^++4). Calc for $C_{16}H_{22}Br_2O_2$: C, 47.29; H, 5.42. Found: C, 46.76; H, 5.08.

III_d: UV λ max (EtOH): 288 nm. IR(KB_r): 1645 (quinone), 1587 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.25-1.70 (18H, m), 2.30 (3H,s) , 2.70 (2H, t) ppm. Mass ($^m/_z$): 432 (M^+), 434 (M^++2), 436 (M^++4). Calc for $C_{18}H_{26}Br_2O_2$: C, 49.77; H, 5.99. Found: C, 49.53; H, 5.54 .

III_e: UV λ max (EtOH): 288 nm. IR(KB_r): 1648 (quinone), 1588 (C=C) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.25-1.70 (22H, m), 2.30 (3H, s), 2.70 (2H, t) ppm. Mass ($^m/_z$): 460 (M^+), 462 (M^++2), 464 (M^++4). Calc for $C_{20}H_{30}Br_2O_2$: C, 51.95; H, 6.49. Found: C, 51.55; H, 6.61.

Synthesis of 3-bromo-2-methoxy-5-methyl-6-alkyl-1, 4-benzoquinone (IV_{a-e}): (**1**) 2-methoxy-5-methyl-1, 4-benzoquinone----$ZnCl_2$ (27.2g, 0.2 mol) was placed in 100 ml of methanol, which was then heated to 60° C . Toluquinone (24g, 0.2 mol) was added with stirring, then heated to reflux for 1h. The solution was cooled and stirred overnight at 0-5° C. The yellow needle cryst als was precipitated and collected, which was washed with cold methanol to give a yield of 30%, m.p. 172-173°C (ref. 11, 172-173°C). (**2**) 2-methoxy-5-methyl-6-alkyl-1, 4-benzoquinone----2-methoxy-5-methyl-1, 4-benzoquinone (1.52g, 10 mmol) was dissolved in 10 ml of acetic acid, which was then heated to 90°C. Alkyl peroxide ($[RCO_2]_2$, 20 mmol in 6 ml of CH_3CO_2H) was added dropwise with stirring at 90° C for 1h, and continued to stir for 2h at 90°C. The solution was then cooled to room temperature and stirred overnight. After diluting with 40 ml of water, the mixture was extracted with 100 ml of ether. The ether layer was washed with a diluted $NaHCO_3$ solution and water, and dried over Na_2SO_4, which then concentrated in *vacuo*. The concentrate was purified by silica gel plates which developed by a hexane-ether (3.5:1). The yellow band of R_f 0.4~0.5 was collected and eluted with ether to give a yield about 15% of product. (**3**) 3-bromo-2-methoxy-5-methyl-6-alkyl-1, 4-benzoquinone---- 2-methoxy-5-methyl-6-alkyl-1, 4-benzoquinone (2 mmol) was dissolved in 12 ml of acetic acid. Bromine (0.35g, 2.2 mmol) was added slowly to the stirred solution at room temperature, which continued to stir for 2h. The acetic acid was removed from solution in *vacuo*. The residue was dissolved in a small amount of ether, which was applied to the silica gel plates developed by a hexane-ether (3.5:1) to give near quantitative of additive compound (5-cyclohexene-1, 4-dione, 2, 3-dibromo-2-methoxy-5-methyl-6-alkyl), which was used immediately in the next step. The additive compound was placed in 50 ml of ether which was cold in ice-bath. $(C_2H_5)_2NH$ (0.29 g, 4 mmol in 10ml of ether) was added dropwise with stirring for 10 min at about O° C and continued to stir for 10 min at 0~5° C. It was warmed to room temperature and continually stirred for 30 min. The ether layer was separated and washed with water, then dried over Na_2SO_4. After removing ether in *vacuo*, the residue was applied to the silica gel plates developed by a hexane-ether (3.5:1). A yellow band of R_f 0.5~ 0.6 was collected to give a yield about 40% of IV_{a-e}.

IV_a: UV λ_{max} (EtOH): 284 nm. IR (KB_r): 1640 (quinone), 1582 (C=C), 1250 (C-O) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t, $CH_2(CH_2)_3$ *CH_3*), 1.12-1.60 (6H, m, CH_2 (*CH_2*)$_3CH_3$), 2.05 (3H, s, *CH_3*), 2.50 (2H, t, *CH_2* $(CH_2)_3$ CH_3), 4.14 (3H, s, *OCH_3*) ppm. Mass ($^m/_z$): 300 (M^+), 302 (M^++2). Calc for $C_{13}H_{17}BrO_3$: C, 51.83; H, 5.65. Found: C, 51.57; H, 5.30.

IV_b: UVλ max (EtOH): 284 nm. IR(KB_r): 1642 (quinone), 1582 (C=C), 1248 (C-O) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.12-1.60 (10H, m), 2.05 (3H, s), 2.50 (2H, t), 4.13 (3H, s)

ppm. Mass (m/z): 328(M^+), 330 (M^++2). Calc for $C_{15}H_{21}B_rO_3$: C, 54.71; H, 6.38. Found: C, 54.92; H, 5.93.

IV_c: UV λ max (EtOH): 284 nm. IR (KB_r): 1642 (quinone), 1585 (C=C), 1250 (C-O) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.12-1.60 (14H, m), 2.05 (3H, s), 2.50 (2H, t), 4.13(3H, s) ppm. Mass (m/z): 356(M^+), 358 (M^++2). Calc for $C_{17}H_{25}B_rO_3$: C, 57.14; H, 7.00. Found: C, 56.68; H, 7.30.

IV_d: UV λ max (EtOH): 284 nm. IR (KB_r): 1640 (quinone), 1582 (C=C), 1250 (C-O) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H, t), 1.12-1.60 (18H, m), 2.05 (3H, s), 2.50 (2H, t), 4.13 (3H, s) ppm. Mass (m/z): 384 (M^+), 386 (M^++2). Calc for $C_{19}H_{29}B_rO_3$: C, 59.22; H. 7.53. Found C, 59.61; H, 7.08.

IV_e: UV λ_{max} (EtOH): 284 nm. IR(KB_r): 1644 (quinone), 1582 (C=C), 1250 (C-O) cm^{-1}. ^{1}HNMR ($CDCl_3$): 0.90 (3H,t), 1.12-1.60 (22H, m), 2.05 (3H, s), 2.50 (2H, t), 4.13 (3H, s) ppm. Mass (m/z): 412 (M^+), 414 (M^++2). Calc for $C_{21}H_{33}B_rO_3$: C, 61.01; H, 7.99. Found: C, 60.82; H, 7.86.

REFERENCES

1. Trebst, A., Harth, E. and Draber, W., Z. Naturforsch, 1970, 25B 1157-1159.
2. Loschen, G. and Azzi, A, FEBS Lett., 1974, 41, 115-117.
3. Degli Esposti, M., Rotilio, G. and Lenaz, G., Biochim. Biophys. Acta, 1984, 767, 10-20
4. Hauska, G., Hart, E., Gabellim, N. and Lockan, W., Biochim. Biophys. Acta, 1983, 726, 97-133.
5. Malkin, R, FEBS Lett, 1981, 131, 169-172.
6. Judith, M. and Peter, B.H., Chem. Soc. Perkin II, 1981, 32-41.
7. Smith, B., J. Am. Chem. Soc., 1941, 63, 616.
8. Gu, L.Q., Liu, B.L. and Zhang, J.L., Youji Huaxue (Chinese), 1986, 463-465.
9. Gu, L.Q. and Xu, J.X., ibid. 1984, 414-422.
10. March, J. "Advance Org. Chem.", p. 529-530, McGraw-Hill Book Co., 1968, New York.
11. Gu, L.Q., Yu, L. and Yu, C.A., Biochem. Biophys. Res. Commun., 1983, 113 , 477-482.
12. Berger, S. and Rieker, A., in "The Chemistry of the Quinoid Compounds", p. 164, (Ed. by Patai, S.), John Wiley & Sons, London, 1974.
13. King, T.E., J. Biol. Chem., 1961, 236, 2342-2346.
14. Yu, C.A. , Yu. L. and King, T.E., J. Biol. Chem., 1974, 249, 4905-4910.
15. "CRC Handbook of Data on Org. Compounds", (Ed. by Weast, R.C. and Grasselli, J.G.)p. 1649,3355, 3428, 2nd Ed. , CRC Press, Inc., 1989, Boca Raton, Florida.
16. "CRC Handbook of Chemistry and Physics" (Ed. by Weast, R.C.) (1984-1985), C-165, CRC Press, Inc., Boca Raton, Florida.

Acknowledgement -- The work is partially supported by grants from NIH-GM-16769 and NIH-HB-R37-12576, and Chinese National Science Foundation.