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Ubiquinone and Related Compounds. XXXVIII.[1] Biological Oxidation of Ubiquinone

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Ubiquinone-2 (**1**) was oxidized to 6-[7-carboxy-3-methyl-(2*E*,6*E*)-2,6-octadienyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (**2**) and 6-[6,7-dihydroxy-3,7-dimethyl-(*E*)-2-octenyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (**3**) by the 6000 ×*g* supernatant of guinea-pig liver homogenate in the presence of nicotinamide-adenine dinucleotide (NAD)- and dihydronicotinamide-adenine dinucleotide phosphate (NADPH)-generating systems.

Keywords——ubiquinone-2; 6-[7-carboxy-3-methyl-(2*E*,6*E*)-2,6-octadienyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone; 6-[6,7-dihydroxy-3,7-dimethyl-(*E*)-2-octenyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone; ω-oxidation

Previously,[2] we isolated several new metabolites (I—IV, Chart 1) of ubiquinone-7 (Q-7) from the tissues (liver, adrenals, and ovaries) and urine of rats, to which Q-7 had been administered orally, using ultraviolet (UV) absorption at 275 nm (specific for the quinone ring) as the marker for the metabolites. From the structural point of view, it was concluded

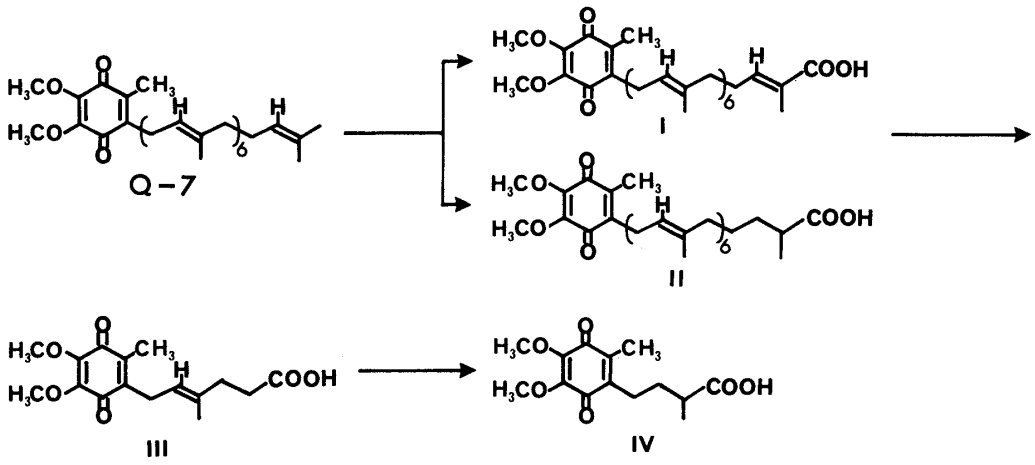

Chart 1

that the terminal (*E*)-methyl group[3] in the multiprenyl side chain of Q-7 was initially oxidized to give I and II, and subsequent β-oxidation produced III and IV. Since an ω-carboxylic acid derivative of ubiquinone-9 (Q-9) was detected in the tissues of rats fed a normal diet without specific addition of Q-7, we assumed that intrinsic Q-9 could also be metabolized into the ω-carboxylate in a manner similar to that of exogenous Q-7.

In the study reported here, we investigated the metabolites of ubiquinone using a cell-free system obtained from a homogenate of guinea-pig liver to clarify which terminal methyl group is oxidized. Since ubiquinone-2 (Q-2, **1**) has the necessary structural requirements and is

relatively soluble in aqueous solution, we used **1** as a substrate for this experiment. According to the procedure of Wakabayashi and Shimazono,[4] **1** was incubated in the $6000 \times g$ supernatant of guinea-pig liver homogenate in the presence of nicotinamide-adenine dinucleotide (NAD)- and dihydronicotinamide-adenine dinucleotide phosphate (NADPH)-generating systems. The metabolites were extracted with ethyl acetate and the extract was subjected to thin layer chromatography (TLC). In addition to the band of **1**, three bands were detected by UV irradiation of the thin layer. Each band was extracted with ethyl acetate to give three reddish oils (A—C). The metabolites B and C showed molecular peaks at $m/z = 348$ (B) and 352 (C), respectively, in addition to characteristic fragment peaks of ubiquinone, 235 (chromenylium) and 197 (benzylium)[5] in their mass spectra (MS), suggesting that they are an ω-carboxy derivative (**2**) and a dihydroxy derivative (**3**), respectively. They were shown to be identical with synthetic standard compounds by high-performance liquid chromatography (HPLC, Fig. 1) under conditions such that (*E*)- and (*Z*)-isomers of the methyl ester of III could be separated.[6] Oil A did not seem to originate from **1** because peaks at $m/z = 235$ and 197 were not observed in its MS.

The authentic metabolites B and C were synthesized as shown in Charts 2 and 3.

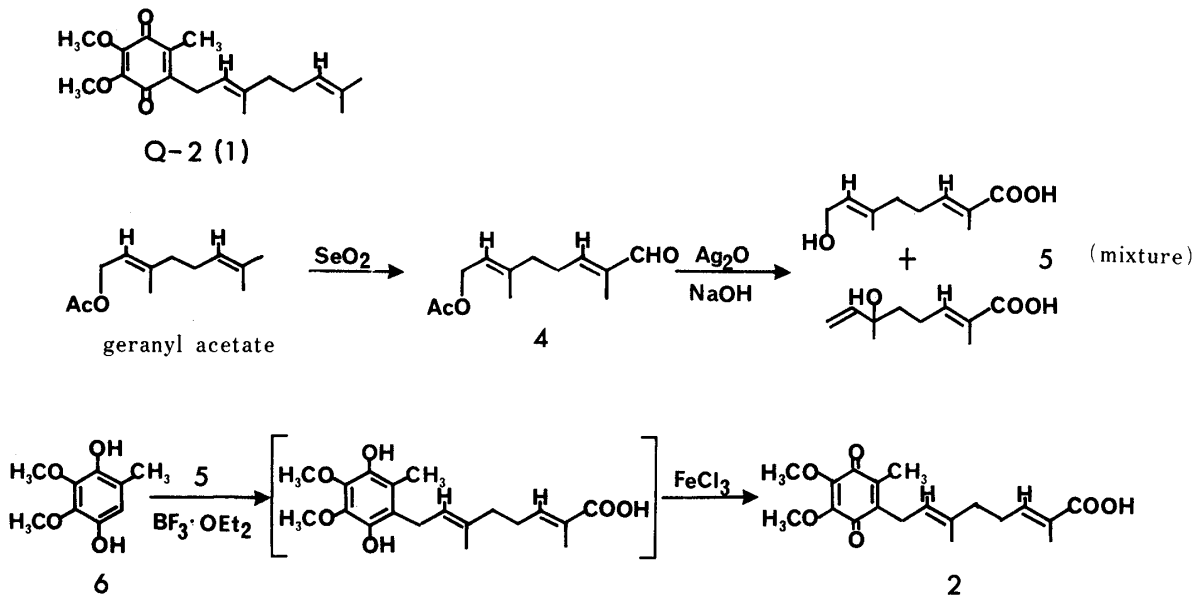

Chart 2

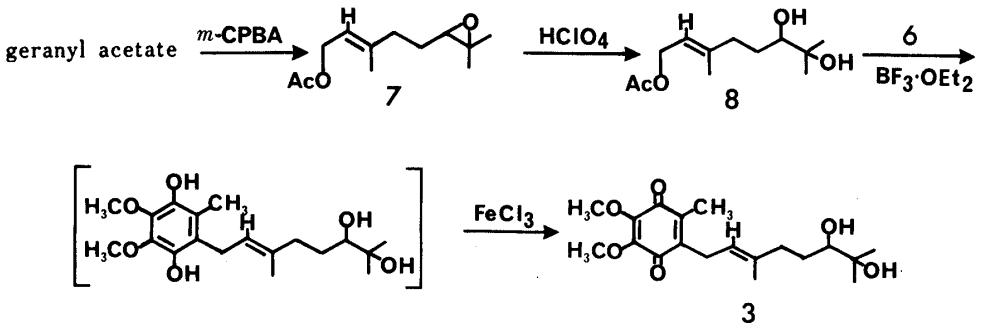

Chart 3

Oxidation of geranyl acetate with selenium oxide gave an aldehyde (**4**) by regio-specific oxidation of the terminal (*E*)-methyl group. The aldehyde **4** was oxidized with silver oxide in the presence of sodium hydroxide to give a mixture (**5**) of 8-hydroxy-2,6-dimethyl-2,6-

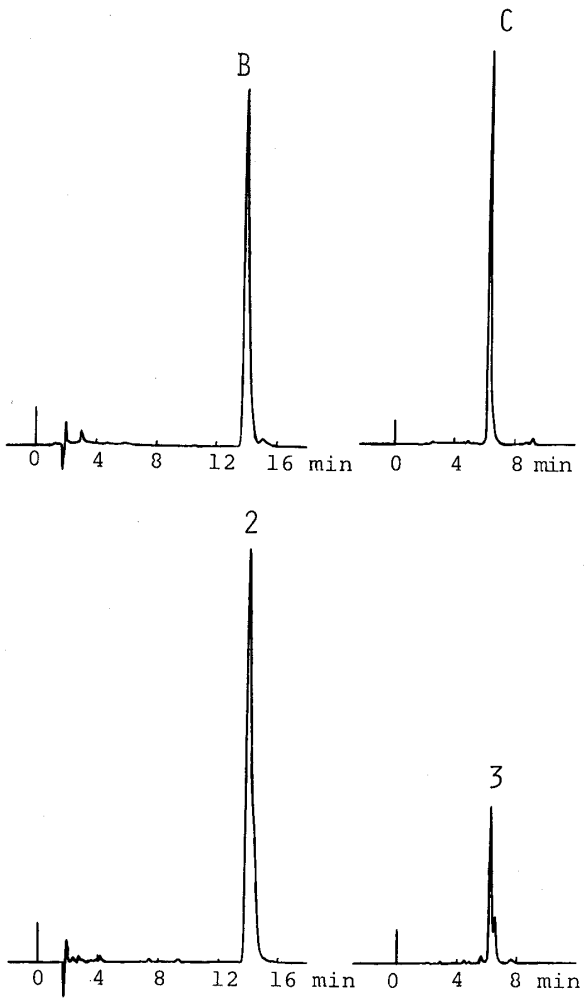

Fig. 1. Comparison of Metabolites B and C with the Synthetic Compounds **2** and **3** by High-Performance Liquid Chromatography

A solution of a sample in acetonitrile was injected to a Hibar LiChrosorb RP-18 (5 μm) column (4 × 250 mm, Merck) using a Reodyne injector and eluted with acetonitrile–0.05 M acetic acid (4 : 5) at flow rate of 1 ml/min using a Waters 6000A pump. Absorbance at 275 nm was monitored with a Waters 481 LC spectrophotometer.

octadienoic acid and its allylic isomer. The mixture (**5**), without separation, was treated with 2,3-dimethoxy-5-methylhydroquinone (**6**) in the presence of borontrifluoride etherate, and the resulting product was purified by TLC. The structure of the product was confirmed to be 6-[7-carboxy-3-methyl-(2*E*,6*E*)-2,6-octadienyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (**2**) from the chemical shifts, $\delta = 1.76$ [(*Z*)-CH_3 on 3-C] and 6.80 (6-H which is *Z* to the carboxyl group) in its proton nuclear magnetic resonance spectrum (^{1}H-NMR).[7] The result obtained in the biological oxidation of Q-2 supports our assumption that the ω-oxidation of ubiquinone occurs at the sterically less-hindered terminal (*E*)-methyl group.[2]

Oxidation of geranyl acetate with an equimolar amount of *m*-chloroperbenzoic acid gave an epoxide (**7**) which was subsequently led to the dihydroxy derivative (**8**) by treatment with perchloric acid. Compound **8** was condensed with **6** to give *dl*-6-[6,7-dihydroxy-3,7-dimethyl-(*E*)-2-octenyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (**3**). Synthetic **3** is a racemic compound with respect to the 6-carbon, and the steric configuration of enzymatically produced C remains to be studied.

The significance of enzymatically formed C in the metabolism of ubiquinone homologs will be clarified in the future.

Experimental

Q-2 (**1**) and 2,3-dimethoxy-5-methyl-1,4-benzoquinone (**6**) were synthesized in the authors' laboratories by the methods described in the literature.[8] UV spectra were recorded in EtOH with a Hitachi EPS-3T spectrophotometer,

and MS were taken with a JMS-OISC mass spectrometer (Japan Electron Optics). ^{1}H-NMR spectra were obtained on Varian HA-100 and EM-390 spectrometers with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given as δ values (ppm): s, singlet; d, doublet; t, triplet; m, multiplet. Male Hartley guinea-pigs weighing 460 g were used to isolate the liver. Geranyl acetate (Wako Pure Chemical), silica gel plates (Silica Gel 60F-254, Merck), NAD^+ (Boehringer Mannheim), $NADP^+$ (Oriental Yeast), nicotinamide (Wako Pure Chemical), and *dl*-isocitric acid (Sigma) were commercial products.

Biological Oxidation of Ubiquinone-2 (1)——The 6000 × ***g*** liver supernatant fraction was obtained by centrifuging a 20% (w/v) homogenate of guinea-pig liver (21.5 g) in Krebs–Ringer bicarbonate buffer, pH 7.6, at 6000 × ***g***. The reaction mixture consisted of 300 μmol of nicotinamide, 12.5 μmol of **1** in acetone (1 ml), 10 μmol of NAD^+, 5 μmol of $NADP^+$, 18 μmol of $MnCl_2$, 200 μmol of isocitrate, and 10 ml of 6000 × ***g*** supernatant fraction in a total volume of 37.5 ml of Krebs–Ringer bicarbonate buffer. Incubation was carried out by shaking the reaction mixture at 37 °C for 3 h in an atmosphere of air.

Isolation of Biological Oxidation Products (A, B and C)——The reaction mixture was extracted three times with AcOEt (each 10 ml). The combined extracts were washed with water (10 ml), dried on Na_2SO_4 and evaporated to dryness *in vacuo*. The residue was chromatographed on a silica gel plate (20 × 20 cm) with CCl_4–AcOEt (1 : 1) as the developing solvent. Four bands, Rf = 0.88 (**1**), 0.61 (A), 0.36 (B), and 0.21 (C) were detected under irradiation from a UV lamp (252 nm). Each band was extracted with AcOEt and the resulting extracts were worked up in the usual manner to give **1**, A, B, and C, respectively.

Geranial Acetate (4)——SeO_2 (2.3 g) was added dropwise to a solution of geranyl acetate (2 g) in EtOH (20 ml) under reflux, and the mixture was further refluxed for 1.5 h. The reaction solution was evaporated to dryness *in vacuo*. The resulting residue was purified on a silica gel column (2 × 30 cm) with CCl_4–acetone (5 : 1) to obtain a colorless oil, bp 110 °C (0.2 mmHg). Yield 2 g (82%). NMR ($CDCl_3$) δ: 9.44 (1H, s, CHO), 6.45 (1H, t, CH=), 5.4 (1H, t, CH=), 4.57 (2H, d, CH_2OCO), 2.6—2.2 (4H, m, CH_2C=), 2.03 (3H, s, CH_3CO), 1.75 (6H, s, CH_3C=). *Anal.* Calcd for $C_{12}H_{18}O_3$: C, 68.54; H, 8.63. Found: C, 68.01; H, 8.81.

8-Hydroxy-2,6-dimethyl-2,6-octadienoic Acid and Its Allylic Isomer (5)——A 2 N NaOH solution (5 ml) was added dropwise to a mixture of **4** (1.7 g), $AgNO_3$ (2 g), H_2O (4 ml), and EtOH (6 ml) with stirring at room temperature. After being stirred for 3 h, the reaction mixture was filtered. The filtrate was extracted with *n*-hexane. The aqueous layer was acidified with 5% HCl, then extracted three times with Et_2O (each 10 ml). The extracts were worked up in the usual manner to give **5** as a colorless oil. Yield 300 mg (20%).

6-[7-Carboxy-3-methyl-(2*E*,6*E*)-2,6-octadienyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (2)——A solution of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (270 mg) in Et_2O (10 ml) was shaken twice with 10% $Na_2S_2O_4$ (each 10 ml). The Et_2O layer was worked up in the usual manner, and the resulting residue (**6**) was dissolved in dioxane (3 ml). A solution of **5** (270 mg) and 45% $BF_3 \cdot Et_2O$ (0.5 ml) in dioxane (3 ml) was added dropwise to the ice-cooled solution of **6** under a stream of N_2 with stirring. The mixture was stirred for a further 2 h at room temperature, poured into H_2O (10 ml) and then extracted three times with Et_2O (10 ml, 5 ml × 2). The Et_2O layer was oxidized with a solution of $FeCl_3$ (1 g) in 50% MeOH (10 ml), washed with H_2O (10 ml), and then extracted three times with 5% Na_2CO_3 (10 ml, 5 ml × 2). After it had been acidified with dil. HCl, the aqueous layer was extracted with Et_2O (10 ml, 5 ml × 2), then the extracts were worked up in the usual manner to give **2** as a reddish oil. Yield 46 mg (9%). UV λ_{max}^{EtOH} nm ($E_{1\,cm}^{1\%}$): 275 (524). NMR ($CDCl_3$) δ: 6.8 (1H, t, CH=), 5.0 (1H, t, CH=), 4.0 (6H, s, CH_3O), 3.2 (2H, d, ring-CH_2), 2.4—2.05 (4H, m, CH_2C=), 2.0 (3H, s, ring-CH_3), 1.8 (3H, s, =CCH_3), 1.76 (3H, s, CH_3C=). MS *m/z*: 348 (M^+), 235 (chromenylium), 197 (benzylium). COO

***dl*-6,7-Epoxy-3,7-dimethylocta-2-enyl Acetate (7)**——A solution of *m*-chloroperbenzoic acid (*m*-CPBA) (1.4 g) in $CHCl_3$ (25 ml) was added dropwise to a solution of geranyl acetate (1.4 g) in $CHCl_3$ (50 ml) under ice-cooling with stirring, and the mixture was stirred for a further 30 min. The reaction mixture was extracted twice with 5% Na_2CO_3 (50, 20 ml), then washed with H_2O (20 ml). The mixture was dried with Na_2SO_4, and the $CHCl_3$ was evaporated off. The resulting oil was purified on a silica gel column (2 × 30 cm) with *n*-hexane–AcOEt (5 : 2), and the fraction corresponding to **7** was worked up in the usual manner to give a colorless oil, bp 90—92 °C (0.2 mmHg). Yield 1.1 g (73%). NMR ($CDCl_3$) δ: 5.4 (1H, t, CH=), 4.6 (2H, d, CH_2OCO), 2.7 (1H, t, CH–O), 2.4—2.1 (4H, m, CH_2), 2.03 (3H, s, CH_3CO), 1.73 (3H, s, CH_3C=), 1.30, 1.25 (6H, s, CH_3C–O). *Anal.* Calcd for $C_{12}H_{20}O_3$: C, 67.89; H, 9.50. Found: C, 67.52; H, 9.50.

***dl*-6,7-Dihydroxy-3,7-dimethylocta-2-enyl Acetate (8)**——An aliquot of 60% $HClO_4$ (1 ml) was added to a solution of the epoxide (**7**) (2.1 g) in 10% 1,2-dimethoxyethane (50 ml), and the mixture was stirred for 2 h at 30 °C. The reaction mixture was evaporated to dryness *in vacuo* and the resulting residue was dissolved in $CHCl_3$–Et_2O (1 : 1) (60 ml). The solution was washed twice with H_2O (each 10 ml), and then worked up in the usual manner. The residue was purified on a silica gel column (2 × 30 cm) with AcOEt to give **7** as a colorless oil. Yield 1.8 g (78%). NMR ($CDCl_3$) δ: 5.2 (1H, t, CH=), 4.6 (2H, d, CH_2OCO), 3.3 (1H, t, CH–O), 2.3–2.15 (4H, m, CH_2), 2.05 (3H, s, CH_3CO), 1.72 (3H, s, CH_3C=), 1.2, 1.18 (6H, s, CH_3C–O).

***dl*-6-[6,7-Dihydroxy-3,7-dimethyl-(*E*)-2-octenyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone (3)**——2,3-Dimethoxy-5-methyl-1,4-benzoquinone (1 g) was reduced with $Na_2S_2O_4$ as described above and worked up in the usual manner to give **6**. Then 45% BF_3–Et_2O (1 ml) was added to a solution of **6** in dioxane (5 ml), and a solution of **8**

(1.8 g) in dioxane (10 ml) was added dropwise under ice-cooling within 30 min. The mixture was stirred for 30 min, then poured into H_2O (20 ml). The aqueous solution was extracted three times with Et_2O (each 30 ml). The Et_2O layer was oxidized with a solution of $FeCl_3$ (2 g) in 50% MeOH (50 ml) with stirring. The Et_2O layer was separated, and the aqueous layer was extracted twice with Et_2O (each 20 ml). The combined Et_2O extracts were worked up in the usual manner. The resulting residue was purified on a silica gel column (3 × 30 cm) with AcOEt. The fraction which showed $Rf=0.27$ on TLC using CCl_4–AcOEt (1 : 1) as a developing solvent was further purified by preparative TLC to give a yellow oil. Yield 28 mg. UV λ_{max}^{EtOH} nm ($E_{1\,cm}^{1\%}$): 275 (370). NMR ($CDCl_3$) δ: 5.0 (1H, t, CH=), 4.0 (6H, s, CH_3O), 3.36 (1H, t, CH–O), 3.2 (2H, d, ring-CH_2), 2.0 (3H, s, ring-CH_3), 2.4—2.1 (2H, m, $CH_2C=$), 1.78 (3H, s, $CH_3C=$), 1.6—1.3 (2H, m, CH_2C–O), 1.2, 1.18 (6H, s, CH_3C–O). MS *m/z*: 352 (M^+), 235 (chromenylium), 197 (benzylium).

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