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Synthesis and Biological Activities of 2,3-Dimethyl-1,4-benzoquinones Having Alkylthio and Arylthio Side Chains

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New 2,3-dimethyl-1,4-benzoquinones having an alkylthio or arylthio side chain at the 5-position and two alkylthio side chains at the 5- and 6-position were synthesized as possible antimetabolites of coenzyme Q. These compounds were tested for inhibition of coenzyme Q in mitochondrial succinoxidase and reduced nicotinamide adenine dinucleotide (NADH)-oxidase systems, and were found to show greater inhibition of the NADH-oxidase system than of the succinoxidase system. 5,6-Di-octylthio-2,3-dimethyl-1,4-benzoquinone showed greater inhibitory activities than 5-alkylthio-2,3-dimethyl-1,4-benzoquinones. 5-Arylthio-2,3-dimethyl-1,4-benzoquinones showed potent inhibitory activities towards both enzyme systems.

Keywords——coenzyme Q analog; 5-alkylthio-2,3-dimethyl-1,4-benzoquinone; 5-arylthio-2,3-dimethyl-1,4-benzoquinone; succinoxidase; NADH-oxidase; 5,6-dialkylthio-2,3-dimethyl-1,4-benzoquinone

Coenzyme Q (ubiquinone, CoQ) has a 2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone structure, and has been reported to play an important role in the electron transport system in the mitochondria. Many kinds of coenzyme Q analogs that act as antagonists have been synthesized.[1-10] They inhibit succinoxidase and reduced nicotinamide adenine dinucleotide (NADH)-oxidase *in vitro* and show antitumor activity *in vivo*.[4,5] Alkyl- or arylthio derivatives of 2,3-dimethoxy-1,4-benzoquinone,[6,7] 5-methyl-2,3-dimethoxy-1,4-benzoquinone,[6,8] 2-hydroxy-1,4-naphthoquinone,[9] and 6-hydroxy-quinolinequinone[10] were obtained by the reaction of quinones with thiols in ethanol. Studies of the biological activities of these analogs have indicated that an alkylthio side chain with a long carbon chain and an arylthio side chain are the effective substituents of CoQ antagonists. This paper describes the synthesis and biological activities of new 2,3-dimethyl-1,4-benzoquinones having an alkylthio or arylthio side chain at the 5-position and two alkylthio side chains at the 5- and 6-positions.

Results and Discussion

Synthesis of 2,3-Dimethyl-1,4-benzoquinones Having Alkylthio and Arylthio Side Chains

New 5-alkylthio and 5-arylthio-2,3-dimethyl-1,4-benzoquinones were prepared by treat-

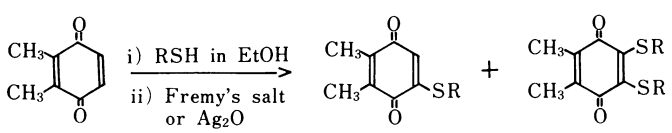

Chart 1

ing 2 eq of 2,3-dimethyl-1,4-benzoquinone (I) in ethanol with a hexane solution of 1 eq of the alkyl or arylthiol by the method shown in Chart 1. The addition of the thiol to the quinone (I) proceeded readily, and the reaction mixture containing the alkylthio- or arylthio-hydroquinone was oxidized with Fremy's salt to give the quinone (II—IV, VI, or VII). The

TABLE I. Effects of Quinones on Succinoxidase and NADH-Oxidase Activities in Beef Heart Mitochondria

Compd. (No.)	Succinoxidase			NADH-oxidase		
	Concentration[a]	Relative enzyme activity[b]	Antimetabolite CoQ index[c]	Concentration[a]	Relative enzyme activity[b]	Antimetabolite CoQ index[c]
None	—	100	—	—	100	—
Standard	6	55		4	61	
inhibitor[d]	8	56	5	6	54	4
	10	49		8	41	
5-R*S*-2,3-Dimethyl-1,4-benzoquinones						
R						
n-$C_{18}H_{37}$ (II)	500	95		100	95	
			>287	200	88	>291
				500	78	
n-$C_{12}H_{25}$ (III)	500	84		250	51	
	1000	83	>575	500	42	151
				1000	38	
n-C_8H_{17} (IV)	250	73		10	76	
	500	62	552	50	70	105
	1000	49		250	42	
				400	10	
Di-*n*-C_8H_{17} (V)	100	69		50	83	
	500	54	345	100	65	87
	1000	40		250	28	
β-$C_{10}H_7$ (VI)	100	65		10	62	
	200	51	132	50	27	10
	500	45		100	12	
	1000	32		500	16	
C_6H_5 (VII)	10	82		25	74	
	50	62		50	30	
	100	47	49	100	14	20
	500	7				
5-R*S*-2,3-Dimethoxy-1,4-benzoquinones[e]						
R						
n-$C_{18}H_{37}$	500	96		500	99	
β-$C_{10}H_7$	1000	91	>425	1000	98	>567
	16	68		8	84	
	20	59	11	16	50	9
	28	46		24	33	
	40	32				
2-R*S*-1,4-Naphthoquinones[f]						
R						
n-$C_{18}H_{37}$	100	99	>37	100	100	>49
β-$C_{10}H_7$	80	78		20	85	
	120	64	>73	30	72	28
	200	57		60	48	

a) nmol in a flask. *b*) Percentage of specific activity in the presence of inhibitor to that of the control. *c*) The mark (>) means that antimetabolite CoQ index is greater than the number shown. *d*) 7-*n*-Dodecylthio-6-hydroxy-5,8-quinolinequinone. *e*) Ref. 6. *f*) Ref. 9.

reactions gave overall yields of 34—48% based on the amount of I. 5,6-Di-*n*-octylthio-2,3-dimethyl-1,4-benzoquinone (V) was also obtained from the reaction of I and *n*-octanethiol under similar conditions.

Inhibition of Succinoxidase and NADH-Oxidase Systems

The newly synthesized quinones were evaluated in mitochondrial succinoxidase and NADH-oxidase systems for inhibition of coenzyme Q_{10} (CoQ_{10}). 7-*n*-Dodecylthio-6-hydroxy-5,8-quinolinequinone was used as a standard inhibitor. To compare the inhibitory activities of the quinones, the inhibitory activities are expressed as antimetabolite CoQ_{10} indices[3] for approximately 50% inhibition of enzyme activity. This index is calculated on the basis of nmol of the inhibitor per nmol of CoQ_{10}. The results are summarized in Table I. It can be seen that these quinones caused a slightly greater inhibition of the NADH-oxidase system than of the succinoxidase system. A dependence of the inhibitory effects for both enzyme systems on the length of the alkylthio side chain was observed, and the 5-*n*-octylthio group was slightly more effective in both enzyme systems than *n*-dodecylthio and *n*-octadecylthio groups. Moreover, 5,6-di-*n*-octylthio-2,3-dimethyl-1,4-benzoquinone (V) showed more potent inhibitory activities than 5-alkylthio-2,3-dimethyl-1,4-benzoquinones (II—IV). Two 5-arylthio-2,3-dimethyl-1,4-benzoquinones (VI, VII) exhibited potent inhibitory activities towards both enzyme systems. On the other hand, in order to evaluate the effect of the presence of the 2,3-dimethyl group on the activities, the inhibitory effects of II and VI were compared with those of 5-R*S*-2,3-dimethoxy-1,4-benzoquinones[6] and 2-R*S*-1,4-naphthoquinones[9] (Table I). Table I shows that VI and 5-*β*-naphthylthio-2,3-dimethoxy-1,4-benzoquinone caused greater inhibitions of both enzyme systems than 2-*β*-naphthylthio-1,4-naphthoquinone. In the case of *n*-octadecylthio analogs, all three kinds of quinones showed weak inhibitory activities in both enzyme systems. From these results, it is considered that the 5-arylthio-2,3-dimethyl-1,4-benzoquinone moiety is effective for the inhibition of succinoxidase and NADH-oxidase. Further investigations should be done on shorter-chain homologs of 5,6-dialkylthio-2,3-dimethyl-1,4-benzoquinone.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus, and are uncorrected. Infrared (IR) spectra were taken in KBr with a Hitachi 260-30 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were measured with a Hitachi R-22 NMR spectrometer in $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given as δ values (ppm): s, singlet; t, triplet; br, broad; m, multiplet. Mass spectra (MS) were measured with a Hitachi M-60 mass spectrometer. Beef heart mitochondria were isolated by the usual procedures.[13] The final mitochondrial pellet, which was a mixture of heavy and light particles, was suspended in 0.25 M sucrose and was used immediately or kept frozen until used. Phospholipid micelles were prepared by sonication of commercial soybean phospholipids (Asolectin)[14] and used instead of mitochondrial phospholipids. Protein was determined by the Lowry method.[15] The amount of CoQ_{10} in the mitochondrial preparation was determined by the modified Craven's assay[16] after extraction with pentane.[17] The mitochondria contained 2.61 nmol of CoQ_{10}/mg of mitochondrial protein.

Synthesis of 5-Alkylthio-2,3-dimethyl-1,4-benzoquinones (II, III)——A solution of *n*-octadecanethiol (590 mg) in *n*-hexane (20 ml) was added dropwise to a solution of I (277 mg) in EtOH (20 ml) at room temperature under stirring. After being stirred for 3 h, the reaction mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel with *n*-hexane–benzene (1 : 1) as the eluent. The first eluate afforded di-*n*-octadecyldisulfide (424 mg). The second eluate was concentrated under reduced pressure and diluted with MeOH (10 ml). A solution of Fremy's salt (2.4 g), 1 N sodium acetate (1.4 ml) and H_2O (40 ml) was added to the MeOH solution. After being stirred for 20 min, the reaction mixture was extracted with Et_2O. The extract was concentrated to a volume of 20 ml and Ag_2O was added in order to oxidize the residual hydroquinone. The insoluble material was removed by filtration. The filtrate and the third eluate were combined and evaporated to dryness under reduced pressure. The residue was recrystallized from *n*-hexane to afford 5-*n*-octadecylthio-2,3-dimethyl-1,4-benzoquinone (II). Yield 424 mg (36.2%). NMR ($CDCl_3$) δ: 0.87 (3H, s, CH_2–$C\underline{H}_3$), 1.0—1.8 (32H, br, $(CH_2)_{16}$), 2.02 (6H, s, CH_3 on the ring), 2.73 (2H, t, SCH_2), 6.32 (1H, s, H on the ring). Compound I (367 mg) and *n*-dodecanethiol (544 mg) were

TABLE II. Physicochemical Data for 5-R*S*-2,3-dimethyl-1,4-benzoquinones

Compd. No.	R	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	MS (M^+)	IR (cm^{-1}, KBr)
II	n-$C_{18}H_{37}$	36.2	78.5—79	$C_{26}H_{44}O_2S$	74.23 (74.06)	10.45 10.70)	420	2920 (C–H) 1660 (C=O)
III	n-$C_{12}H_{25}$	46.4	69—70	$C_{20}H_{32}O_2S$	71.38 (71.56)	9.58 9.79)	336	2910 (C–H) 1660 (C=O)
IV	n-C_8H_{17}	34.7	61.5—62	$C_{16}H_{24}O_2S$	68.53 (68.33)	8.63 8.74)	280	2920 (C–H) 1660 (C=O)
V[a)]	Di-n-C_8H_{17}	6.2	31—31.5	$C_{24}H_{40}O_2S$	67.88 (67.64)	9.49 9.63)	424	2920 (C–H) 1650 (C=O)
VI	β-$C_{10}H_7$	48.2	123—124	$C_{18}H_{14}O_2S$	73.45 (73.25	4.79 4.73)	294	1665 (C=O) 1575 (C=C)
VII	C_6H_5	46.5	90—91	$C_{14}H_{12}O_2S$	68.83 (68.84	4.95 4.68)	244	1650 (C=O) 1575 (C=C)

a) 5,6-Di-*n*-octylthio-2,3-dimethyl-1,4-benzoquinone.

reacted in a manner similar to that described for the synthesis of II to afford 5-*n*-dodecylthio-2,3-dimethyl-1,4-benzoquinone (III). However, the oxidation procedure with Ag_2O was unnecessary in this case. Yield 422 mg (46.4%). NMR ($CDCl_3$) δ: 0.87 (3H, s, CH_2–$C\underline{H}_3$), 1.0—1.8 (20H, br, $(CH_2)_{10}$), 2.02 (6H, s, CH_3 on the ring), 2.73 (2H, t, SCH_2), 6.32 (1H, s, H on the ring). The physicochemical data are summarized in Table II.

Synthesis of 5,6-Dialkylthio-2,3-dimethyl-1,4-benzoquinone (V)——A solution of *n*-octanethiol (350 mg) in *n*-hexane (10 ml) was added dropwise to a solution of I (280 mg) in EtOH (20 ml) at room temperature under stirring. The reaction mixture was treated in the usual manner and the residue was purified by column chromatography on silica gel with *n*-hexane–benzene (1 : 1) as the eluent. The eluates were separated into three fractions; fraction 1 (colorless), fraction 2 (brown color), fraction 3 (brown color). Fraction 1 was concentrated to dryness to give di-*n*-octyldisulfide. Fraction 2 was concentrated under reduced pressure and the residue was purified by column chromatography on alumina with *n*-hexane–benzene (3 : 2) as the eluent. The eluate was concentrated under reduced pressure and the residue was recrystallized from 80% EtOH to afford V. Yield 539 mg (6.2%). NMR ($CDCl_3$) δ: 0.87 (6H, s, CH_3), 1.0—1.8 (24H, br, $(CH_2)_6$), 2.01 (6H, s, CH_3), 2.73 (2H, t, SCH_2), 6.34 (1H, s, H on the ring). Fraction 3 was concentrated under reduced pressure and the residue was recrystallized from petroleum ether to afford 5-*n*-octylthio-2,3-dimethyl-1,4-benzoquinone (IV). Yield 200 mg (34.7%). NMR ($CDCl_3$) δ: 0.87 (3H, s, CH_2–$C\underline{H}_3$), 1.0—1.8 (12H, br, $(CH_2)_6$), 2.01 (6H, s, CH_3 on the ring), 2.73 (2H, t, SCH_2), 6.34 (1H, s, H on the ring). The physicochemical data are summarized in Table II.

Synthesis of 5-Arylthio-2,3-dimethyl-1,4-benzoquinones (VI, VII)——A solution of β-naphthalenethiol (257 mg) in Et_2O (10 ml) was added dropwise to a solution of I (199 mg) in EtOH (10 ml) at room temperature under stirring. The mixture was stirred for 3 h until the solution became colorless. The reaction mixture was treated in the usual manner and the residue was purified by column chromatography on silica gel with benzene–*n*-hexane (1 : 1) as the eluent. The eluate was concentrated under reduced pressure and the residue was recrystallized from *n*-hexane to afford VI. Yield 207 mg (48.2%). NMR ($CDCl_3$) δ: 1.96 (3H, s, CH_3), 2.04 (3H, s, CH_3), 5.84 (1H, s, H), 7.3—8.2 (7H, m, $C_{10}H_7$). Compound I (241 mg) and benzenethiol (231 mg) were reacted in a manner similar to that described for the synthesis of VI to afford 5-phenylthio-2,3-dimethyl-1,4-benzoquinone (VII). Yield 201 mg (46.5%). NMR ($CDCl_3$) δ: 1.99 (3H, s, CH_3), 2.05 (3H, s, CH_3), 5.84 (1H, s, H), 7.45 (5H, s, C_6H_5). The physicochemical data are summarized in Table II.

Inhibition of Mitochondrial Succinoxidase and NADH-Oxidase Systems——Succinoxidase and NADH-oxidase activities were determined manometrically in a Gilson differential respirometer.[13)] A total of 2.6 ml of the reaction mixture in the main compartment of each 15 ml flask contained: 1.0 ml of 0.1 M Tris–HCl buffer (pH 7.6); 0.5 ml of 1 M sucrose; 0.1 ml of 0.8mM ethylenediaminetetraacetic acid (EDTA); 0.05 ml of Asolectin (20 mg/ml); 0.25 mg of the standard inhibitor dissolved in ethanol; 0.1 ml of 2% cytochrome c; mitochondrial enzyme (0.667 mg of protein for the succinoxidase assay and 0.659 mg of protein for the NADH-oxidase assay). Then, 0.2 ml of 0.75 M succinate or 0.075 M NADH was put in the side arm and 0.2 ml of 6 N KOH was put in the center well. The reaction was initiated by addition of the substrate from the side arm into the reaction mixture, and the activity was determined at 30 °C.

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