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Synthesis, Metabolism, and *in Vitro* Biological Activities of 6-(10-Hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (CV-2619)-Related Compounds[1)]

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Demethyl derivatives (**3a** and **3b**) of 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (**1**, CV-2619), demethyl derivative (**4**) of the metabolite **2**-4, and compounds (**13**, **20**, **21**, **23a** and **23b**) in which the hydroxyalkyl chain of **1** is modified, were synthesized for metabolic studies and evaluation of their biological activities. In rats, **13** and *trans*-**23a** were metabolized more slowly than **1**. These compounds, except for the demethyl derivatives (**3a**, **3b** and **4**), showed an electron transfer effect comparable to that of **1** in the succinate oxidation system. Compounds **13** and **23b** inhibited the lipid peroxidation of rat liver homogenate.

Keywords——CV-2619; demethyl derivative; hydroxyalkyl chain modification; metabolism; succinate oxidation system; lipid peroxidation; 1,4-benzoquinone

6-(10-Hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (**1**, CV-2619)[3)] has been reported to suppress the onset of renal and cerebral vascular disorders in stroke-prone spontaneously hypertensive rats (SHRSP).[4)] It also partially alleviates the abnormal energy metabolism induced by cerebral ischemia.[5)] We have examined some *in vitro* biological activities of **1**, and found that both an electron transfer activity in the terminal respiratory chain of mitochondria and an antioxidant activity in the mitochondrial and microsomal membranes are correlated with the above effects of **1** on cerebrovascular disorders.[6)] In the previous study of the metabolism of **1** in animals and humans, side-chain-oxidized metabolites **2**-*n* ($n=4$, 6, 8 and 10) and their hydroquinone glucuronides and sulfates as well as the hydroquinone glucuronide and sulfate of the original compound (**1**) have been found in plasma and urine (Chart 1).[7)] Metabolites (**3** and **4**) probably formed by demethylation of the 2- or 3-methoxy group of **1** and **2**-4 have also been detected.[8)]

In this study, we synthesized **3a** and **4** from 3-hydroxy-2-methoxy-5-methyl-1,4-benzoquinone (**6**),[9)] a mixture of **3a** and **3b** from **1**, and some derivatives of **1**, and evaluated their *in vitro* biological activities. The metabolic behavior of these compounds in rats is also described.

Experimental

Melting points were measured with a Yanagimoto micro melting point apparatus, and are uncorrected. Infrared (IR) spectra were obtained with Hitachi EPI-S2 and 215 spectrophotometers. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were run on Varian HA-100, T-60 and A-60A spectrometers with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given as δ values (ppm): s, singlet; d, doublet; t, triplet; br, broad; m, multiplet. Ubiquinone-10 was isolated from whale heart muscle,[10)] and ubiquinone-2 and -3 were synthesized in our laboratory.[11)] Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Sigma, and other chemical reagents from Wako Pure Chemical Industries. Protein concentrations were determined by the Lowry method.[12)]

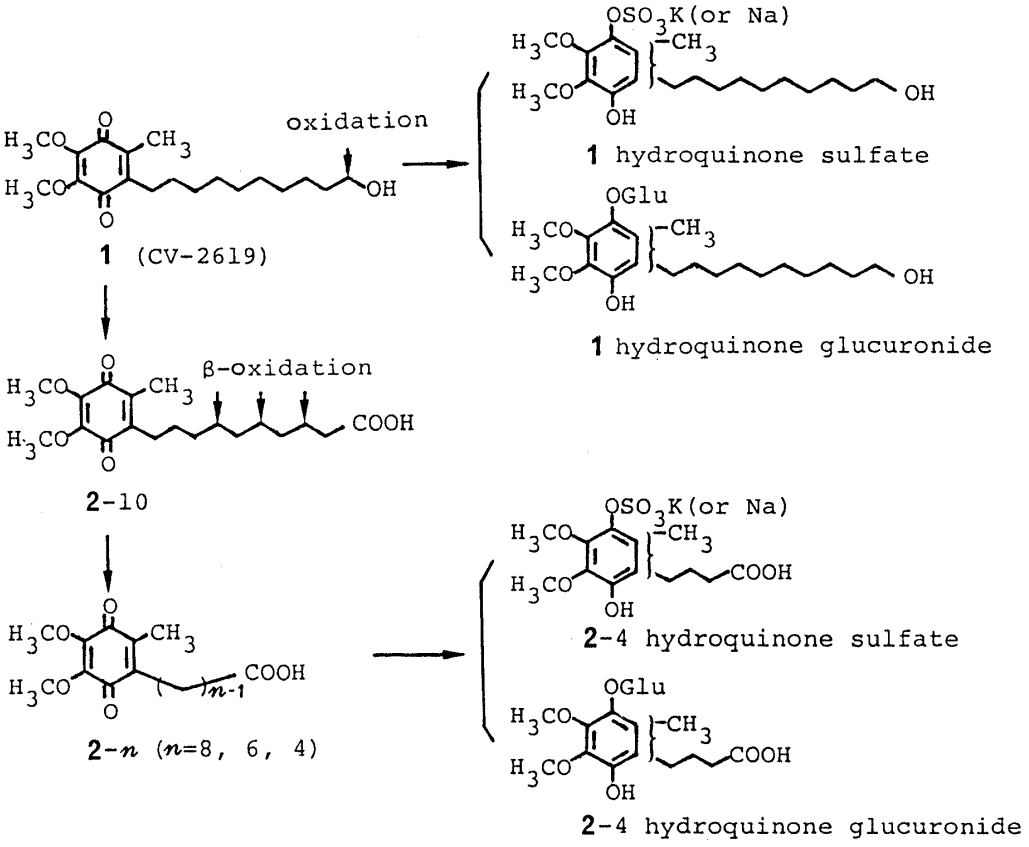


Chart 1. Proposed Metabolic Pathway of **1** (CV-2619)

3-Hydroxy-6-(10-hydroxydecyl)-2-methoxy-5-methyl-1,4-benzoquinone (3a), 2-Hydroxy-6-(10-hydroxydecyl)-3-methoxy-5-methyl-1,4-benzoquinone (3b), and 6-(10-Hydroxydecyl)-2(and 3)-methoxy-5-methyl-1,4-benzoquinone (5)——1) A suspension of $LiAlH_4$ (20 g) in dioxane (300 ml) was refluxed for 2 h. A solution of 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (**1**, 10 g) in dioxane (350 ml) was added portionwise to the suspension at room temperature over a period of 4 h. The mixture was refluxed for 40 min, then excess $LiAlH_4$ was decomposed with cold H_2O and 3 N HCl. The mixture was extracted with AcOEt, and the extract was worked up in the usual way. The residue was dissolved in MeOH, a solution of $FeCl_3$ (40 g) in H_2O (100 ml) was added to the methanolic solution, and the whole was stirred. The reaction mixture was extracted with AcOEt, the extract was worked up in the usual way and the residue was purified by column chromatography on silica gel. The first fraction eluted with $CHCl_3$ gave **5**, the second fraction with $CHCl_3$–EtOH (50 : 1, v/v) gave a mixture of **3a** and **3b**, and the third fraction (same solvent) gave **3a**. Each fraction was rechromatographed on silica gel and recrystallized from AcOEt–hexane to give **5** (0.43 g) as yellow needles, a mixture of **3a** and **3b** (1.2 g, 12.5%) as dark-red needles, and **3a** (0.85 g) as dark-red needles. **3a**: ^{1}H-NMR (d_6-benzene) δ: 1.26 (16H, br, CH_2), 1.70 (3H, s, CH_3 on the ring), 2.28 (2H, t, CH_2 on the ring), 3.40 (2H, t, CH_2O), 3.77 (3H, s, OCH_3), 6.40 (1H, br, OH). **3a** and **3b**: mp 90—97 °C, ^{1}H-NMR (d_6-benzene) δ: 1.26 (16H, br, CH_2), 1.71 and 1.74 (3H, s, CH_3 on the ring), 2.28 and 2.23 (2H, t, CH_2 on the ring), 3.42 (2H, t, CH_2O), 3.78 (3H, s, OCH_3). **5**: ^{1}H-NMR (d_6-benzene) δ: 1.26 (16H, br, CH_2), 1.80 and 1.88 (3H, s, CH_3 on the ring), 2.34 (2H, m, CH_2 on the ring), 2.94 (3H, s, OCH_3), 3.44 (2H, t, CH_2O), 5.54 (1H, s, the ring H).

2) 6-(10-Acetoxydecyl)-3-hydroxy-2-methoxy-5-methyl-1,4-benzoquinone (**9**) was dissolved in a mixed solution of MeOH (55 ml) and concentrated HCl (0.2 ml), and the mixture was allowed to stand at room temperature for 12 h. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in AcOEt (100 ml). The solution was washed with saturated aqueous NaCl and dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was recrystallized from AcOEt–hexane, giving **3a** as dark-red needles. Yield 900 mg.

4-(3-Hydroxy-2-methoxy-5-methyl-1,4-benzoquinon-6-yl)butyric Acid (4)——Diglutaryl peroxide (**10**, 13.4 g) was added portionwise to a solution of 3-hydroxy-2-methoxy-5-methyl-1,4-benzoquinone (**6**, 2.9 g)[9] in toluene (60 ml) at 95—100 °C over a period of 2 h. After being stirred at 95—100 °C for 0.5 h, the reaction mixture was diluted with AcOEt. The organic layer was washed with saturated aqueous NaCl and extracted with saturated aqueous $NaHCO_3$. The alkaline solution was acidified with 3 N HCl and extracted with AcOEt. The extract was worked up in the usual way and the residue was subjected to column chromatography on silica gel (containing 10% H_2O, 60 g) with hexane–

AcOEt–AcOH (70 : 30 : 1, v/v) as the eluent. The solvent was evaporated off and the residue was recrystallized from Et_2O–hexane, giving **4** as red-purple needles. Yield 240 mg. ^{1}H-NMR ($CDCl_3$) δ: 1.50—2.10 (2H, m, CH_2), 2.00 (3H, s, CH_3 on the ring), 2.50 (4H, m, $C\underline{H}_2CH_2C\underline{H}_2CO$), 4.00 (3H, s, OCH_3).

Bis(11-acetoxyundecanoyl) Peroxide (8)——Ice-H_2O was added to a solution of 11-acetoxyundecanoyl chloride[13] (**7**, 10.5 g) in Et_2O (50 ml). Na_2O_2 (4 g) was added portionwise to the mixture. After being stirred vigorously, the reaction mixture was diluted with H_2O and extracted with Et_2O. The extract was washed with H_2O and dried over $CaCl_2$. Evaporation of the solvent gave **8** as a white wax. Yield 8.6 g. IR ν_{max}^{nujol} cm^{-1}: 1780 (COOOCO). Without purification, **8** was used for the next step.

6-(10-Acetoxydecyl)-3-hydroxy-2-methoxy-5-methyl-1,4-benzoquinone (9)——Compound **8** (8.75 g) was added portionwise to a solution of **6** (1.5 g) in toluene (10.8 ml) at 85—95 °C over a period of 5.5 h. After being stirred for 0.5 h, the reaction mixture was diluted with H_2O and extracted with Et_2O. The extract was washed successively with H_2O, saturated aqueous $NaHCO_3$, NaCl and H_2O, and dried over Na_2SO_4. The solvent was evaporated off and the residue was subjected to column chromatography on silica gel. The product was recrystallized from AcOEt–hexane, giving **9** as dark-red needles. Yield 733 mg. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (16H, m, CH_2), 2.02 (6H, s, CH_3 on the ring, $OCOCH_3$), 2.48 (2H, t, CH_2 on the ring), 4.06 (3H, s, OCH_3), 4.08 (2H, t, CH_2O).

11-(2-Hydroxy-3,4-dimethoxy-6-methylphenyl)-2-methylundecan-2-ol (12)——A solution of CH_3I (4.9 g, 0.035 mol) in Et_2O (17.3 ml) was added dropwise to a suspension of Mg (0.92 g, 0.038 mol) in Et_2O (6 ml) over a period of 1 h. After being warmed at 40 °C for 40 min, the mixture was diluted with tetrahydrofuran (THF) (50 ml). A solution of methyl 10-(2-hydroxy-3,4-dimethoxy-6-methylphenyl)decanoate (**11**, 4.99 g)[3] in THF (50 ml) was added to the reaction mixture at room temperature over a period of 1.5 h and the whole was warmed at 40 °C for 30 min. The mixture was decomposed with aqueous NH_4Cl and H_2O, and extracted with Et_2O (300 ml). The extract was worked up in the usual way and the residue was subjected to column chromatography on silica gel with $CHCl_3$ as the eluent. Evaporation of the solvent gave **12** as a colorless oil. Yield 2.38 g (47.9%). ^{1}H-NMR ($CDCl_3$) δ: 1.18 (6H, s, CH_3), 1.32 (16H, br, CH_2), 2.17 (3H, s, CH_3 on the ring), 1.40—1.64 (2H, m, CH_2 on the ring), 3.80 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 6.02 (1H, s, OH on the ring), 6.17 (1H, s, the ring H).

6-(10-Hydroxy-10-methylundecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (13)——A mixture of **12** (2.335 g), Fremy's salt (35 g) and sodium acetate (12 g) in MeOH–H_2O (5 : 2 v/v, 210 ml) was stirred at 50 °C for 14 h. H_2O (100 ml) was added to the mixture and the MeOH was evaporated off. The mixture was extracted with AcOEt (200 ml), and the extract was worked up in the usual way. The residue (2.115 g) was subjected to column chromatography on silica gel with $CHCl_3$ as the eluent and gave **13** as an orange oil. Yield 1.716 g. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (6H, s, CH_3C), 1.32 (16H, br, CH_2), 1.98 (3H, s, CH_3 on the ring), 2.17 (1H, s, OH), 2.38—2.60 (2H, m, CH_2 on the ring), 3.95 (6H, s, OCH_3).

***p*-Substituted Phenylalkanoic Acids (14a, 14b, 15a, 15b and 16)**——Compounds **14a** and **14b** were obtained by Clemmensen reduction of *p*-methoxybenzoylalkanoic acids. Demethylation of **14a** and **14b** with 48% HBr in AcOH gave **15a** and **15b**, respectively. **16** was prepared by acetylation of **15b** with Ac_2O. The melting points and elemental analysis data of **14a**, **14b**, **15a**, **15b** and **16** are given in Table I.

***trans*- and *cis*-6-(4-Hydroxycyclohexyl)hexanoic Acids (*trans*- and *cis*-17a)**——1) **15a** (2 g) was hydrogenated over 5% Rh–C (500 mg) in EtOH (100 ml) at room temperature under 10.1 kg/cm^2 of H_2 for 70 min. After removal of the catalyst, the solvent was evaporated off and the residue was subjected to column chromatography on silica gel. From the first fraction eluted with $CHCl_3$–EtOH (50 : 1, v/v), 6-cyclohexylhexanoic acid[14] (365 mg, 19.2%) was obtained as a by-product. The fractions eluted with $CHCl_3$–EtOH (50 : 2, v/v) successively gave *cis*-**17a** (392 mg, 19.0%), a mixture of *cis* and *trans*-**17a** (922 mg, 44.8%) and *trans*-**17a** (237 mg, 11.5%). *cis*-**17a** was recrystallized from Et_2O–ligroin as colorless needles and *trans*-**17a** from EtOH–H_2O as colorless needles. 2) **15a** (20 g) was hydrogenated over 5% Ru–C (3 g) in 80% EtOH (500 ml) at 100—109 °C under 109 kg/cm^2 of H_2 for 75 min. The reaction mixture was worked up in a manner similar to that described above to give *trans*-**17a** (4.9 g, 23.8%) and a mixture of *trans*- and *cis*-**17a** (12.35 g, 60.0%).

***trans*- and *cis*-7-(4-Hydroxycyclohexyl)heptanoic Acids (*trans*- and *cis*-17b)**——**15b** (20 g) was hydrogenated over 5% Ru–C (3 g) in 80% EtOH at 102—104 °C under 115 kg/cm^2 of H_2 for 1 h. After removal of the catalyst, the solvent was evaporated off and the residue was dissolved in Et_2O. Crude *trans*-**17b** was crystallized by trituration with ether, and recrystallization from EtOH–H_2O and then isopropanol gave pure *trans*-**17b** (6.98 g, 34.0%) as colorless needles. The ether washing from the crystallization of *trans*-**17b** was diluted with ligroin to give crude *cis*-**17b**, which was recrystallized from Et_2O-petroleum ether to give *cis*-**17b** (8.64 g, 42.0%) as colorless needles.

***trans*-6-(4-Acetoxycyclohexyl)hexanoic Acid (*trans*-18a)**——A solution of *trans*-**17a** (5 g) and *p*-TsOH (10 mg) in Ac_2O (25 ml) was heated to 60—70 °C for 1 h. The excess Ac_2O was removed and the residue was diluted with H_2O. The mixture was stirred at room temperature for 24 h, warmed at 50—60 °C for 5 h and poured into ice-H_2O. Recrystallization of the precipitates from EtOH–H_2O gave *trans*-**18a** (4.93 g, 82.4%) as colorless needles.

***trans*-7-(4-Acetoxycyclohexyl)heptanoic Acid (*trans*-18b)**——*trans*-**18b** was obtained from *trans*-**17b** in a manner similar to that used for the synthesis of *trans*-**18a**.

The experimental data for *trans*-, *cis*-**17a**, *trans*-, *cis*-**17b**, *trans*-**18a** and *trans*-**18b** are summarized in Table II.

2,3-Dimethoxy-6-[5-(4-methoxyphenyl)pentyl]-5-methyl-1,4-benzoquinone (20)——A mixture of **14a** (3.7 g) and

$SOCl_2$ (5 ml) was stirred at room temperature overnight. The excess $SOCl_2$ was removed and the residue was dissolved in petroleum ether (30 ml), then ice-H_2O (15 ml) was added. Na_2O_2 (3 g) was added portionwise under vigorous stirring at 0 °C. The mixture was extracted with $CHCl_3$ and the extract was worked up in the usual way, giving bis[6-(4-methoxyphenyl)hexanoyl] peroxide (3.6 g) as a white solid. This peroxide was added portionwise to a solution of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (**19**, 1.48 g) in AcOH (30 ml) at 95 °C over a period of 3 h. The mixture was heated at 120 °C for 1 h and the AcOH was removed *in vacuo*. The residue was extracted with Et_2O and the extract was worked up in the usual way. The residue was subjected to column chromatography on silica gel with CCl_4–AcOEt (19 : 1, v/v) as the eluent. Evaporation of the solvent gave **20** as an orange oil. Yield 0.461 g. ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.80 (6H, br, CH_2), 1.98 (3H, s, CH_3 on the ring), 2.27—2.67 (4H, m, CH_2 on the ring), 3.77 (3H, s, OCH_3), 3.97 (6H, s, OCH_3), 6.70 (2H, d, the ring H), 7.00 (2H, d, the ring H).

6-[6-(4-Hydroxyphenyl)hexyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (21)——A mixture of **16** (10 g) and $SOCl_2$ (14 ml) was warmed at 70 °C for 1.5 h. Removal of the excess $SOCl_2$ left the crude acid chloride (11.2 g). Under ice-cooling, 3.5 ml of 30% H_2O_2 was added dropwise to a solution of the acid chloride in Et_2O (75 ml), followed by addition of 7.2 ml of pyridine. After being stirred at room temperature for 1 h, the ethereal solution was worked up in the usual way to give bis[7-(4-acetoxyphenyl)heptanoyl] peroxide (8.96 g) as a white solid. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1810 (COOOCO), 1750 (ester). This peroxide was added portionwise to a solution of **19** (3.11 g) in AcOH at 95—100 °C over a period of 2 h and the mixture was kept at 95 °C for 2 h. The reaction mixture was worked up in the usual way, giving an orange oil which was subjected to column chromatography on silica gel with CCl_4–AcOEt (20 : 1, v/v) as the eluent. Removal of the solvent gave 6-[6-(4-acetoxyphenyl)hexyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (2.1 g) as an orange oil. This product was dissolved in MeOH (55 ml) containing concentrated HCl (0.3 ml) and the solution was kept at room temperture for 4 h. The solvent was removed and the resulting residue was purified by column chromatography on silica gel using $CHCl_3$–Et_2O (99 : 1, v/v) as the eluent to obtain **21** as orange crystals. Recrystallization from hexane–Et_2O gave **21** as orange needles. Yield 0.83 g. ^{1}H-NMR ($CDCl_3$) δ: 1.00—1.80 (8H, m, CH_2), 2.17 (3H, s, CH_3 on the ring), 2.17—2.67 (4H, m, CH_2 on the ring), 3.98 (6H, s, OCH_3), 5.10 (1H, s, OH), 6.63 (2H, d, J=8 Hz, the ring H), 6.93 (2H, d, J=8 Hz, the ring H).

***trans*-6-[5-(4-Acetoxycyclohexyl)pentyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (*trans*-22a)**——Bis[*trans*-6-(4-acetoxycyclohexyl)hexanoyl] peroxide was obtained from *trans*-**18a** in a manner similar to that used for bis[6-(4-methoxyphenyl)hexanoyl] peroxide. This peroxide (4.8 g) was condensed with **19** (1.7 g) in AcOH (30 ml) in a manner similar to that described in the synthesis of **20**. Recrystallization from petroleum ether gave *trans*-**22a** as orange crystals. Yield 0.814 g. ^{1}H-NMR ($CDCl_3$) δ: 1.02—1.88 (17H, m, CH_2, CH), 2.02 (3H, s, CH_3 on the ring), 2.44 (2H, t, CH_2 on the ring), 3.99 (6H, s, OCH_3), 4.64 (1H, m, CH–O).

***trans*-6-[5-(4-Hydroxycyclohexyl)pentyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (*trans*-23a)**——*trans*-**22a** (0.415 g) was dissolved in MeOH (10 ml) containing concentrated HCl (0.056 ml) and the solution was kept at room temperature for 24 h. The solvent was removed and the resulting residue was worked up in the usual manner. The residue was subjected to column chromatography on silica gel with CCl_4–AcOEt (23 : 2, v/v) as the eluent. Recrystallization from hexane–Et_2O gave *trans*-**23a** as orange needles. Yield 0.195 g. ^{1}H-NMR ($CDCl_3$) δ: 0.97—1.90 (17H, m, CH_2, CH), 2.00 (3H, s, CH_3 on the ring), 2.43 (2H, t, CH_2 on the ring), 3.53 (1H, br, CH–O), 3.98 (6H, s, OCH_3).

***cis*-6-[5-(4-Hydroxycyclohexyl)pentyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (*cis*-23a)**——A mixture of *trans*- and *cis*-**17a** was acetylated in a manner similar to that used for *trans*-**18a**. A mixture of *trans*- and *cis*-**18a** was converted to an acid chloride, then to a peroxide. Condensation of the peroxide (6.9 g) with **19** (1 g) followed by hydrolysis was done in a manner similar to that used for *trans*-**23a**. The crude product was purified by column chromatography using silica gel with CCl_4–AcOEt (9 : 1, v/v) as the eluent. The solvent was removed and the residue was recrystallized from hexane–Et_2O to obtain *cis*-**23a** as orange needles. Yield 0.465 g. ^{1}H-NMR ($CDCl_3$) δ: 1.13—1.70 (17H, br, CH_2, CH), 2.02 (3H, s, CH_3 on the ring), 2.47 (2H, t, CH_2 on the ring), 3.98 (7H, s, OCH_3, CH–O). From the second fraction, *trans*-**23a** was obtained. Yield 0.194 g.

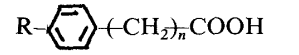
***trans*-6-[6-(4-Hydroxycyclohexyl)hexyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (*trans*-23b)**——Diacyl peroxide (5.9 g) obtained from *trans*-**18b** was condensed with **19** (1.82 g) in a manner similar to that used for the synthesis of **21**. The product was hydrolyzed and recrystallized from petroleum ether–Et_2O to give *trans*-**23b** as orange needles. Yield 0.885 g. ^{1}H-NMR ($CDCl_3$) δ: 1.00—2.10 (19H, m, CH_2, CH), 2.00 (3H, s, CH_3 on the ring), 2.42 (2H, t, CH_2 on the ring), 3.47 (1H, br, CH–O), 3.97 (6H, s, OCH_3).

***cis*-6-[6-(4-Hydroxycyclohexyl)hexyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (*cis*-23b)**——Diacyl peroxide (26.5 g) obtained from *cis*-**18b** was condensed with **19** (4.95 g) in a manner similar to that used for the synthesis of **21**. The product was hydrolyzed and recrystallized from hexane–Et_2O to give *cis*-**23b** as orange needles. Yield 1.82 g. ^{1}H-NMR ($CDCl_3$) δ: 1.27—1.68 (17H, m, CH_2, CH), 2.01 (3H, s, CH_3 on the ring), 2.45 (2H, t, CH_2 on the ring), 3.99 (7H, s, OCH_3, CH–O).

The physicochemical data of quinone compounds are shown in Table III.

Metabolic Experiment——(i) *In Vitro*: The liver of a male Sprague-Dawley rat (168 g body weight) was isolated and sliced into 0.3 mm sections with a McIlwan tissue chopper. The sliced tissue (200 mg) was suspended in phosphate-saline buffer (10 ml).[15] After the flask had been shaken at 37 °C for 1 min, glucose (final concentration

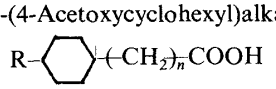
TABLE I. Analytical Data for *p*-Substituted Phenylalkanoic Acids



Compound No.	R	*n*	mp (°C)	Lit. mp (°C)	Formula	Analysis (%) Calcd (Found)	
						C	H
14a	OCH_3	5	46—48	46—47[a]			
14b	OCH_3	6	67—72	69[b]			
15a	OH	5	106—108	107—108[a]			
15b	OH	6	87—89		$C_{13}H_{18}O_3$	70.24	8.16
						(70.31	8.20)
16	$OCOCH_3$	6	63—65		$C_{15}H_{20}O_4$	68.16	7.63
						(68.06	7.83)

a) D. Papa, E. Schwenk and H. Hankin, *J. Am. Chem. Soc.*, **69**, 3018 (1947). *b*) H. Stetter and W. Dierichs, German Patent 915085 (1954).

TABLE II. Physicochemical Data for *trans*- and *cis*-(4-Hydroxycyclohexyl)alkanoic Acids (*trans*-, *cis*-**17a, b**) and *trans*-(4-Acetoxycyclohexyl)alkanoic Acids (*trans*-**18a, b**)



Compound No.	R	*n*	mp (°C)	Recryst. solvent	Formula	Analysis (%) Calcd (Found)	
						C	H
trans-**17a**	OH	5	127—129	EtOH–H_2O	$C_{12}H_{22}O_3$	67.25	10.35
						(67.48	10.64)
cis-**17a**	OH	5	83—85	Et_2O–ligroin	$C_{12}H_{22}O_3$	67.25	10.35
						(67.47	10.53)
trans-**17b**	OH	6	126—128	iso-PrOH–Et_2O	$C_{13}H_{24}O_3$	68.38	10.59
						(68.56	10.36)
cis-**17b**	OH	6	75—77	Et_2O–petroleum ether	$C_{13}H_{24}O_3$	68.38	10.59
						(68.56	10.36)
trans-**18a**	$OCOCH_3$	5	82—84	EtOH–H_2O	$C_{14}H_{24}O_4$	65.59	9.44
						(65.76	9.45)
trans-**18b**	$OCOCH_3$	6	66—68	AcOEt–hexane	$C_{15}H_{26}O_4$	66.63	9.69
						(66.81	9.83)

Compound No.	Solvent	^{1}H-NMR δ ppm –CH_2– –CH–	CH_2COO (2H, t)	>CH–O– (1H, br)	$OCOCH_3$ (3H, s)
trans-**17a**	DMSO-d_6	0.90—1.80 (17H, m)	2.18	3.33	
cis-**17a**	DMSO-d_6	1.25 (17H, br)	2.18	3.77	
trans-**17b**	DMSO-d_6	0.73—1.92 (19H, m)	2.15	3.27	
cis-**17b**	DMSO-d_6	1.23 (19H, br)	2.17	3.70	
trans-**18a**	$CDCl_3$	0.90—1.70 (17H, m)	2.32	4.60	2.00
trans-**18b**	$CDCl_3$	0.82—2.13 (19H, m)	2.35	4.65	2.03

TABLE III. Physicochemical Data for Quinone Compounds

Compound No.	Method[a]	Yield (%)	mp (°C)	Recryst. solvent	Formula	Analysis (%) Calcd (Found) C	H	IR ν_{max}[b] cm^{-1} OH (COOH) [Ester]	quinone
3a	A	90.0	98—99	AcOEt–hexane	$C_{18}H_{28}O_5$	66.64	8.70	3350	1630, 1610
	B	8.9				(66.40	8.79)		
4	C, A[c]	5.5	106—108	Et_2O–hexane	$C_{12}H_{14}O_6$	56.69	5.55	(1700)	1640, 1630
						(56.88	5.52)		
5	B	4.7	96—107	AcOEt–hexane	$C_{18}H_{28}O_4$	70.10	9.15	3500	1660, 1640
						(70.33	9.20)		1600
9	C	22.4	59—61	AcOEt–hexane	$C_{20}H_{30}O_6$	65.55	8.25	[1730]	1630, 1610
						(65.47	8.31)		
13	D	70.0	Oil		$C_{21}H_{34}O_5$	68.82	9.35	3500	1660, 1650
						(68.63	9.27)		1610
20	C	15.8	Oil		$C_{21}H_{26}O_5$	70.37	7.31	—	1660, 1650
						(70.32	7.25)		1610
21	C, A[c]	13.5	87—88	Et_2O–hexane	$C_{21}H_{26}O_5$	70.37	7.31	3500	1660, 1640
						(70.09	7.38)		1610
trans-**22a**	C	22.9	33—34	Petroleum ether	$C_{22}H_{32}O_6$	67.32	8.22	[1730]	1670, 1650
						(67.59	8.17)		1610
trans-**23a**	A	52.6	64—65	Et_2O–hexane	$C_{20}H_{30}O_5$	68.54	8.63	3500	1660, 1640
						(68.78	8.33)		1610
cis-**23a**	C, A[c]	24.2	49—51	Et_2O–hexane	$C_{20}H_{30}O_5$	68.54	8.63	3500	1640, 1610
						(68.70	8.73)		
trans-**23b**	C, A[c]	22.2	69—71	Et_2O–petroleum ether	$C_{21}H_{32}O_5$	69.20	8.85	3500	1660, 1640
						(69.26	8.66)		1610
cis-**23b**	C, A[c]	18.4	33.5—35.5	Et_2O–hexane	$C_{21}H_{32}O_5$	69.20	8.85	3500	1660, 1640
						(69.32	8.56)		1610

a) Methods: A, hydrolysis of an ester compound; B, reaction with $LiAlH_4$; C, condensation with diacyl peroxide; D, oxidation with Fremy's salt. *b*) IR spectra were recorded in KBr pellets except for oily compounds (film). *c*) Method C followed by method A.

9.7 mM) and lecithin emulsion (1 ml) containing **1** (4.92 mg) were added to the flask. The mixture was shaken at 37 °C for 1 h and the reaction was stopped by adding 70% perchloric acid (0.5 ml). The reaction mixture was extracted by the method described in Fig. 1 and the metabolites were measured by high-performance liquid chromatography (HPLC). Apparatus: Shimadzu LC-2 pump, with SPD-2A ultraviolet (UV) absorbance detector. Waters RCM-100 radial compression, Radial pack cartridge C_{18} (8 mm i.d. × 10 cm). Mobile phase: H_2O–dioxane–isopropanol–AcOH (80 : 35 : 35 : 0.29, v/v) for the detection of **2**-10 and H_2O–dioxane–isopropanol–AcOH (70 : 10 : 10 : 0.25, v/v) for **2**-4. The flow rates were 0.65 ml/min for **2**-10 and 0.7 ml/min for **2**-4. Detection was achieved by UV absorption measurement at 280 nm. Retention times: 15.2—16.6 min for **2**-10, 10.9—11.2 min for **2**-4.

(ii) *In Vivo*: Compounds **1**, **13** and *trans*-**23a** were given *per os* to rats (Sprague-Dawley, male, 6 weeks old) at a dosage of 100 mg per kg. Blood samples were taken from the abdominal aorta at 0.5 h after the administration and the livers and kidneys were isolated. The extraction procedure is shown in Fig. 1. When **1** was given to rats, the liver homogenate was fractionated to obtain mitochondria. Analysis was done by HPLC. Metabolite **2**-4 was analyzed under the conditions described above, and **1**, **13** and *trans*-**23a** were determined under the following conditions. Column: Waters, Radial pack B cartridge (5 mm i.d. × 10 cm). Mobile phase: 5% isopropanol–hexane for **1** and *trans*-**23a**, and 1.7% isopropanol–hexane for **13**. Retention times: **1**, 12.4 min (flow rate 1.4 ml/min); **13**, 13.6 min (flow rate 1.8 ml/min); and *trans*-**23a**, 13.1 min (flow rate 1.4 ml/min).

Restoration of Mitochondrial Oxidation——Succinate oxidase activity was assayed by the procedure described previously.[6b] Beef heart mitochondria were treated with acetone[16] to obtain acetone-treated beef heart mitochondria (A-BHM). Oxygen consumption rates were measured with a Clark oxygen electrode (Gilson oxygraph, type K-IC) and the mean value of two or more measurements was determined. These activities are given in terms of ng atom oxygen/min per mg protein. Test compounds were added to a suspension of mitochondrial preparations in the form of an aqueous solution containing five to ten times their weight of a detergent, OP-10 (Nikko Kogyo).

Lipid Peroxidation——Livers of female Sprague-Dawley rats, 6—8 weeks old, were isolated and minced with a razor. The minced tissue was homogenized in ice-cold buffered potassium chloride (0.15 M KCl, 5 mM Tris-maleate,

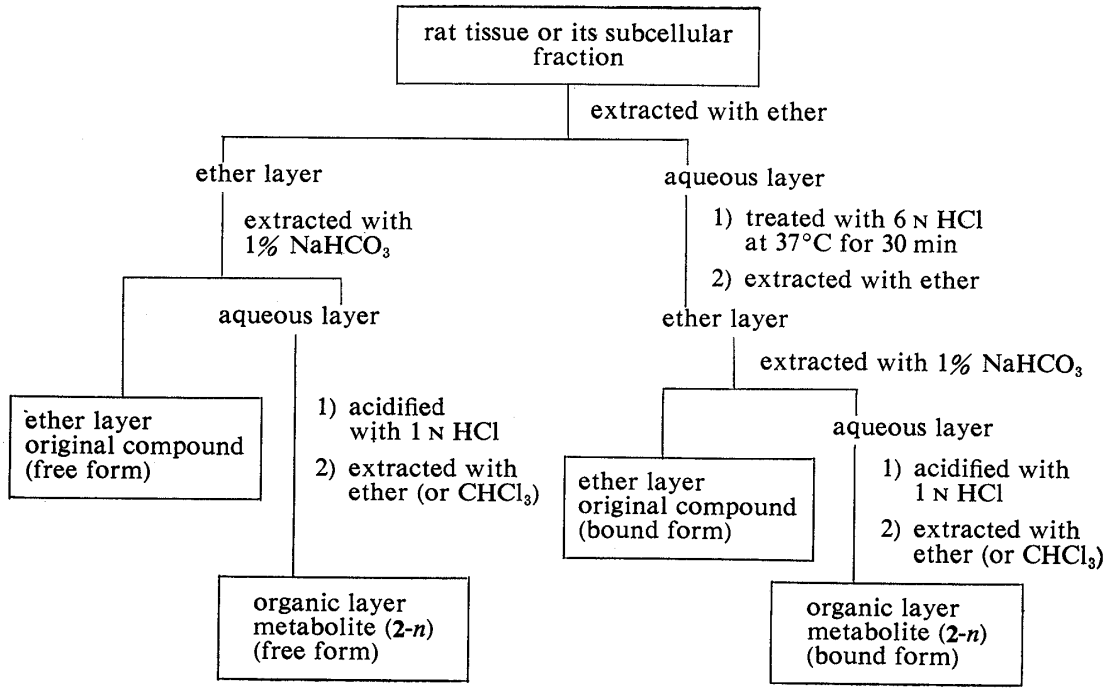


Fig. 1. Extraction of Tissue and Its Subcellular Fractions from Rats Orally Administered with **1** and Related Compounds

pH 7.4) using a Teflon-glass homogenizer. Homogenates were assayed by a procedure described previously.[6d] The amount of lipid peroxide was determined as the value of thiobarbituric acid-reactive substance (TBARS).

Results

Chemistry

Preparation of a mixture of 2- and 3-hydroxy derivatives of ubiquinones by irradiation with light or reduction with $LiAlH_4$ has been reported.[17] Their structures were established by the ^1H-NMR chemical shifts of the protons on the ring methyl and methylene substituents in benzene or toluene as a solvent.[18] In the present study, we found that a mixture of 2- and 3-hydroxy derivatives (**3a** and **3b**) was also obtained by the reduction of **1** with $LiAlH_4$. In this reaction, we also isolated a small amount of **5**. Compound **5** was also a mixture of 2- and 3-demethoxy derivatives of **1** (Chart 2). On the other hand, the pure 3-hydroxy derivative (**3a**) was synthesized by condensation of 3-hydroxy-2-methoxy-5-methyl-1,4-benzoquinone (**6**) with diacyl peroxide (**8**) obtained from 11-acetoxyundecanoyl chloride (**7**), followed by hydrolysis of the resulting acetate (**9**). Similarly, the 3-demethyl derivative (**4**) of **2**-4 was prepared from **6** and diglutaryl peroxide (**10**) (Chart 2).

Derivatives of **1** having a secondary or a tertiary hydroxyalkyl chain were also synthesized. Thus, the reaction of the ester (**11**) with methyl magnesium iodide gave **12**, which, through oxidation of the phenol ring to quinone, afforded the tertiary hydroxy homolog (**13**) of **1** (Chart 3).

p-Substituted phenylalkanoic acids (**14**—**16**, Table I) were prepared as starting materials for the synthesis of the compounds with modified hydroxyalkyl chains (**20**, **21**, **22**, **23a**, **b**). Mixtures of *trans* and *cis* isomers of 6-(4-hydroxycyclohexyl)hexanoic acid (**17a**) and of 7-(4-hydroxycyclohexyl)heptanoic acid (**17b**) were obtained from 6-(4-hydroxyphenyl)-hexanoic acid (**15a**) and 7-(4-hydroxyphenyl)heptanoic acid (**15b**), respectively, by catalytic reduction using rhodium–carbon or ruthenium–carbon. These products were separated by silica gel column chromatography followed by recrystallization, or only by recrystalliza-

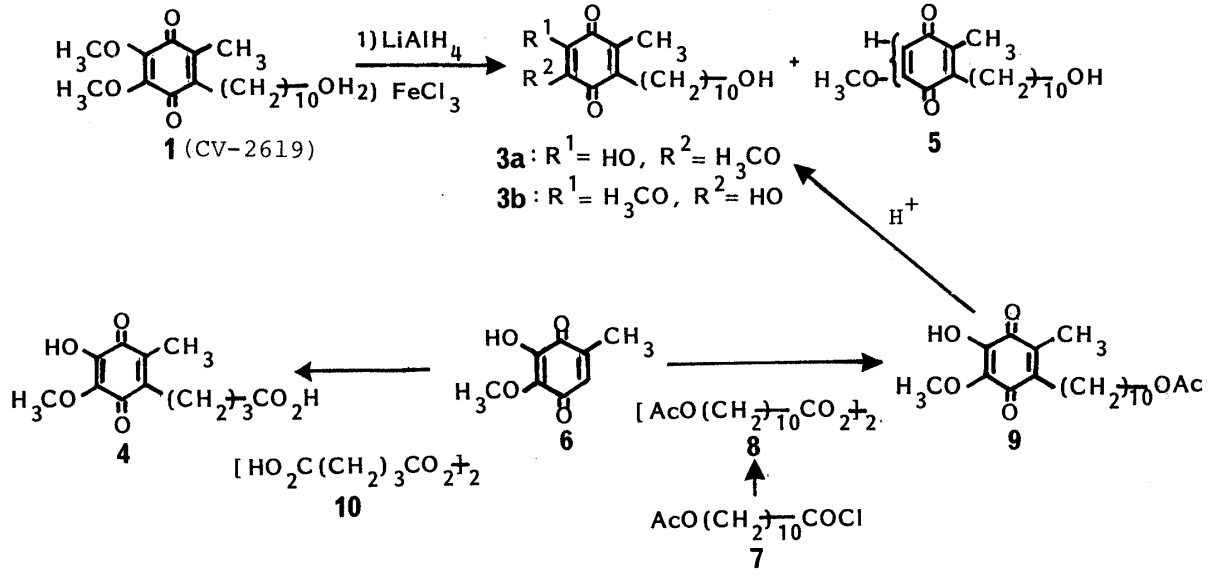


Chart 2

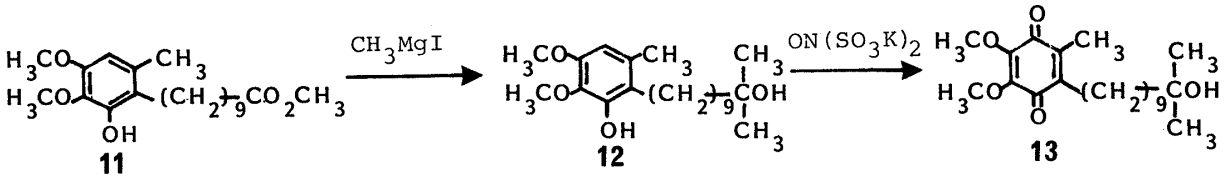


Chart 3

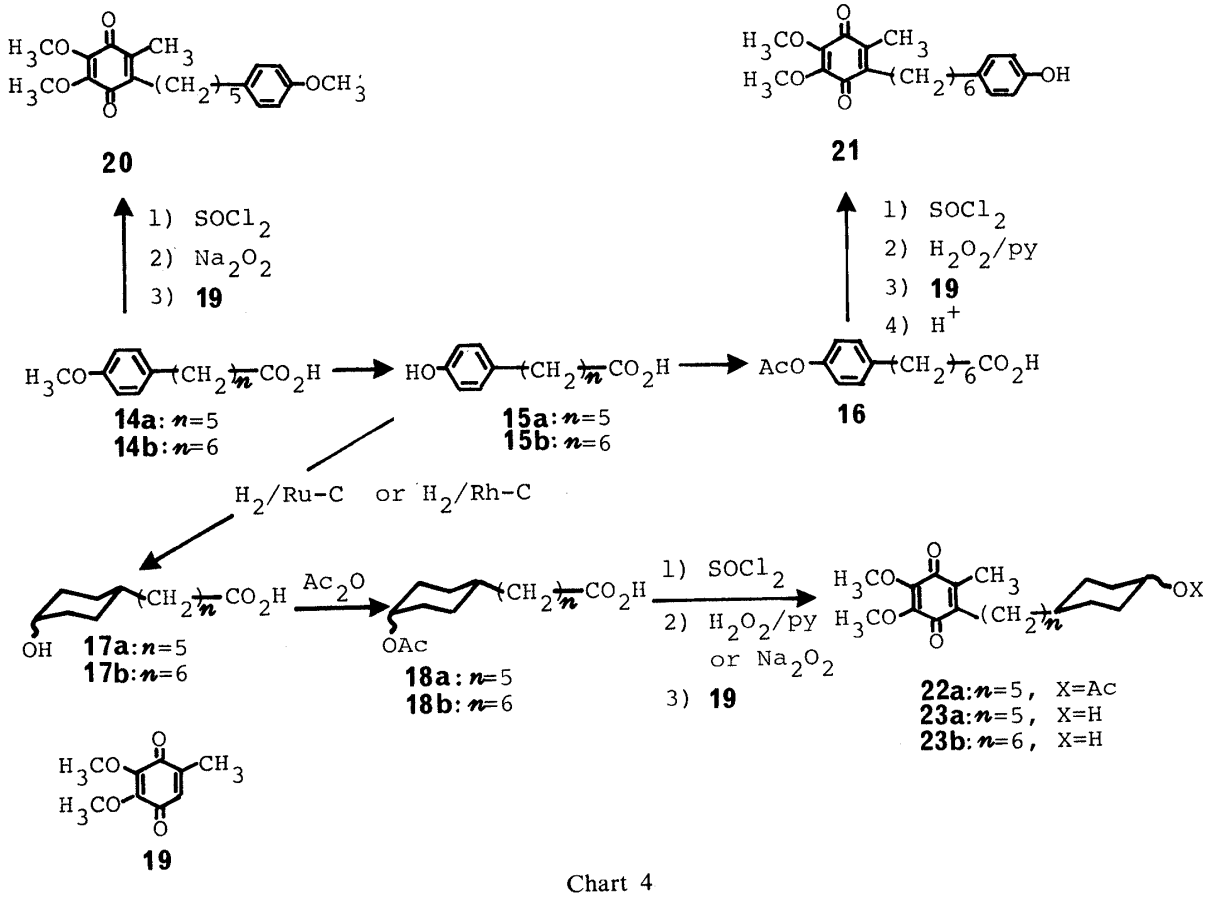


Chart 4

tion (Table II).

The compounds *trans*-**17a** and *trans*-**17b** were acetylated to give *trans*-**18a** and *trans*-**18b**, respectively. The carboxylic acids **14a**, and *trans*-**18a** gave the quinone compounds (**20**, and *trans*-**22a**, respectively) as shown in Chart 4. Hydrolysis of *trans*-**22a** yielded *trans*-**23a**. The condensation of **19** with a stereoisomeric mixture of **18a** followed by hydrolysis gave a mixture of *trans*- and *cis*-**23a** which could be separated by silica gel column chromatography. The diacyl peroxides prepared from **16**, *trans*-**18b** and *cis*-**18b**, were condensed with **19** and then hydrolyzed to yield **21**, *trans*-**23b**, and *cis*-**23b**, respectively (Chart 4).

Metabolism in Rats

Generally, drugs which are absorbed through the gastrointestinal tract after oral administration are transferred *via* the portal vein to the liver and metabolized. We therefore incubated **1** with rat liver slices at 37 °C for 1 h and extracted the reaction mixture as shown in

TABLE IV. Conversion of **1** to **2**-10 by Incubation with Rat Liver Slices[a]

1 (mg)	Liver slices (mg)	Products[b] (μg) **2**-10	**2**-4
0	202	ND	ND
0	232	ND	ND
4.92	209	48	ND
4.92	220	42	ND

a) Incubation was performed in phosphate buffer, pH 7.4, in the presence of glucose at 37 °C for 1 h, and the mixture was extracted with ether as described in Fig. 1. *b*) The amount of product was determined by HPLC. Details are given in Experimental. ND, not detected. Detection limit; 0.1 μg/g tissue.

TABLE V. **2**-4 (Bound Form) in Tissues of Rats Orally Administered **1** (100 mg/kg)[a]

Fraction[b]	Liver		Kidney		
Rat	No. 1	No. 2	No. 1	No. 2	No. 3
Homogenate	10.1[c]	20.1	40.9	19.3	52.0
Mitochondria	1.7	—	0.7	—	—
Supernatant	6.7	—	32.6	—	—

a) Tissues were extracted as described in Fig. 1. *b*) Fractionation was done as described in Experimental. *c*) μg/g tissues. These were determined by HPLC as described in Experimental. —, not done.

TABLE VI. **13** and *trans*-**23a** in Liver and Plasma of Rats Orally Administered these Compounds (100 mg/kg each)[a]

Compound	Form	Liver		Plasma	
Rat		No. 1	No. 2	No. 1	No. 2
13	Free	2.03[b]	3.69	ND	ND
	Bound	—	—	—	—
trans-**23a**	Free	1.47	0.56	ND	ND
	Bound	33.2	14.3	1.51	0.81

a) Liver or plasma was extracted as described in Fig. 1. *b*) μg/g tissue or ml of plasma. These values were determined by HPLC. —, not done because of instability of **13** on treatment with HCl. ND, not detected.

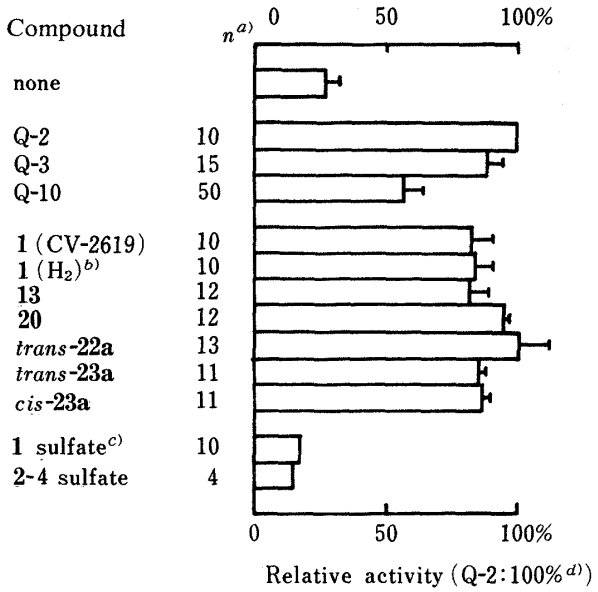


Fig. 2. Restoration of Succinate Oxidase Activity of Acetone-Treated Beef Heart Mitochondria (A-BHM) with Q-Homologs and Related Compounds (10 nmol/mg protein) at 25 °C

a) Carbon number of side chain. *b*) Hydroquinone of **1**. *c*) Hydroquinone 4-sulfate. *d*) The activity of Q-2 was 104.3±4.7 ng atom oxygen/min per mg protein.

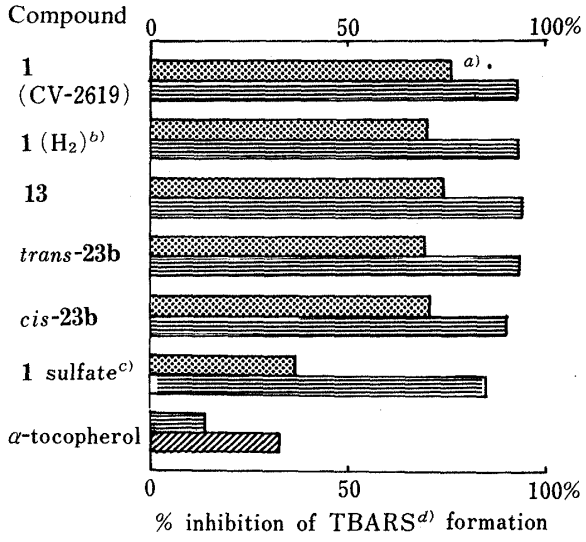


Fig. 3. Effects of **1** and Related Compounds on Lipid Peroxidation of Rat Liver Homogenate

a) ▨, 1×10^{-5} M; ▤, 1×10^{-4} M; ▨, 2×10^{-4} M. *b*) Hydroquinone of **1**. *c*) Hydroquinone 4-sulfate. *d*) TBARS: Thiobarbituric acid-reactive substance.

Fig. 1. The quantities of **2**-4 and **2**-10 in each extract were analyzed by HPLC (Table IV). The results confirmed the metabolic conversion of **1** into **2**-10 in liver, but no **2**-4 was detected in this *in vitro* test, in contrast to the results of the *in vivo* test.[7] Therefore, **1** was administered orally to rats and the extracts of each rat liver were analyzed. The conjugate form of **2**-4 was detected in liver and kidney mainly in the supernatant fraction, while unchanged **1** was below the limit of detection (0.1 μg/g tissue) (Table V).

From the results of these preliminary tests, we investigated the metabolic fate of the tertiary alcohol derivative (**13**) and *trans*-**23a** in rats after oral administration. The unchanged **13** and *trans*-**23a**, and the conjugate form of *trans*-**23a** were detected in the extract of liver. Furthermore, the conjugate form of *trans*-**23a** was detected in rat plasma (Table VI).

In Vitro Biological Activity

The electron transfer effect of **1**-related compounds on succinate oxidation was investigated using A-BHM. All the compounds, except for demethyl derivatives, had a prominent restoring effect on succinate oxidase activity, as shown in Fig. 2. On the other hand, hydroquinone sulfates of **1** and **2**-4 had no effect.

We also investigated the effects of compounds **13** and **23b** on lipid peroxidation. These compounds showed comparable inhibitions of NADPH/Fe^{2+}-dependent lipid peroxidation of rat liver homogenate to **1** and its hydroquinone (Fig. 3). α-Tocopherol and hydroquinone sulfate of **1** showed weak inhibition of peroxidation, but the hydroquinone sulfate of **2**-4 exhibited no antioxidant activity.

Discussion

The presumed metabolites of **1**, demethylated derivatives **3a**, **3b** and **4**, were synthesized

as standards for metabolic studies in rats. However, our preliminary experiment did not reveal such metabolites in tissues of rats. The main metabolic pathway of **1** was found to be oxidative degradation of the hydroxyalkyl chain (Chart 1). Therefore, we thought that inhibition of the oxidation of the hydroxy group and subsequent β-oxidation would prolong the maintenance of the plasma level and activity of **1**.

In advance of the metabolic study, we investigated the metabolism of **1** using rat liver slices and also tested for the original compound and the metabolites in the tissues after oral administration. The formation of **2**-10 from **1** in rat liver slices was confirmed, but the main metabolite (**2**-4) in rats[7] could not be detected (Table IV). The experiment with liver slices seemed not to be a suitable model for the study of the metabolism of **1**. Thus, when **1** was administered orally, the original compound was not detected but a conjugate of **2**-4 formed by β-oxidation of **2**-10 was found in the supernatant fraction from liver and kidney (Table V). Based on these preliminary results, we decided to use HPLC analysis of rat liver extracts after oral administration. Compounds **13** and *trans*-**23a** and a conjugate form of *trans*-**23a** were detected in the extract (Table VI), showing that these derivatives were less susceptible to metabolic degradation than **1**.

We next studied the effect of derivatives of **1** on electron transfer activity and lipid peroxidation *in vitro*. The prepared derivatives (**13**, **20**, *trans*-**22a**, *trans*-, and *cis*-**23a**) showed equipotent effects to **1** on the succinate oxidase system in A-BHM (Fig. 2). Compounds **13** and **23b** inhibited the production of lipid peroxide in the homogenate of rat liver (Fig. 3). Thus, the side chain modification did not affect the *in vitro* biological profile of **1** but prolonged the blood level. The derivatives described in this report may have a lipophilicity similar to that of **1**, based on the *Rf* values on paraffin-treated thin-layer chromatography. This is consistent with our previous finding that the partition coefficient correlates with the succinate oxidase activity.[6b,19]

The *in vivo* pharmacological activities of terminal carbon-modified derivatives of **1**, especially compounds **13**, **23a** and **23b**, seem to be interesting from the viewpoint of long duration of action.

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References and Notes

1) We dedicate this report to Professor Th. Wieland on his seventy-fifth birthday.
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