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Effects of 6-(ω-Substituted Alkyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinones and Related Compounds on Mitochondrial Succinate and Reduced Nicotinamide Adenine Dinucleotide Oxidase Systems

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2,3-Dimethoxy-5-methyl-1,4-benzoquinones having an ω-hydroxyalkyl or ω-aminoalkyl group at the 6-position were synthesized. The effects of these compounds and related compounds on the respiratory system of ubiquinone-depleted mitochondrial preparations were investigated. The compounds with an alkyl side chain of 10 to 13 carbon atoms showed rather high restoration activity on antimycin-sensitive succinate oxidation in acetone-treated beef heart mitochondria. This activity was correlated with their partition coefficients. Among these compounds, 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (V-10, idebenone) was selected for further testing to determine its effect on the respiratory system of injured canine brain mitochondrial preparation, because this compound showed prominent activity in the system described above. When V-10 was added to acetone-treated canine brain mitochondria (A-CBM), antimycin- and KCN-sensitive succinate oxidation were restored to the level observed in the freeze-stored canine brain mitochondria (CBM). V-10 also restored the reduced nicotinamide adenine dinucleotide (NADH) oxidation of pentane-treated canine brain mitochondria and submitochondrial particles (P-CBM and P-CBSM). Metabolites (I-4, I-10) of V-10 in human and animals showed no restoration activity in either respiratory enzyme system.

Keywords——ubiquinone; ubiquinone metabolite; ubiquinone metabolite-related compound; idebenone; beef heart mitochondria; canine brain mitochondria; succinate oxidase system; NADH oxidase system; acetone-treated mitochondria; pentane-treated mitochondria

Ubiquinone (Q) plays an important role in the energy-conserving system of cells as a proton carrier through the Q cycle.[1] Human and animals obtain Q homologs (Q-*n*) from their diet, as well as biosynthetically; Q obtained exogenously is transported into the mitochondrial inner membrane where it functions in the energy-conserving system.[2]

Previously, we investigated the effects of Q metabolites (Q acid-I and Q acid-II) and 6-(ω-carboxyalkyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinones (I-*n*, Table I) on succinate and reduced nicotinamine adenine dinucleotide (NADH) oxidation in Q-depleted beef heart mitochondrial preparations. The respiratory activities of these compounds were found to depend on the length of the alkyl chain. The carbon numbers (*n*) of 16 to 18 were optimal for the activities of I-*n*.[3]

In this report, we describe the synthesis of 6-alkyl-2,3-dimethoxy-5-methyl-1,4-benzoquinones having a hydroxy or amino group on the terminal carbon of the alkyl chain (Table I) and the determination of their restoration activity on the respiratory system in Q-depleted mitochondrial preparations. Among these compounds, 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (V-10, idebenone) showed prominent restoration activity on succinate oxidation in beef heart mitochondria. The prophylactic effect of V-10 against cerebrovascular disorders in stroke-prone spontaneously hypertensive rats (SHRSP) has been reported.[4] Therefore, the effects of V-10 and its metabolites (I-4, I-10, XI and XII,

TABLE I. Structures of Quinone and Hydroquinone Compounds

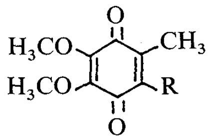
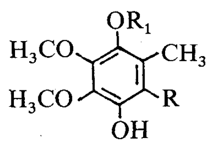
Chemical structure	R	R_1	Abbreviation and compound No.
	$(CH_2CH=C(CH_3)CH_2)_nH$		Q-*n*
	$CH_2CH=C(CH_3)CH_2CH_2COOH$		Q acid-I
	$CH_2CH_2CH(CH_3)COOH$		Q acid-II
	$(CH_2)_{n-1}COOH$		I-*n* (*n*=3, 4, 6, 8, 10, 11, 13, 16, 18, 22)
	$(CH_2CH=C(CH_3)CH_2)_nOH$		II-*n* (*n*=2, 3)
	$CH_2CH=C(CH_3)(CH_2)_3OH$		III
	$CH_2CH_2CH(CH_3)CH_2OH$		IV
	$(CH_2)_nOH$		V-*n* (*n*=1—4, 6, 10—13, 18, 20, 22)
	$(CH_2)_nOCOCH_3$		VI-*n* (*n*=6, 10—12)
	$(CH_2)_nOCH_3$		VII-*n* (*n*=1, 4, 10)
	$(CH_2)_n$N(morpholino)O		VIII-*n* (*n*=1, 10)
	$(CH_2)_nN(CH_3)_2$		IX-*n* (*n*=1, 4, 10)
	$(CH_2)_{10}OH$	H	X
	$(CH_2)_{10}OH$	SO_3K	XI
	$(CH_2)_3COOK$	SO_3K	XII

Table I)[5] on the respiratory system in injured canine brain mitochondria were investigated in order to elucidate the mechanism of activity of V-10 against cerebrovascular disorders.

Experimental

Melting points were measured with a Yanagimoto micro melting point apparatus, and are uncorrected. Nuclear magnetic resonance (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. Q-10 and Q-7 were isolated from whale heart muscle and cells of *Candida utilis*, respectively.[6] Q metabolites, I-*n*, III, IV, V-*n* (*n*=4—22), XI and XII, were synthesized in our laboratories by the methods described in previous reports.[7] Acetone, pentane (Wako Pure Chemical, special grade), OP-10 (Nikko Kogyo), cytochrome c, NADH (Sigma) and antimycin A (ICN Pharmaceuticals) were purchased. Rotenone was kindly supplied by Prof. H. Fukami (Kyoto University). Beef hearts were obtained within 1 h after slaughter. Canine brains were obtained from male or female mongrel dogs (about 10 kg weight). Mitochondria and submitochondrial particles were isolated by the usual procedures,[8] stored at $-20\,^{\circ}C$ after suspension in 0.25 M sucrose or lyophilization, and thawed or suspended in 0.25 M sucrose just before use. These preparations were treated with acetone or pentane in the usual way[9] to obtain acetone-treated heavy beef heart mitochondrial and canine brain mitochondrial preparations (A-HBHM, A-CBM) and pentane-treated beef heart submitochondrial, canine brain mitochondrial and canine brain submitochondrial preparations (P-BHSM, P-CBM, P-CBSM). Protein was determined by using the Folin–Ciocalteu reagent.[10] Succinate and NADH oxidase activities were assayed by a procedure described previously.[9] Oxygen consumption rates were measured with a Clark oxygen electrode (Gilson oxygraph, Type K-IC). Test compounds were added to a suspension of mitochondrial preparations in the form of an ethanolic solution for the assay of NADH oxidase, and in

the form of an aqueous solution containing five to ten times their weight of a detergent, OP-10, for the assay of succinate oxidase. The assay data in this paper represent means of duplicate experiments or means ± S.E. of more than triplicate experimens.

6-(8-Hydroxy-3,7-dimethyl-2,6-octadienyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (II-2)——a) 2,3-Dimethoxy-5-methylhydroquinone (XIII, 1.4 g) and 8-hydroxy-2,6-dimethyl-2,6-octadienal (XIV, 1.44 g) were dissolved in dioxane (50 ml) and a mixture of BF_3–ether (4 ml) and dioxane (8 ml) was added to the solution under stirring at room temperature. The mixture was stirred for 90 min and a solution of $FeCl_3$ (15 g) in 83% MeOH (36 ml) was added to the mixture, followed by extraction with AcOEt. The extract was worked up in a usual way and the residue was purified by column chromatography on silica gel (75 g) with benzene as the eluent. The solvent was evaporated off to give 6-(7-formyl-3-methyl-2,6-octadienyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (XV) as an orange oil. Yield 968 mg (38%). *Anal.* Calcd for $C_{19}H_{24}O_5$: C, 68.65; H, 7.28. Found: C, 68.70; H, 7.19. A solution of XV (550 mg) in MeOH (10 ml) was added to a solution of $NaBH_4$ (250 mg) in MeOH (10 ml) at 0 °C. The mixture was stirred for 15 min, then diluted with cold H_2O, and a solution of $FeCl_3$ (5.0 g) in 83% MeOH (24 ml) was added to it. The whole was extracted with AcOEt, the extract was worked up in a usual way and the residue was purified by column chromatography on silica gel (30 g) with CCl_4–AcOEt (3 : 1, v/v) as the eluent. The solvent was evaporated *in vacuo* to give II-2 as an orange oil. Yield 350 mg (63%). *Anal.* Calcd for $C_{19}H_{26}O_5$: C, 68.24; H, 7.84. Found: C, 68.07; H, 7.82. NMR ($CDCl_3$) δ: 1.64 (3H, s, CH_3), 1.75 (3H, s, CH_3), 1.98—2.24 (4H, m, CH_2), 2.02 (3H, s, CH_3 on the ring), 3.17 (2H, d, CH_2 on the ring), 3.99 (8H, s, OCH_3, $\underline{CH_2}OH$), 4.84—5.44 (2H, m, =CH).

b) A solution of the hydroquinone acetate (180 mg) of Q-2 and SeO_2 (106 mg) in 95% EtOH (8 ml) was heated at 75—80 °C for 1 h, at the end of which time the reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in CCl_4, the insoluble materials were removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (10 g) with CCl_4–AcOEt (3 : 1, v/v) as the eluent. The solvent was evaporated off to give 6-(7-formyl-3-methyl-2,6-octadienyl)-2,3-dimethoxy-5-methylhydroquinone diacetate (XVI) as a colorless oil. Yield 27 mg (15%). NMR ($CDCl_3$) δ: 1.64 (6H, s, CH_3), 2.02 (3H, s, CH_3 on the ring), 2.29 (3H, s, $COCH_3$), 2.33 (3H, s, CH_3), 3.21 (2H, d, CH_2 on the ring), 3.83 (6H, s, OCH_3), 5.04 (1H, t, =CH), 6.39 (1H, t, =CH), 9.35 (1H, s, CHO). A solution of XVI (27 mg) in Et_2O (8 ml) was added to a suspension of $LiAlH_4$ (33 mg) in Et_2O (8 ml) under ice-cooling with stirring. The mixture was stirred for 30 min and the excess $LiAlH_4$ was decomposed with cold H_2O. A solution of $FeCl_3$ (1 g) in H_2O (20 ml) was added to the reaction mixture and the whole was stirred for 30 min, then extracted with AcOEt. The extract was worked up in a usual way and the residue was purified by column chromatography on silica gel (10 g) with CCl_4–acetone (5 : 1, v/v) as the eluent. The solvent was evaporated off to give II-2 as an orange oil. Yield 18 mg (83%).

6-(12-Hydroxy-3,7,11-trimethyl-2,6,10-dodecatrienyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (II-3)——A solution of the hydroquinone acetate of Q-3 (580 mg) and SeO_2 (137 mg) in 95% EtOH (50 ml) was heated at 70—80 °C for 6 h, at the end of which time the reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in CCl_4, the insoluble materials were removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was purified by column chromatography on silica gel (30 g) with CCl_4–acetone (10 : 1, v/v) as the eluent. The solvent was evaporated *in vacuo* to give 6-(12-hydroxy-3,7,11-trimethyl-2,6,10-dodecatrienyl)-2,3-dimethoxy-5-methylhydroquinone diacetate (XVII) as a colorless oil. Yield 86 mg (14%). NMR ($CDCl_3$) δ: 1.65, 1.72 (9H, s, CH_3), 2.00—2.20 (8H, m, CH_2), 2.04 (3H, s, CH_3 on the ring), 2.29 (3H, s, $COCH_3$), 2.32 (3H, s, $COCH_3$), 3.20 (2H, d, CH_2 on the ring), 3.81 (6H, s, OCH_3), 3.94 (2H, s, $\underline{CH_2}OH$), 4.8—5.5 (3H, m, =CH). A solution of XVII (86 mg) in Et_2O (5 ml) was added to a suspension of $LiAlH_4$ (34 mg) in Et_2O (4 ml) under ice-cooling. The mixture was stirred for 30 min, then the excess $LiAlH_4$ was decomposed with cold H_2O. A solution of $FeCl_3$ (0.8 g) in H_2O (10 ml) was added to the reaction mixture and the mixture was stirred for 30 min, and extracted with AcOEt. The extract was worked up in a usual way and the residue was purified by column chromatography on silica gel (8 g) with CCl_4–acetone (10 : 1, v/v) as the eluent. The solvent was evaporated off to give II-3 as an orange oil. Yield 26 mg (37%). NMR ($CDCl_3$) δ: 1.66 (6H, s, CH_3), 1.73 (3H, s, CH_3), 1.98—2.26 (8H, m, CH_2), 2.02 (3H, s, CH_3 on the ring), 3.17 (2H, d, CH_2 on the ring), 3.98 (8H, s, OCH_3, $\underline{CH_2}OH$), 4.70—5.55 (3H, m, =CH).

6-(ω-Hydroxyalkyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinones (V-*n*, *n* = 1—3)——a) Diacetoxyacetylperoxide (XIX) [prepared from acetoxyacetyl chloride[11] (28 g) and Na_2O_2] was added portionwise to a solution of 2,3-dimethoxy-5-methyl-1,4-benzoquinone (XVIII, 9 g) in AcOH (60 ml) at 90 °C over a period of 30 min. After being stirred for 2 h, the reaction mixture was subjected to column chromatography in a usual way to afford V-1 acetate (VI-1) as a yellow oil. Yield 963 mg (7.7%). NMR ($CDCl_3$) δ: 2.07 (3H, s, CH_3 on the ring), 2.12 (3H, s, $COCH_3$), 4.03 (6H, s, OCH_3), 5.00 (2H, s, CH_2). VI-1 (963 mg) was hydrolyzed with conc. HCl (0.6 ml) and MeOH (50 ml) at room temperature for 18 h to give V-1 as orange crystals. Yield 364 mg (45.3%). NMR ($CDCl_3$) δ: 2.08 (3H, s, CH_3 on the ring), 2.65 (1H, d, J = 7 Hz, OH), 3.98 (6H, s, OCH_3), 4.48 (2H, d, J = 7 Hz, CH_2).

b) A solution of XIII prepared from XVIII (2.2 g), 37% formalin (2.4 ml) and morpholine (2.4 ml) in dioxane (11 ml) was heated at 100 °C for 2 h. A saturated aqueous solution of NaCl was added to the reaction mixture, and the aqueous solution was extracted with $CHCl_3$. The extract was worked up in a usual way and the product was recrystallized from Et_2O–hexane to give 2,3-dimethoxy-5-methyl-6-morpholinomethylhydroquinone (XX). Yield 2.23 g (65.7%). mp 127—130 °C. *Anal.* Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94. Found: C, 59.66; H, 7.44; N,

TABLE II. Physicochemical Properties and Analytical Data of Quinone Compounds

Compd. No.	mp (°C)	Recryst. solvent	Formula	Analysis (%) Calcd (Found)		
				C	H	N
V-1	53—55	Et_2O–hexane	$C_{10}H_{12}O_5$	56.60	5.70	
				(56.40	5.64)	
V-2	Oil		$C_{11}H_{14}O_5$	58.40	6.24	
				(58.51	6.27)	
V-3	Oil		$C_{12}H_{16}O_5$	59.99	6.71	
				(59.97	6.57)	
VII-1	36	Et_2O–hexane	$C_{11}H_{14}O_5$	58.40	6.24	
				(58.61	6.11)	
VII-4	Oil		$C_{14}H_{20}O_5$	62.67	7.51	
				(62.36	7.45)	
VII-10	Oil		$C_{20}H_{32}O_5$	68.15	9.15	
				(68.18	9.17)	
VIII-1·HCl	163—165	MeOH–Et_2O	$C_{14}H_{19}NO_5 \cdot HCl$	52.91	6.34	4.41
				(52.80	6.48	4.35)
VIII-10·HCl	101—103	MeOH–Et_2O	$C_{23}H_{37}NO_5 \cdot HCl$	62.22	8.63	3.16
				(61.98	8.33	3.03)
IX-1·HCl	141—143	MeOH–Et_2O	$C_{12}H_{17}NO_4 \cdot HCl$	52.27	6.58	5.08
				(52.06	6.58	4.93)
IX-4·HCl	125	MeOH–Et_2O	$C_{15}H_{23}NO_4 \cdot HCl$	56.69	7.61	4.41
				(56.50	7.50	4.40)
IX-10·HCl	Powder	MeOH–Et_2O	$C_{21}H_{35}NO_4 \cdot HCl \cdot 1/10\,H_2O$	62.47	9.04	3.47
				(62.13	8.66	3.41)

5.00. XX (1.73 g) was converted to 2,5-diacetoxy-1-acetoxymethyl-3,4-dimethoxy-6-methylbenzene (XXI) by treatment with Ac_2O (20 ml), AcOH (2 ml), AcONa (2 g) and a small amount of Zn powder at 130—150 °C for 7 h. Yield 1.80 g (72.9%). *Anal.* Calcd for $C_{16}H_{20}O_8$: C, 56.46; H, 5.92. Found: C, 56.38; H, 5.99. NMR ($CDCl_3$) δ: 2.05 (3H, s, $COCH_3$), 2.17 (3H, s, CH_3 on the ring), 2.32 (6H, s, $OCOCH_3$ on the ring), 3.78 (6H, s, OCH_3), 5.03 (2H, s, CH_2). Compound XXI (7.4 g) was reduced with $LiAlH_4$ and the resulting hydroquinone compound was oxidized with $FeCl_3$ to afford V-1. Yield 2.2 g (49%).

c) 2,5-Diacetoxy-3,4-dimethoxy-6-methylphenylacetaldehyde[7a] (XXII, 397 mg) was reduced with $LiAlH_4$ and the resulting hydroquinone derivative was oxidized with $FeCl_3$ to afford V-2 as an orange oil. Yield 205 mg (70.9%). NMR ($CDCl_3$) δ: 2.05 (3H, s, CH_3), 2.67 (2H, t, $J=7$ Hz, CH_2 on the ring), 3.12 (1H, br, OH), 3.68 (2H, t, $J=6$ Hz, CH_2O), 3.96 (6H, s, OCH_3).

d) The hydroquinone derivative of the methyl ester (XXIII) of I-3[7a] was reduced with $LiAlH_4$ and then oxidized with $FeCl_3$ to afford V-3 as an orange oil.

The experimental data for V-*n* ($n=1$, 2, 3) are summarized in Table II.

2,3-Dimethoxy-6-(ω-methoxyalkyl)-5-methyl-1,4-benzoquinone (VII-*n*)——a) Solvolysis of XXI with HCl–MeOH followed by oxidation with $FeCl_3$ yielded orange-red crystals of 2,3-dimethoxy-6-methoxymethyl-5-methyl-1,4-benzoquinone (VII-1). NMR ($CDCl_3$) δ: 2.08 (3H, s, CH_3 on the ring), 3.33 (3H, s, OCH_3), 3.97 (6H, s, OCH_3), 4.28 (2H, s, CH_2).

b) A mixture of 60% NaH in mineral oil (0.3 g) and CH_3I (0.56 ml) in 1,2-dimethoxyethane (DME) (2 ml) was stirred at 40 °C for 15 min. A solution of the hydroquinone derivative of V-4[7a] (500 mg) in DME (2 ml), prepared by the reduction of V-4 with $Na_2S_2O_4$, was added to the above mixture. Further NaH and CH_3I were added at 45—60 °C and the reaction mixture was stirred for 90 min. The reaction mixture was diluted with dil. HCl and the organic phase was separated. The aqueous phase was extracted with Et_2O. The organic phase and Et_2O extract were combined, treated as usual, and chromatographed on silica gel to yield 1,2,3,4-tetramethoxy-6-(4-methoxybutyl)-5-methylbenzene (XXIV-4). *Anal.* Calcd for $C_{16}H_{26}O_5$: C, 64.40; H, 8.76. Found: C, 64.43; H, 8.49. NMR ($CDCl_3$) δ: 1.43—2.35 (4H, m, CH_2), 2.13 (3H, s, CH_3 on the ring), 2.53 (2H, t, $J=6$ Hz, CH_2 on the ring), 3.30 (3H, s, $CH_2\underline{OCH_3}$), 3.37 (2H, t, $J=6$ Hz, $\underline{CH_2}OCH_3$), 3.73 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.87 (6H, s, OCH_3). AgO (500 mg) and 6 N HNO_3 (1.2 ml) were added to a solution of XXIV-4 (400 mg) in tetrahydrofuran (THF, 20 ml) and the mixture was stirred at 0 °C for 5 min. Further AgO (500 mg) and 6 N HNO_3 (0.5 ml) were added to the reaction mixture. After being stirred for 10 min, the reaction mixture was diluted with H_2O and extracted with AcOEt. The

extract was treated as usual and chromatographed on silica gel. The fraction eluted with CCl_4–AcOEt (19 : 1) afforded 2,3-dimethoxy-6-(4-methoxybutyl)-5-methyl-1,4-benzoquinone (VII-4) as an orange oil. Yield 189 mg (49%). NMR ($CDCl_3$) δ: 1.37—1.67 (4H, m, CH_2), 2.00 (3H, s, CH_3 on the ring), 2.48 (2H, t, J=6 Hz, CH_2 on the ring), 3.33 (3H, s, $CH_2O\underline{CH_3}$), 3.38 (2H, t, J=5 Hz, $\underline{CH_2}OCH_3$), 3.98 (6H, s, OCH_3).

c) 1,2,3,4-Tetramethoxy-6-(10-methoxydecyl)-5-methylbenzene (XXIV-10) was obtained in a manner similar to that used for XXIV-4. *Anal.* Calcd for $C_{22}H_{38}O_5$: C, 69.07; H, 10.01. Found: C, 69.09; H, 10.17. NMR ($CDCl_3$) δ: 1.13—1.72 (16H, m, CH_2), 2.15 (3H, s, CH_3 on the ring), 2.50 (2H, br, CH_2 on the ring), 3.32 (3H, s, $CH_2O\underline{CH_3}$), 3.33 (2H, t, J=6 Hz, $\underline{CH_2}OCH_3$), 3.75 (6H, s, OCH_3), 3.83 (6H, s, OCH_3). Compound XXIV-10 was reacted in a manner similar to that described for VII-4 to give 2,3-dimethoxy-6-(10-methoxydecyl)-5-methyl-1,4-benzoquinone (VII-10) as an orange oil. Yield 52 mg (46%). NMR ($CDCl_3$) δ: 1.10—1.67 (16H, m, CH_2), 2.02 (3H, s, CH_3 on the ring), 2.43 (2H, br, CH_2 on the ring), 3.32 (3H, s, $CH_2O\underline{CH_3}$), 3.33 (2H, t, J=5 Hz, $\underline{CH_2}OCH_3$), 3.98 (6H, s, OCH_3).

The experimental data for VII-*n* (n=1, 4, 10) are summarized in Table II.

2,3-Dimethoxy-5-methyl-6-(ω-morpholinoalkyl)-1,4-benzoquinone (VIII-*n*)——a) Compound XX described in section b) on the synthesis of V-1 was oxidized with Ag_2O in a mixture of Et_2O–dioxane (5 : 2). The reaction mixture was treated in a usual way and the product was recrystallized from AcOEt–hexane to yield 2,3-dimethoxy-5-methyl-6-morpholinomethyl-1,4-benzoquinone (VIII-1). mp 60—62 °C. NMR ($CDCl_3$) δ: 2.10 (3H, s, CH_3 on the ring), 2.42 (4H, t, J=4 Hz, NCH_2), 3.37 (2H, s, CH_2 on the ring), 3.62 (4H, t, J=4 Hz, OCH_2), 4.00 (6H, s, OCH_3). The free base of VIII-1 was converted to the hydrochloride in a usual manner to give VIII-1·HCl as orange needles.

b) A solution of V-10 (3.38 g) in dry dichloromethane (20 ml) was added to a suspension of pyridinium chlorochromate (3.3 g) and AcONa (500 mg) in dry dichloromethane (20 ml) at room temperature. The mixture was stirred for 1 h, then further pyridinium chlorochromate (1 g) and AcONa (500 mg) were added, and the whole was stirred for 1.5 h, then poured into ice–H_2O. The aqueous solution was extracted with AcOEt. The extract was treated in a usual way and subjected to chromatography on silica gel. The product was recrystallized from AcOEt–hexane to afford orange-yellow needles of XXV. mp 43—44 °C. Yield 1.3 g (38.7%). *Anal.* Calcd for $C_{19}H_{28}O_5$: C, 67.83; H, 8.39. Found: C, 67.73; H, 8.27. NMR ($CDCl_3$) δ: 1.33 (14H, br, CH_2), 1.98 (3H, s, CH_3 on the ring), 2.23—2.67 (4H, m, CH_2 on the ring and $\underline{CH_2}CHO$), 3.95 (6H, s, OCH_3), 9.62 (1H, s, CHO). Compound XXV (900 mg) was added portionwise over a period of 1 h at 60—80 °C to a solution of morpholine formate (430 mg) in MeOH (5 ml) prepared from equimolar amounts of morpholine and formic acid.

Further morpholine formate (200 mg) was added and the mixture was stirred for 1 h. The reaction mixture was poured into ice–H_2O. The aqueous solution was extracted with AcOEt. The extract was treated as usual and chromatographed on silica gel. From the fraction eluted with hexane–AcOEt (4 : 1), VIII-10 (430 mg, 41.9%) was obtained as an orange oil. This oil was converted into orange crystals of the hydrochloride (VIII-10·HCl) by treatment with HCl–MeOH. Yield 240 mg (51.2%). NMR ($CDCl_3$) δ: 1.34 (16H, br, CH_2), 1.98 (3H, s, CH_3 on the ring), 2.45 (2H, t, J=5 Hz, CH_2 on the ring), 3.00—3.50 (6H, m, NCH_2), 3.95 (10H, s, OCH_3 and OCH_2).

The experimental data for VIII-*n* (n=1, 10) are summarized in Table II.

6-(ω-Dimethylaminoalkyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (IX-*n*)——a) A mixture of 40% aqueous dimethylamine (1.2 ml) and 10% aqueous formalin (3.3 ml) was added to a solution of XIII (1.84 g; prepared from XVIII) in dioxane (15 ml) at 80—90 °C, and the whole was stirred for 3 h, then more of the mixture (0.5 ml) of dimethylamine and formalin was added. The reaction mixture was stirred at the same temperature for 1 h, then poured into ice–H_2O. The aqueous solution was extracted with $CHCl_3$. The $CHCl_3$ solution was extracted with dil. HCl. The acidic solution was made alkaline and extracted with $CHCl_3$, the extract was concentrated, and then the residue was dissolved in Et_2O (40 ml) and stirred with Ag_2O (2 g). The reaction mixture was treated as usual. The resulting oil was dissolved in $CHCl_3$, and the $CHCl_3$ solution was extracted with dil. HCl. The aqueous solution was made alkaline with $NaHCO_3$ and extracted with $CHCl_3$. The solvent was evaporated off to give IX-1 as an orange oil. NMR ($CDCl_3$) δ: 2.10 (3H, s, CH_3 on the ring), 2.23 (6H, s, NCH_3), 3.30 (2H, s, CH_2 on the ring), 4.00 (6H, s, OCH_3). HCl–MeOH was added to an Et_2O solution of IX-1 and the mixture was concentrated. Recrystallization of the residue from MeOH–Et_2O gave orange crystals of IX-1·HCl. Yield 1.45 g (52.6%).

b) A solution of the hydroquinone derivative (2.3 g; prepared from XXV in a usual manner) in MeOH (8 ml) was added to a solution of dimethylamine formate (900 mg) in MeOH (8 ml) at 60 °C. The mixture was heated at 80—90 °C for 3 h, then further dimethylamine formate (400 mg) was added, and the whole was stirred for 30 min, then poured into ice–H_2O. The aqueous solution was made acidic with dil. HCl and extracted with Et_2O to remove unreacted starting material. The aqueous solution was extracted with $CHCl_3$. The residue (2.5 g) obtained upon removal of the solvent was dissolved in H_2O (50 ml) and shaken with a solution of $FeCl_3$ (3 g) in H_2O (20 ml). The aqueous solution was extracted with $CHCl_3$ and purified by column chromatography on silica gel to give IX-10·HCl as a yellow powder. Yield 1.97 g (71.6%). NMR ($CDCl_3$) δ: 1.33 (16H, br, CH_2), 2.00 (3H, s, CH_3 on the ring), 2.40 (2H, m, CH_2 on the ring), 2.85 (6H, s, NCH_3), 2.93—3.23 (2H, m, CH_2N), 3.93 (6H, s, OCH_3).

c) A mixture of 6-(3-carboxypropyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (I-4, 1 g), Zn powder (1.22 g), Ac_2O (10 ml) and pyridine (1 ml) was stirred at room temperature overnight. The insoluble precipitates were removed by filtration, and the filtrate was concentrated *in vacuo* then diluted with H_2O. After being stirred overnight, the aqueous solution was extracted with AcOEt. The solvent was removed and the resulting oil was chromatographed on

silica gel. Recrystallization from AcOEt–hexane gave 4-(2,5-diacetoxy-3,4-dimethoxy-6-methylphenyl)butyric acid (XXVI) as colorless needles. Yield 475 mg (36.0%). mp 125—127 °C. *Anal.* Calcd for $C_{17}H_{22}O_8$: C, 57.62; H, 6.26. Found: C, 57.59; H, 6.21. Thionyl chloride (2 ml) was added to XXVI (323 mg) and the mixture was stirred at room temperature for 1 h and then at 80 °C for 1 h. The excess of $SOCl_2$ was removed *in vacuo* to yield the crude acid chloride derivative of XXVI. The product was subjected to the next step without further purification. A solution of the acid chloride derivative of XXVI in benzene was added under ice-cooling to a solution of dimethylamine in benzene. After being stirred at room temperature for 18 h, the reaction mixture was diluted with H_2O. The aqueous solution was extracted with AcOEt and worked up in a usual manner to give 4-(2,5-diacetoxy-3,4-dimethoxy-6-methylphenyl)-*N*,*N*-dimethylbutanamide (XXVII). The reduction of XXVII with $LiAlH_4$ followed by oxidation afforded IX-4. HCl–MeOH was added to a solution of IX-4 in MeOH. IX-4·HCl was obtained as orange needles from MeOH–Et_2O. NMR ($CDCl_3$) δ: 1.40—1.88 (4H, m, CH_2), 2.02 (3H, s, CH_3 on the ring), 2.20—3.31 (4H, m, CH_2), 2.78 (6H, s, NCH_3), 3.98 (6H, s, OCH_3).

The experimental data for IX-*n* ($n=1$, 4, 10) are summarized in Table II.

Results

New compounds tested in this study were synthesized by the methods shown in Charts 1 and 2. II-2 and II-3 were prepared by selective oxidation of the terminal methyl group of the isoprenyl chain of Q-2 and Q-3, respectively. Since the synthesis of V-1 from glycolic acid gave only a low yield, a morpholino methyl derivative (XX) obtained by the Mannich reaction of the hydroquinone derivative of XVIII was acetylated, reduced and then oxidized to obtain V-1. V-2 was obtained by the reduction of the phenylacetaldehyde compound (XXII) with lithium aluminum hydride followed by oxidation. V-3 was synthesized in the usual way[7a] from the methyl ester of I-3. The methyl ether, VII-1, was obtained by methanolysis of XXI, followed by oxidation with $FeCl_3$, and VII-4 (as well as VII-10) was obtained by the methylation of the corresponding hydroxy derivative, followed by oxidation with argentic oxide. Morpholino derivatives (VIII-*n*) were obtained by the oxidation of an intermediate (XX) obtained by the Mannich reaction as described above, or by the Leukart–Wallach reaction of the aldehyde derivatives (XXV). Dimethylamino derivatives (IX-1 and IX-10) were synthesized by the Mannich reaction of XVIII, and the Leukart–Wallach reaction of XXV, respectively. The amide derivative (XXVII) obtained from the carboxylic acid derivative (XXVI) was reduced, then oxidized to obtain IX-4.

Lester and Fleischer[9a] reported that succinate oxidation in acetone-treated heavy beef heart mitochondria (A-HBHM) was specifically restored by Q homologs (Q-*n*). Thereafter, Szarkowska,[9b] and Ernster *et al.*[9c] reported that NADH oxidation in pentane-treated

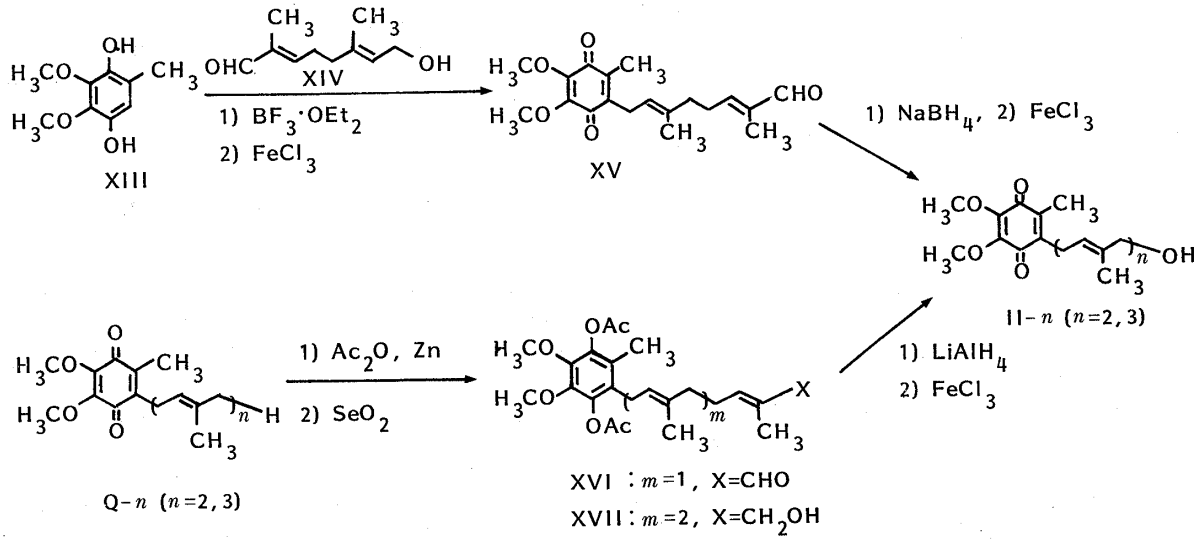


Chart 1

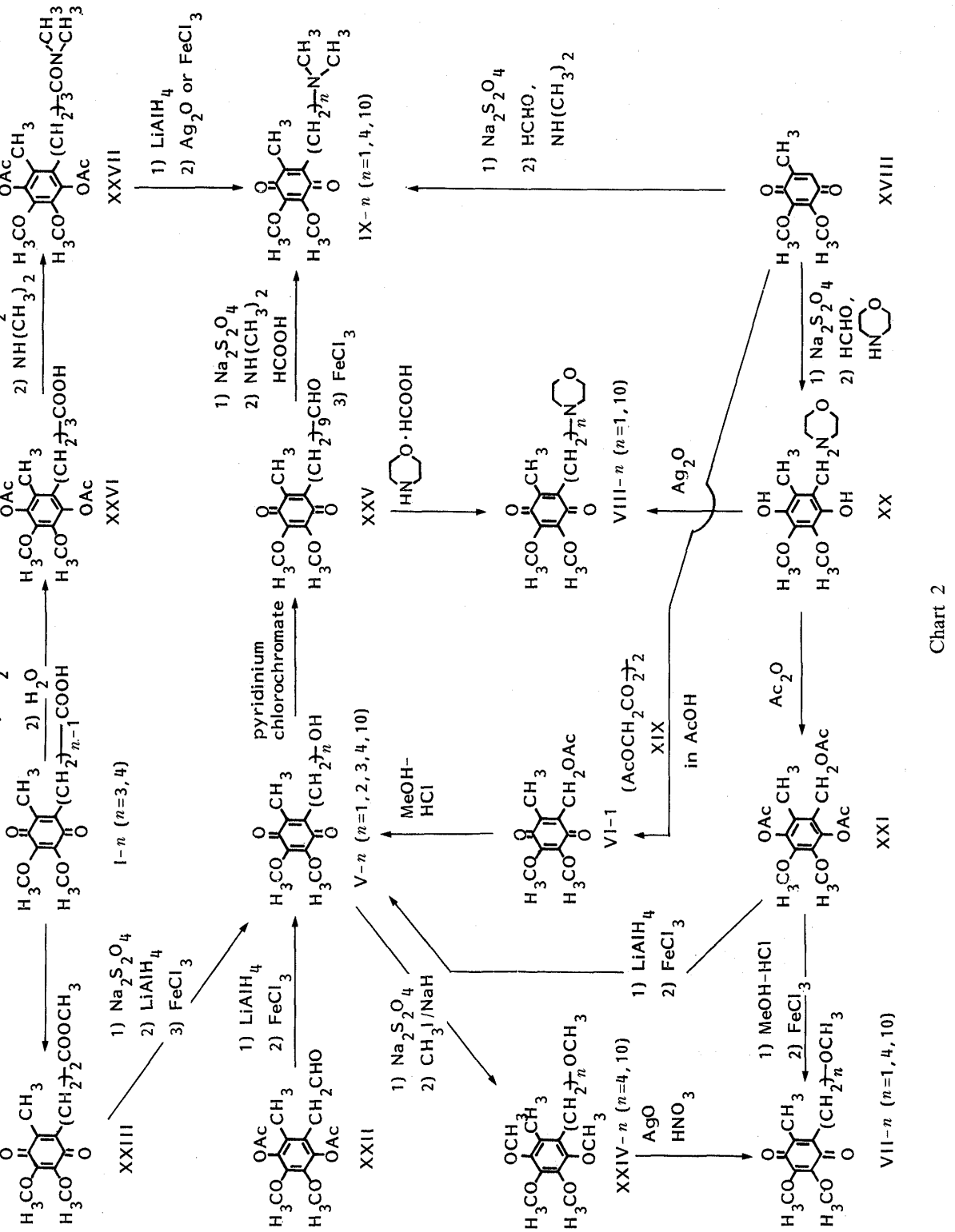


Chart 2

HBHM (P-HBHM) and pentane-treated beef heart submitochondrial particles (P-BHSM) was restored by the addition of Q.

When added to A-HBHM in the form of an aqueous solution containing a detergent, Q- or Q metabolite-related compounds (II-2, II-3, III and IV) which have a hydroxy group at the terminal methyl group of the prenyl chain showed activity to restore succinate oxidation. This activity was antimycin-sensitive, which indicates mediation through the activity of cytochrome b region. The restoration activities depended upon the carbon number (*n*) of the alkyl chain, and the order of the activity at a concentration of 10 nmol/mg protein was II-3

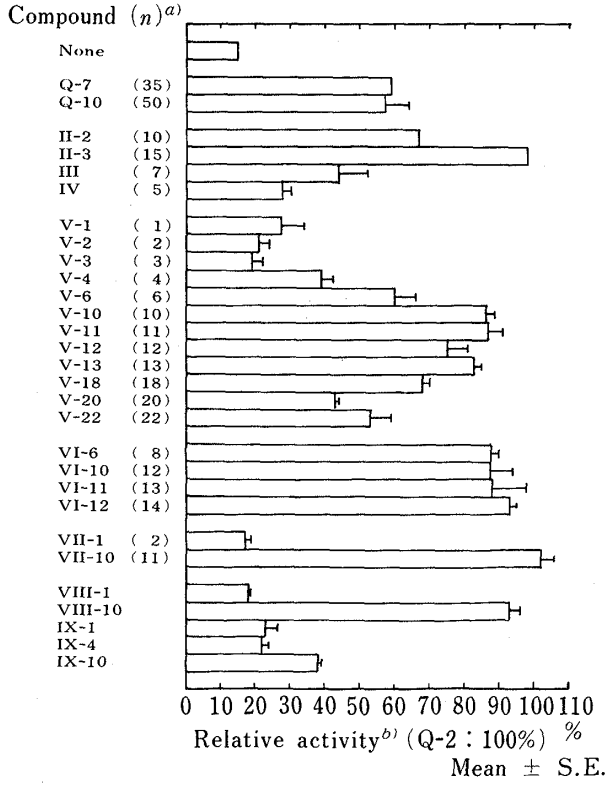


Fig. 1. Restoration of Succinate Oxidase Activity in Acetone-Treated Heavy Beef Heart Mitochondrial Preparation (A-HBHM) with V-*n* and Related Compounds

Rates of oxygen consumption were measured with a Clark oxygen electrode as described in Experimental. The reaction mixture consisted of 200 mM sucrose, 10 mM Tris–HCl (pH 7.4), 20 mM KCl, 3 mM $MgCl_2 \cdot 6H_2O$, 50 μM ethylenediaminetetraacetate·2Na (EDTA·2Na), 100 μg of cytochrome c, 2.5 mM potassium succinate, 1.0 mg protein of A-HBHM and a solution of a test compound (each 10 nmol/mg protein) containing OP-10, or a solution of OP-10 (50 μg) as a control. Final volume, 2 ml; temperature, 25—27 °C.

a) *n*: Carbon number of side chain. *b*) To compare the activities of various related compounds, Q-2 was assayed as the standard in every three to five assays, and the activity was expressed as per cent of activity of Q-2. The activity of Q-2 was 110.38 ± 5.20 ng atom oxygen/min per mg protein.

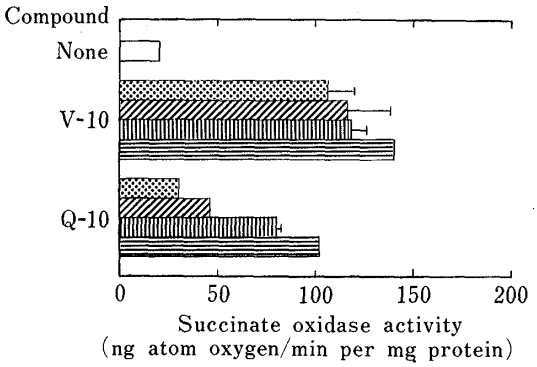


Fig. 2. Restoration of Succinate Oxidase Activity in Acetone-Treated Heavy Beef Heart Mitochondrial Preparation (A-HBHM) with V-10 and Q-10

Assay details were as described in Fig. 1. The oxidase activity of lyophilized heavy beef heart mitochondria was 154 ng atom oxygen/min per mg protein. ▒: 1.3×10^{-6} M, ▨: 2.5×10^{-6} M, ▥: 5×10^{-6} M, ▤: 1×10^{-5} M.

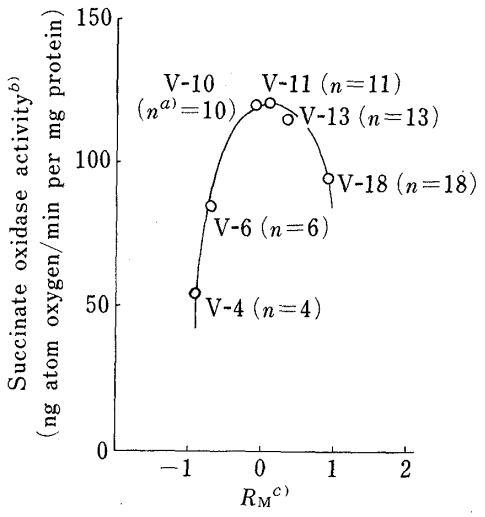


Fig. 3. The Relationship between Succinate Oxidase Activity of V-*n* and the R_M Values

a) Carbon number of side chain. *b*) Assay details were as described in Fig. 1. *c*) $R_M = \log(1/Rf - 1)$. *Rf* values were determined by paraffin-treated thin layer chromatography developed with acetone–H_2O (1.4 : 1, v/v).

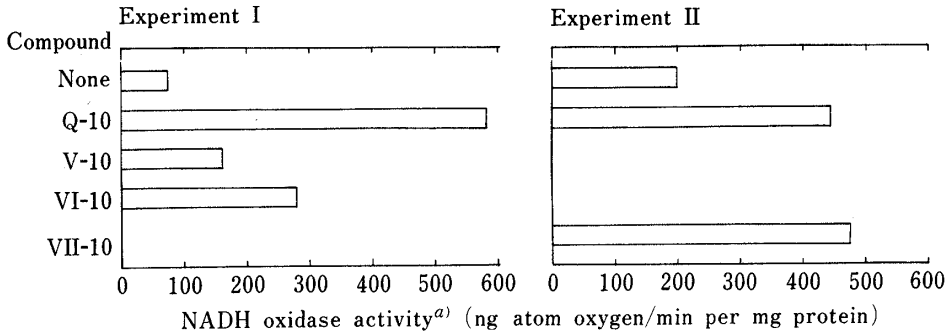


Fig. 4. Restoration of NADH Oxidase Activity in Pentane-Treated Beef Heart Submitochondrial Particles (P-BHSM) with Q-10, V-10 and Related Compounds [each 5×10^{-5} M (Expt. I), 5×10^{-6} M (Expt. II)]

Rates of oxygen consumption were measured with a Clark oxygen electrode as described in Experimental. The reaction mixture consisted of 167 mM sucrose, 50 mM Tris–HCl (pH 7.4), 100 μg of cytochrome c, 0.5 mM NADH, 0.25 mg protein of P-BHSM and an ethanol solution of a test compound. Final volume, 2 ml; temperature, 30 °C.

a) The NADH oxidase activities of lyophilized beef heart submitochondrial particles were 491 ± 34 (Expt. I) and 544 (Expt. II) ng atom oxygen/min per mg protein.

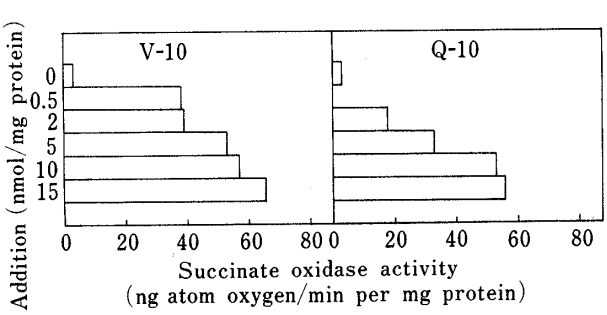


Fig. 5. Restoration of Succinate Oxidase Activity in Acetone-Treated Canine Brain Mitochondrial Preparation (A-CBM) with Various Concentrations of V-10 and Q-10

The reaction mixture consisted of 167 mM sucrose, 50 mM Tris–HCl (pH 7.4), 100 μg of cytochrome c, 2.5 mM potassium succinate, 1.0 mg protein of A-CBM and a solution of a test compound containing OP-10, or a solution of OP-10 (50 μg) as a control. Final volume, 2 ml; temperature, 30 °C.

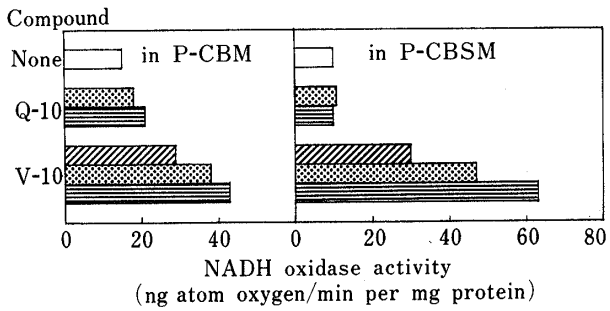


Fig. 6. Restoration of NADH Oxidase Activity in Pentane-Treated Canine Mitochondrial Preparations (P-CBM and P-CBSM) with V-10 and Q-10

Assay details were as described in Fig. 4, except for protein; 1 mg protein of P-CBM or 0.6 mg protein of P-CBSM was added to the reaction mixture. ▨: 1.3×10^{-5} M, ▩: 2.5×10^{-5} M, ▤: 5.0×10^{-5} M.

($n=15$) > II-2 ($n=10$) > III ($n=7$) > IV ($n=5$) (Fig. 1). The hydroxyalkyl analogs (V-*n*), their acetates (VI-*n*) and methyl ethers (VII-*n*) as well as the morpholino derivatives (VIII-*n*) also showed restoration activity (Fig. 1). The optimal carbon numbers (*n*) for the restoration of the activity were 10 to 13. The restoration by these compounds was stronger than those by I-*n*.[3] The dimethyl amino compounds (IX-*n*) slightly restored the activity. The activity of V-10 was higher than that of Q-10, especially at low concentrations (Fig. 2). We determined the R_M values[12] of V-*n* as the index of lipophilicity in order to investigate the relation between restoration activity and partition coefficient. The optimal carbon numbers (*n*) of V-*n* for the activity were correlated with the R_M values (Fig. 3), as observed in the case of I-*n*.[3]

The restroration of NADH oxidase activity with V-10 and its derivatives was investigated by using P-BHSM. The restoration activities of VII-10 and VI-10 were 80% and 50%, respectively, of that of the untreated preparation (Fig. 4).

Then, we investigated the effects of V-10 and its metabolites (I-10, I-4, XI and XII)[5] on

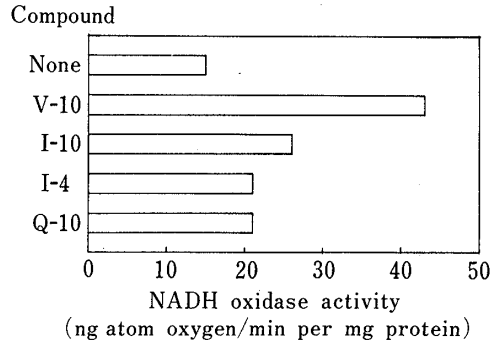


Fig. 7. Restoration of NADH Oxidase Activity in Pentane-Treated Canine Brain Mitochondrial Preparation (P-CBM) with Q-10, V-10 and Related Compounds (each 100 nmol/mg protein)

Assay details were as described in Fig. 4 except for protein content of the mitochondrial preparation; 1 mg protein of P-CBM was used instead of 0.25 mg protein of P-BHSM.

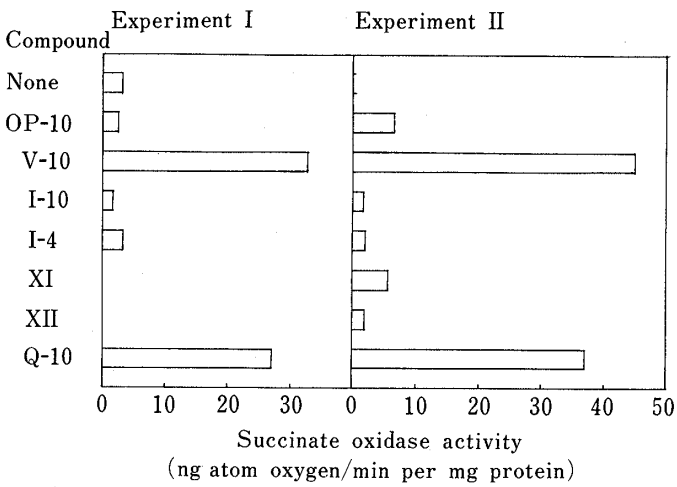


Fig. 8. Restoration of Succinate Oxidase Activity in Acetone-Treated Canine Brain Mitochondrial Preparation (A-CBM) with Q-10, V-10 and Related Compounds (each 10 nmol/mg protein)

Assay details were as described in Fig. 5.

the respiratory system in injured canine brain mitochondria to elucidate the mechanism of their activity against cerebrovascular disorders. The succinate oxidation in the acetone-treated canine brain mitochondria (A-CBM) was restored with V-10, and the restroration activity was higher than that of Q-10 at low concentrations (Fig. 5), as in the case of A-HBHM. This restoration activity was inhibited by antimycin A and KCN, indicating that the activity was mediated through the cytochrome system. V-10 also restored NADH oxidation in pentane-treated canine brain mitochondrial preparations (P-CBM, P-CBSM) (Fig. 6), though these activities were hardly inhibited by rotenone and inhibitors which are specific for the oxidoreductase of the cytochrome system. Metabolites of V-10 tested did not show substantial restoration activities on either oxidase system of injured canine brain mitochondrial preparations (Figs. 7 and 8).

Discussion

The structure–activity relation of Q has been investigated.[13] The 2,3-dimethoxy-5-methyl-1,4-benzoquinone moiety is essential for the mitochondrial electron transport activity, and the short chain homologs of Q showed strong activity in the succinate oxidase system but not in the NADH oxidase system. Lenaz *et al.*[14] considered that, unlike the long chain homologs, the short chain homologs are less able to reach the active site of NADH oxidase because they are less hydrophobic. Further, the hydroquinones of the short chain homologs are hardly oxidized by ubiquinol-cytochrome c reductase, especially in the NADH oxidase system.[15] In an attempt to obtain compounds that have electron transport activity, we synthesized 2,3-dimethoxy-5-methyl-1,4-benzoquinones having an ω-hydroxyalkyl or ω-aminoalkyl group at the 6-position (Table I), and found that their electron transport activities in succinate oxidation of A-HBHM were greater than that of Q-10 especially at low concentrations (Figs. 1 and 2). These activities were found to be correlated with hydrophobicity (shown as R_M values in Fig. 3). On the other hand, we could not find such a correlation in the case of the NADH oxidase activity of P-BHSM (data not shown). The

structural requirement for the mitochondrial NADH oxidation remains to be studied, but it can at least be said that hydrophobicity is not the only factor important for the restoration of NADH oxidation in P-BHSM. *In vivo* activity of VIII-10 is interesting, because water-soluble Q analogs which have electron transport activity *in vitro* have not yet been reported.

Nagaoka *et al.*[4] searched for drugs which would improve cellular energy metabolism in the brain, using SHRSP in addition to the conventional screening method, and they found that V-10 protects against cerebrovascular disorders. Therefore we investigated the effects of V-10 and its metabolites on the respiratory system in the brain tissue using canine brain mitochondria (CBM). As in the cases of HBHM and BHSM, we treated CBM and CBSM with organic solvents to obtain injured mitochondrial preparations. V-10 substantially restored not only the succinate oxidase activity (Fig. 5) of A-CBM, but also the NADH oxidase activity (Fig. 6) of P-CBM and P-CBSM, and in this respect it differs markedly from the short chain homologs of Q. The restoration of NADH oxidase activity in P-CBM or P-CBSM with V-10 was not fully sensitive to several inhibitors of the NADH oxidase system, and the role of V-10 in this system remains to be studied. V-10, when added to the mitochondrial preparation, is reduced to its hydroquinone form by both mitochondrial oxidases.[16] From these results, it is considered that the protective effects of V-10 on cerebrovascular disorders are due to its restoration activities on the succinate and NADH oxidase systems of injured brain mitochondria, in addition to the antioxidant activity[17] of the hydroquinone (X) of V-10 against membranous lipid peroxidation.

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