chromatogd on 60 g of silica gel (100–200 mesh) and eluted with EtOAc (500 ml). The solvent was removed and the residue was recrystd from hexane to give colorless crystals: 0.27 g (42%); mp 87–88°; nmr δ 2.56 (s, 3 H, 4-CH_3), 5.05 (d, 2 H, CH_2). *Anal.* ($C_{11}H_{11}NO$) C, H, N.

3-Methylisoquinoline-1-carboxaldehyde (5). Compd **3** (0.38 g, 2.2 mmoles) was dissolved in 20 ml of C_6H_6 and 0.35 g (4.0 mmoles) of MnO_2 was added. The mixt was refluxed for 4 hr and filtered, and the C_6H_6 was removed to yield **5**. Recrystn from petr ether gave colorless needles: 0.27 g (72%); mp 71–72°; nmr δ 10.22 (s, 1 H, CHO), 2.71 (s, 3 H, 3-CH_3). *Anal.* ($C_{11}H_9NO$) N.

4-Methylisoquinoline-1-carboxaldehyde (13) was synthesized by the same procedure as **5**, oxidizing **12** with MnO_2. Recrystn from petr ether yielded colorless crystals in 70% yield: mp 59–60°; nmr δ 10.21 (s, 1 H, CHO), 2.68 (s, 3 H, 4-CH_3). *Anal.* ($C_{11}H_9NO$) N.

5-Methylisoquinoline-1-carboxaldehyde (16). Compd **15** (0.32 g, 2 mmoles) was dissolved in 25 ml of dioxane and 0.22 g (2 mmoles) of SeO_2 was added slowly. The mixt was refluxed for 2.5 hr and filtered, dioxane was removed, and the residue was extd with dil HCl. The acid layer was filtered and made alk with $NaHCO_3$, and the resulting ppt was collected, washed (H_2O), and dried. Crystn from hexane (Norit) yielded colorless fibrous material: 0.25 g (72%); mp 108–109°; nmr δ 10.28 (s, 1 H, CHO), 8.81 (d, 1 H, J = 5.7 Hz, H-3), 8.18 (dd, 1 H, J = 5.5 and 0.8 Hz, H-4), 2.69 (t, 3 H, 5-CH_3). *Anal.* ($C_{11}H_9NO$) N.

Thiosemicarbazones. The thiosemicarbazones were prepd by treating alcoholic solns of the corresponding carboxaldehydes with an aq soln of thiosemicarbazide contg a few drops of dil AcOH. Relevant data concerning these compds are listed in Table II.

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References

(1) (a) R. W. Brockman, J. R. Thomson, M. J. Bell, and H. E. Skipper, *Cancer Res.*, 16, 167 (1956); (b) F. A. French and E. J. Blanz, Jr., *J. Med. Chem.*, 9, 585 (1966).
(2) W. A. Creasey, K. C. Agrawal, K. K. Stinson, and A. C. Sartorelli, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 29, 908 (1970).
(3) W. Brockman, R. W. Sidwell, G. Arnett, and S. Shaddix, *Proc. Soc. Exp. Biol. Med.*, 133, 609 (1970).
(4) (a) K. C. Agrawal, B. A. Booth, and A. C. Sartorelli, *J. Med. Chem.*, 11, 700 (1968); (b) K. C. Agrawal and A. C. Sartorelli, *ibid.*, 12, 771 (1969); (c) K. C. Agrawal, R. J. Cushley, W. J. McMurray, and A. C. Sartorelli, *ibid.*, 13, 431 (1970).
(5) F. A. French, and E. J. Blanz, Jr., *Cancer Res.*, 25, 1454 (1965).
(6) K. C. Agrawal and A. C. Sartorelli, *J. Pharm. Sci.*, 57, 1948 (1968).
(7) (a) A. C. Sartorelli, *Biochem. Biophys. Res. Commun.*, 27, 26 (1967); (b) A. C. Sartorelli, *Pharmacologist*, 9, 192 (1967).
(8) E. C. Moore, M. S. Zedeck, K. C. Agrawal, and A. C. Sartorelli, *Biochemistry*, 9, 4492 (1970).
(9) (a) B. A. Booth, E. C. Moore, and A. C. Sartorelli, *Cancer Res.*, 31, 228 (1971); (b) E. C. Moore, B. A. Booth, and A. C. Sartorelli, *ibid.*, 31, 235 (1971).
(10) (a) F. A. French and E. J. Blanz, Jr., *ibid.*, 26, 1638 (1966); (b) F. A. French and E. J. Blanz, Jr., *Gann*, 2, 51 (1967).
(11) E. Späth, F. Berger, and W. Kuntara, *Ber.*, 63B, 134 (1930).
(12) M. M. Robison and B. L. Robison, *J. Amer. Chem. Soc.*, 80, 3443 (1958).
(13) V. J. Traynelis and R. F. Martello, *ibid.*, 80, 6590 (1958).
(14) (a) W. Mathes and W. Sauermilch, *Ber.*, 90, 758 (1957); (b) S. Furukawa and Y. Kuroiwa, *Chem. Pharm. Bull.*, 3, 232 (1955).
(15) A. C. Sartorelli, K. C. Agrawal, and E. C. Moore, *Biochem. Pharmacol.*, 20, 3119 (1971).

Specificity of Inhibition of Coenzyme Q-Enzyme Systems by Lipoidal Benzoquinone Derivatives†

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5-Substituted 2,3-dimethoxy-6-phytyl-1,4-benzoquinones were found to inhibit mitochondrial NADH-oxidase and succinoxidase systems from beef heart. The most effective group in the 5 position was OH for the 6-phytyl derivatives. The 5-Cl and 5-Br derivatives were less inhibitory than the 5-OH derivatives, and in diminishing degree. The 5-MeO derivative was essentially noninhibitory. 6-Alkyl- and 6-isoprenyl-2,3-dimethoxy-5-hydroxy-1,4-benzoquinones were similarly evaluated. Inhibition of the NADH-oxidase system was greatest when the hydroxyquinone possessed a side chain of from 16 and 17 C. Inhibition of the succinoxidase system was relatively nonspecific in respect to the side chain. The succinoxidase system was generally more sensitive to most of the benzoquinones tested than was the NADH-oxidase system.

Mitochondrial reconstruction[1,2] and spectrophotometric investigations on the kinetics of coenzyme Q turnover during electron transport[3] are responsible for the view that coenzyme Q participates in the primary electron transport sequence. Coenzyme Q has a widespread distribution in biological systems[4] including the malarial parasite.[5-8] Mammalian succinoxidase and NADH-oxidase systems have been extensively studied and may be considered representative of the coenzyme Q electron transport sequences.

Preliminary structure-activity investigations demonstrated that mitochondrial succinoxidase activity was inhibited by various antimalarial naphthoquinone analogs, and the activity was restored by coenzyme Q and its derivatives.[9] Next, chloroquine and a new napthoquinone antimalarial, 2-ω-cyclohexyloctyl-3-hydroxy-1,4-naphthoquinone, were shown to inhibit beef heart mitochondrial succinoxidase systems.[10] Again, the inhibitory action of antimalarial agents was reversed by coenzyme Q.

These findings link one kind of inhibition of mitochondrial electron transport at the coenzyme Q loci to chemotherapy of malaria. CoQ_8 is the dominant CoQ of *Plasmodium*.[5-8] A series of benzoquinones structurally related to known antimalarial naphthoquinones, which were also succinoxidase inhibitors,[9] have been synthesized.[11] These benzoquinones also have structural resemblance to coenzyme Q. They represent potential antagonists of coenzyme Q function in mitochondrial electron transport; hence, they also represent potential antimalarial activity.

Separate sites for the function of coenzyme Q in the succinoxidase and NADH-oxidase systems in beef heart[12,13] and yeast[13,14] mitochondria have been implicated by studies on the structural specificity of coenzyme Q. These investigations demonstrated that the function of coenzyme Q in the

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†Coenzyme Q. 141.

succinoxidase system has little specificity for the various isoprenologs, but the activity of coenzyme Q in the NADH-oxidase system is directly proportional to the length of the isoprenoid side chain. This different organic structural specificity for the succinoxidase and NADH-oxidase systems will surely influence the capacity of various coenzyme Q analogs to inhibit each of the enzyme systems. Consequently, an investigation of the structure-inhibition relationships was conducted on the recently synthesized benzoquinones, and the data are herein described. This investigation was designed to evaluate the inhibition of various 2,3-dimethoxy-5-hydroxy-6-alkyl-1,4-benzoquinones, particularly the analogous 5-MeO-, 5-Cl-, and 5-bromo-6-phytyl-substituted quinones, on beef heart mitochondrial succinoxidase and NADH-oxidase activities.

Experimental Section

"Heavy beef heart mitochondria" (HBHM) were isolated[15] and lyophilized as described.[2] Portions of the lyophilized HBHM were extd with pentane[2] to remove the coenzyme Q. The coenzyme Q deficient mitochondria were reconstructed by adding coenzyme Q and asolectin (a soybean phospholipid). The activities of the lyophilized unextd and the extd-reconstructed mitochondrial succinoxidase and NADH-oxidase enzyme systems were detd manometrically in the absence and presence of the various benzoquinones to be tested. Coenzyme Q and the various compds were added in EtOH, and the concn of EtOH was maintained in the flasks at 0.1 ml of EtOH per 3 ml of reaction mixt. Asolectin was added to serve as a carrier for the quinones. The mitochondrial protein was detd by the biuret method[16] and was maintained at 0.5–1.0 mg per flask.

The differences in Tables I and II for inhibition of the extracted and reconstructed NADH-oxidase and succinoxidase systems between the specific activity (μatoms of O consumed/min per mg of protein) of the coenzyme Q_{10} supplemented (100 nmoles/flask) systems and the extd control was defined as 100% activity. The per cent activity of the assay systems (specific activity for the compd minus the specific activity of the extd control) was calcd from the uninhibited specific activity representing 100%.

The per cent activities in Tables I and II for the lyophilized and unextd NADH-oxidase and succinoxidase systems were detd from the specific activity of the unextd control which was defined as 100%. The flasks also contd 100 nmoles of added coenzyme Q_{10}.

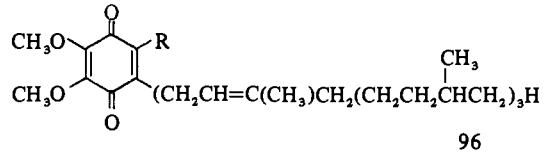
The compd (100 nmoles) was tested in the presence of 100 nmoles of coenzyme Q_{10} to obtain the data in Tables I and II. Each value in Table I and in Table II represents the average of from 2 to 6 assays. The specific activities of the uninhibited extd-reconstructed NADH-oxidase, unextd NADH-oxidase, extd-reconstructed succinoxidase, and unextd succinoxidase ranged from 0.250 to 0.350, 0.400 to 0.650, 0.300 to 0.450, and 0.350 to 0.450 μatom of O consumed per min per mg of protein, respectively.

Results and Discussion

The effect of various 5-substituted 2,3-methoxy-6-phytyl-1,4-benzoquinones on mitochondrial electron transport systems was determined, and the data are in Table I. The 5-OH, 5-Cl, 5-Br, and 5-methoxy-6-phytyl derivatives inhibited 96, 52, 24, and 2% of the controls, respectively. The lyophilized unextracted NADH-oxidase system was similarly affected, but the magnitude of the inhibitions was less.

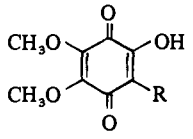
The extracted-reconstructed succinoxidase system was depressed to 17, 49, 79, and 100% of the controls in the presence of the 5-OH, 5-Cl, 5-Br, and 5-methoxy-6-phytyl derivatives, respectively. Similar results were obtained with the unextracted succinoxidase system, although the magnitude of the inhibitions was less. These data demonstrate that the most significant structural change for inhibition of coenzyme Q-enzyme systems is the replacement of the 5-Me group of

Table I. Inhibition of Mitochondrial Succinoxidase and NADH-oxidase by 6-Substituted 2,3-Dimethoxy-6-phytyl-1,4-benzoquinones

R	Inhibition of NADH-oxidase		Inhibition of succinoxidase	
	E and R[a]	L non-E[b]	E and R[a]	L non-E[b]
				
OH	96	62	83	78
Cl	52	36	51	25
Br	24	6	21	24
OCH_3	2	8	0	0

[a]E and R = extracted and reconstructed. [b]L non-E = lyophilized and nonextracted.

Table II. Inhibition of Mitochondrial Succinoxidase and NADH-oxidase by 6-Substituted 2,3-Dimethoxy-5-hydroxy-1,4-benzoquinones

R	Inhibition of NADH-oxidase		Inhibition of succinoxidase	
	E and R[a]	L non-E[b]	E and R[a]	L non-E[b]
				
$(CH_2CH=C(CH_3)CH_2)_2H$ (geranyl)	0	9	56	45
$(CH_2CH=C(CH_3)CH_2)_3H$ (farnesyl)	41	23	76	32
$(CH_2CH=C(CH_3)CH_2)_4H$ (tetraprenyl)	100	62	100	85
$(CH_2CH=C(CH_3)CH_2)_9H$ (solanesyl)	75	39	91	69
$CH_2CH=C(CH_3)CH_2(CH_2CH_2\underset{\displaystyle CH_3}{\underset{\vert}{C}}HCH_2)_3H$ (phytyl)	96	62	83	78
$(CH_2CH_2C(CH_3)HCH_2)_4H$ (dihydrophytyl)	92	49	89	79
$(CH_2)_{16}CH_3$	100	65	100	65
$(CH_2)_{18}CH_3$	67	47	90	76
$(CH_2)_8C_6H_{11}$ (ω-cyclohexyloctyl)	76	39	86	54

[a]E and R = extracted and reconstructed. [b]L non-E = lyophilized and nonextracted.

coenzyme Q with 5-OH. The 5-OH substituted quinone was the most effective inhibitor of all 4 of the CoQ-enzyme systems. The 5-Cl derivative was a more effective inhibitor than the 5-Br derivative. The 5-MeO derivative was essentially noninhibitory in all forms of the enzyme systems.

Since the derivative with the 5-OH group was the most effective inhibitor of these 5-substituted derivatives, the effect of various 6-alkyl-substituted 2,3-dimethoxy-5-hydroxy-1,4-benzoquinones on the activities (HBHM) of NADH-oxidase and succinoxidase was assessed; and the data are in Table II. These data show that the extracted-reconstructed mitochondrial NADH-oxidase activity was not inhibited by the 5-hydroxybenzoquinone containing a 6-geranyl (C_{10}) side chain, but those compounds containing the 6-farnesyl (C_{15}), 6-cyclohexyloctyl (C_{14}), 6-nonadecyl (C_{19}), and 6-solanesyl (C_{45}) groups depressed the enzyme activity to 25-65% of the controls. However, those 5-hydroxybenzoquinones containing the 6-tetraprenyl (C_{20}), 6-phytyl (C_{20}), 6-dihydrophytyl (C_{20}), and 6-heptadecyl (C_{17}) groups inhibited the extracted-reconstructed mitochondrial NADH-oxidase system to 0, 4, 8, and 0% of the controls, respectively.

Similar structure-inhibition relationships were observed in the experiments conducted with preparations of lyophilized HBHM which was not extracted with pentane to remove CoQ_{10}. Again, the most effective inhibitors were the 6-tetraprenyl (C_{20}), 6-phytyl (C_{20}), and 6-heptadecyl (C_{17}) derivatives which inhibited the enzyme activity to 35-40%. The other derivatives caused less than 50% inhibition.

On the basis of these data, an alkyl side chain of 17 or 20 C atoms was more effective for the inhibition of the NADH-oxidase system than were side chains of 14 or 19 C atoms. Branching or degree of unsaturation of the side chain appeared to be of less structural significance than the general length of the side chain.

The specificity for the length of the alkyl side chain which was observed in the studies in NADH-oxidase inhibition was not apparent in the succinoxidase inhibition. The 6-tetraprenyl, 6-solanesyl, 6-phytyl, 6-dihydrophytyl, 6-heptadecyl, 6-nonadecyl, 6-cyclohexyloctyl, and 6-farnesyl derivatives inhibited the activity of the extracted-reconstructed succinoxidase to 0-25% of that of the controls. The 6-geranyl analog inhibited the enzyme activity to 50% of that of the controls. Apparently, the 6-geranyl derivative is too hydrophilic to be functional in electron transfer in the hydrophobic environment associated with the activity of coenzyme Q_{10}.

With the exception of the 6-phytyl and the 6-dihydrophytyl analogs, the extracted-reconstructed succinoxidase system was more sensitive to the various 5-hydroxybenzoquinone analogs than was the comparable NADH-oxidase system.

In the lyophilized and unextracted HBHM, the succinoxidase system appeared to be more sensitive to the various antimetabolites of coenzyme Q than was the NADH-oxidase system.

The finding that the extracted-reconstructed mitochondrial enzyme systems are more sensitive to the analogs of coenzyme Q than are their respective unextracted preparations implies that the site of inhibition of these hydroxybenzoquinones is at the region of coenzyme Q in the electron transport chain, since the inhibitor and coenzyme Q were both present during reconstruction at equal concentration. This interpretation is consistent with the findings of Castelli, *et al.*,[17] that the inhibition by hydroxybenzoquinones of a mitochondrial NADH-oxidase system was reversed by coenzyme Q_2.

The observed greater sensitivity and the lower structural specificity of the succinoxidase as compared to the NADH-oxidase system for the various hydroxybenzoquinones also support the previous conclusion[12-14] that coenzyme Q has separate locations in complexes I and II and, therefore, is situated in two different molecular environments. The greater sensitivity of the succinoxidase system to inhibition may be explained by a greater affinity of the CoQ-site associated with succinoxidase for the hydroxybenzoquinones. These relative inhibitions could also be influenced by a greater affinity for coenzyme Q in the NADH-oxidase than the succinoxidase system.

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References

(1) R. L. Lester and S. Fleischer, *Biochim. Biophys. Acta*, 47, 358 (1961).
(2) L. Szarkowska, *Arch. Biochem. Biophys.*, 113, 519 (1966).
(3) M. Klingenberg and A. Kroger, *Biochem. Mitochondria, Colloq.*, 1966, 11 (1967).
(4) F. L. Crane, *Biochem. Quinones*, 183 (1965).
(5) P. J. Rietz, F. S. Skelton, and K. Folkers, *Int. Z. Vitaminforsch.*, 37, 405 (1967).
(6) F. S. Skelton, P. J. Rietz, and K. Folkers, *J. Med. Chem.*, 13, 602 (1970).
(7) F. S. Skelton, K. D. Lunan, K. Folkers, J. V. Schnell, W. A. Siddiqui, and Q. M. Geiman, *Biochemistry*, 8, 1284 (1969).
(8) J. V. Schnell, W. A. Siddiqui, Q. M. Geiman, F. S. Skelton, K. D. Lunan, and K. Folkers, *J. Med. Chem.*, 14, 1026 (1971).
(9) E. G. Ball, C. B. Anfinsen, and O. Cooper, *J. Biol. Chem.*, 168, 257 (1947).
(10) F. S. Skelton, R. S. Pardini, J. C. Heidker, and K. Folkers, *J. Amer. Chem. Soc.*, 90, 5334 (1968).
(11) J. C. Catlin, R. S. Pardini, G. D. Daves, Jr., J. C. Heidker, and K. Folkers, *ibid.*, 90, 3572 (1968).
(12) G. Lenaz, G. D. Daves, Jr., and K. Folkers, *Arch. Biochem. Biophys.*, 120, 539 (1968).
(13) G. Lenaz, A. Castelli, G. P. Littarru, E. Bertoli, and K. Folkers, *ibid.*, 142, 407 (1971).
(14) A. Castelli, G. Lenaz, and K. Folkers, *Biochem. Biophys. Res. Commun.*, 34, 200 (1968).
(15) P. B. Blair, *Methods Enzymol.*, 10, 78 (1967).
(16) E. Layne, *ibid.*, 3, 47 (1957).
(17) A. Castelli, E. Bertoli, G. P. Littarru, G. Lenaz, and K. Folkers, *Biochem. Biophys. Res. Commun.*, 42, 806 (1971).