1.3 g (45%) of beige flakes: mp 166.0–168.0° (lit.[5] 166–167°); ν_{max} (KBr) 1760 (C=O). *Anal.* ($C_{20}H_{20}O_6$) C, H.

2-Cyclopropyl-6,7-dimethoxy-1-veratryl-3(2*H*)-isoquinolone (5d). Method C. A soln of lactone 8 (10.7 g, 0.03 mole) and cyclopropylamine (5 g, 0.088 mole) in 500 ml of THF was stirred 18 hr at 25°. The volatile material was removed *in vacuo* (85°), and the residue, dissolved in 50 ml of EtOAc, was warmed with excess 5 *N* HCl–EtOH. Evapn of the solvents and recrystn of the residue afforded a yellow solid.

2-Hydroxy-6,7-dimethoxy-1-veratryl-3(2*H*)-isoquinolone Hydrochloride (5g). {2-[3,4-(Dimethoxyphenyl)acetyl]-4,5-dimethoxyphenyl}acetic Acid Oxime. The oxime was prepd[19] by heating 7 with $NH_2OH \cdot HCl$ for 1 hr in aqueous alkali. Recrystn from EtOAc afforded off-white crystals: mp 137–139°. *Anal.* ($C_{20}H_{23}NO_7$) C, H, N. A soln of 7 oxime (2.6 g or 0.0066 mole) in 100 ml of AcOH and 2 ml of 12 *N* HCl was stirred at 25° for 16 hr and concd *in vacuo*. The residue was leached with 100 ml of hot MeOH, and the extracts were evapd to dryness. Recrystn of the residue gave yellow prisms (5g): δ (DMSO-d_6) 3.8–4.0 (12, OCH_3), 4.9 (s, 2, CH_2), 6.9–7.6 (6, ArH). 10.5 (s, 2, OH, H^+).

2-Amino-6,7-dimethoxy-1-veratryl-3(2*H*)-isoquinolone Hydrochloride (5h). A soln of 7 (9.4 g or 0.025 mole), 1.25 g (0.025 mole) of 98% $N_2H_4 \cdot H_2O$, and 150 ml of abs EtOH was refluxed 5 hr. The EtOH was distilled and replaced with 75 ml of *i*-PrOH; 5 *N* HCl–EtOH was added to pH 2, after which the soln was refluxed 1 hr and chilled. The solidified mass, when diluted with Et_2O and filtered, afforded 7.3 g of crude solid which was taken up in hot 0.1 *N* HCl. The soln was filtered and basified with 20% NaOH soln to yield a solid which was purified as the HCl salt to provide light yellow crystals: δ (DMSO-d_6) 3.8–4.0 (12, OCH_3), 5.0 (s, 2, CH_2), 6.9–7.5 (6, ArH), 8.2 (s, 3, NH_3^+).

6,7-Dimethoxy-1-veratrylisoquinolin-3-yl *p*-Bromobenzenesulfonate (6e). A soln of 8.9 g (0.025 mole) of 4, 6.4 g (0.025 mole) of *p*-bromobenzenesulfonyl chloride, 300 ml of $CHCl_3$, and 2.4 g of pyridine was refluxed for 4 hr and filtd (Darco G-60). The filtrate was washed with 150 ml of H_2O, sepd, dried (anhyd K_2CO_3), and evapd *in vacuo* to afford a yellow solid which was recrystd twice from butanone–$(i\text{-Pr})_2O$ to give 9 g (62%) of faintly yellow needles: mp 147–148°; λ_{max} ($CHCl_3$) 332 (ε 5200). *Anal.* ($C_{26}H_{24}BrNO_7S$) C, H, N.

6,7-Dimethoxy-2-methylisoquinoline-1,3,4(2*H*)-trione (11). Eight grams of analytically pure *N*-methyl lactam (5a) was converted to the free base in an open beaker with concd NH_4OH. The bright yellow ppt (mp 165–181°) was taken up in 200 ml of boiling EtOAc and filtered to separate 200 mg (4%) of insol yellow solid. The material is readily soluble in cold, concd H_2SO_4, yielding an orange-brown soln, and dissolves upon heating in dilute Na_2CO_3 to give a colorless soln.[20] One recrystn from CH_3CN afforded material: mp 275–276° (lit.[20] mp 270–271° dec). Ir and nmr spectra are consistent with structure 11. The EtOAc filtrates contained pure 5a (isolated as the HCl salt). *Anal.* ($C_{12}H_{11}NO_5$) C, H, N.

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Antimetabolites of Coenzyme Q. 17. Improved Synthesis of 5-Hydroxy-1,4-benzoquinone Analogs and Their Indices†

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Improvements in the synthesis of 5-hydroxy analogs of coenzyme Q having different aliphatic and isoprenoid substituents in the 6 position, as well as in the purification of these analogs, have been made. New 5-hydroxy analogs have been obtained by these modifications. These eight 2,3-dimethoxy-5-hydroxy-1,4-benzoquinones have the following 6 substituents: decyl-, 5-cyclohexylpentyl-, tetradecyl-, pentadecyl-, nonadecyl-, heneicosyl-, farnesyl-, and phytyl-. They were compared for inhibition of the CoQ_{10} enzymes DPNH-oxidase and succinoxidase. Inhibition is newly defined by an antimetabolite CoQ index which is the number of nmoles of analog/nmoles of CoQ_{10} in the enzyme preparation for 50% inhibition. The antimetabolite CoQ indices of the eight analogs ranged from 5 to 32 for NADH-oxidase and 5 to 14 for succinoxidase showing that these 5-hydroxy analogs are relatively potent antimetabolites of coenzyme Q.

Coenzyme Q has biologically important and indispensable functions in certain organelles of the cell. Its functions in mitochondria as an intrinsic component of the electron transfer processes of respiration and oxidative phosphorylation have now been recognized and studied for over a decade by many investigators. Two enzyme sites for its coenzymatic function in mitochondrial systems are well known. Lenaz, *et al.*,[1] found one site for succinoxidase and a second site for NADH-oxidase. The necessity of coenzyme Q for the interaction of NADH dehydrogenase, succinate dehydro-

†Coenzyme Q. 154.

genase, and cytochrome *b* has been studied by Ernster, *et al.*[2] The essentiality of coenzyme Q for mitochondrial oxidation of α-glycerol phosphate has recently been reported by Salach[3] who studied this enzyme in brain tissue. Salach suggested a separate compartmentation of the CoQ pool for these three mitochondrial oxidase systems.

Nyquist, *et al.*,[4] and Fleischer, *et al.*,[5] reported the presence of coenzyme Q in Golgi fractions of the cell. They[5] found that, on a phospholipid basis, rat liver Golgi and mitochondria have about the same content of CoQ.

Coenzyme Q has been studied by the direct administration for therapeutic objectives to animals having certain disease or nutritional deficiency states. The beneficial treatment with coenzyme Q of genetic muscular dystrophy in mice, that are deficient in coenzyme Q_9,[6] has been demonstrated.[7] The vitamin activity of coenzyme Q has been described for its therapeutic administration to the nutritionally deficient monkey,[8] rat,[9] rabbit,[10] chicken, and turkey[11] and recently to the hamster.[12]

The enzymology of coenzyme Q is also important as a basis for the design and synthesis of analogs which could function as antimetabolites of coenzyme Q. Progress has already been made on the synthesis of analogs; some were found to inhibit *in vitro* the activity of mitochondrial succinoxidase and NADH-oxidase.[13,14] Some of these analogs were also found to have significant antimalarial activity *in vivo* against *Plasmodium berghei*.[15] Since there has been some correlation of inhibition, *in vitro*, and antimalarial activity, *in vivo*, it appears that the usefulness, *in vivo*, of an antimetabolite of coenzyme Q has indeed been achieved and presumably can be extended to greater significance. Consequently, it is of continuing interest to expand studies on antimetabolites of coenzyme Q toward therapeutic activities in several fields of medicinal importance.

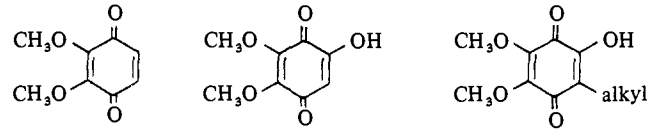
5-Hydroxy analogs of coenzyme Q having a 6-alkyl or 6-isoprenoid side chain were synthesized by Catlin, *et al.*,[16] and some of these compounds were found to inhibit coenzyme Q, *in vitro*. However, these analogs were only prepared on a small scale due to difficulties in the syntheses and in their purification.

Improvements in the synthesis of the 5-hydroxy analogs have now been achieved, because of a need for expanded biological evaluation. The availability of these analogs in greater amounts permitted refinement of the testing for antimetabolite activity which allowed better interpretation of structure-inhibition relationships.

New 5-hydroxy analogs, which have been synthesized and tested *in vitro*, are included in this account.

Organic Synthesis. 2,3-Dimethoxy-1,4-benzoquinone[16,17] was prepared in a yield of 60%. The product which was obtained by reaction of diazotized sulfanilic acid with 2,3-dimethoxyphenol was reduced by $Na_2S_2O_4$, and the aminophenol was extracted by $CHCl_3$ and oxidized immediately by shaking the solvent solution with a 10% solution of $Na_2Cr_2O_7$.

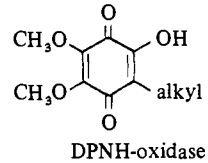
Thiele-Winter acetylation, hydrolysis of the triacetate, and oxidation with Ag_2O gave 2,3-dimethoxy-5-hydroxy-1,4-benzoquinone (II)[17] in a yield of 90%.



I II III

Radical alkylation of II was carried out by thermal decomposition of appropriate diacetyl peroxides.[17] The eight alkylated quinones are listed in Table I.

Table I. Chemical Properties of 6-Alkyl-2,3-dimethoxy-5-hydroxy-1,4-benzoquinones and Their Inhibition of the Activity of DPNH-Oxidase and Succinoxidase



Empirical[a,b] formulas	Alkyl	Mp, °C	DPNH-oxidase Concn[d]	DPNH-oxidase Concn of Inhib/CoQ[e]	DPNH-oxidase Inhibition, %	Succinoxidase Concn[d]	Succinoxidase Concn of Inhib/CoQ[e]	Succinoxidase Inhibition, %
$C_{18}H_{28}O_5$	6-*n*-Decyl	35	17	8	45	21	9.5	55
			26	12	68	63	20	64
						54	25	76
$C_{19}H_{28}O_5$	6-*n*-(5-Cyclohexyl)pentyl	61-62	11	5	57	5	2.3	32
						16	7	60
$C_{22}H_{36}O_5$	6-*n*-Tetradecyl	50-51	22	10	48	12.5	6	10
						17	8	46
$C_{23}H_{38}O_5$	6-*n*-Pentadecyl	55-56	22	10	50	15	7	50
			25	11	66			
			31	14	58			
$C_{27}H_{46}O_5$	6-*n*-Nonadecyl	69-70	80	36	52	25	11	59
			100	45	63	32	14	70
			150	68	78			
$C_{29}H_{50}O_5$	6-*n*-Heneicosyl	54-56	25	11	48	25	11	30
						31	14	50
$C_{23}H_{32}O_5$	6-Farnesyl		25	11	12	12.5	6	11
			50	22	56	25	11	60
			100	45	64	50	22	73
			200	90	72			
$C_{28}H_{46}O_5$[c]	6-Phytyl		38	17	25	20	9	40
			75	34	61	25	11	58
			150	68	80	50	22	80

[a] Analytical results obtained for C and H are within ±0.4% of the theoretical values except when otherwise specified. [b] Verified by mass spectrometry. [c] C: calcd, 72.68; found, 72.54. H: calcd, 10.03; found, 10.96. [d] Concentration of inhibitor in nmoles/mg of mitochondrial protein. [e] Concentration of inhibitor in nmoles/nmoles of CoQ_{10} present in enzymes.

The yield of the product from alkylation was improved 3–5 times by using dry toluene as solvent.

The acid-catalyzed alkylation with the appropriate isoprenoid alcohol was carried out with BF_3–etherate in dioxane.[17,18]

The major contaminants in the synthesis of these benzoquinones were the alkylating alcohols and the alkylating acids. The acids were by-products from thermal decomposition of the peroxides. It was possible to remove most of the contaminating acids by dry-column chromatography over deactivated silica gel.[19] The remaining acidic impurities were removed by distribution between hexane and CH_3CN. Final purification was performed by esterification of residual acids with HCl and MeOH in benzene. The pure benzoquinones could then be extracted from the organic solvent with dilute NaOH solution.

Essentially the same purification steps were used for the 6-isoprenoid-substituted benzoquinones with the exception that the esterification step was not needed.

Experimental Section

Melting points are uncorrected and were determined in a Hoover melting point apparatus. Nmr spectra for compounds 1–8 were obtained in $CDCl_3$ with a Varian A-60A spectrometer and are in accord with the assigned structures. The molecular weights were determined by mass spectrometry (CEC 21-491).

2,3-Dimethoxy-1,4-benzoquinone (I). To 23 g of azo compound obtained by coupling diazotized sulfanilic acid with 2,3-dimethoxyphenol[17] 270 ml of 10% NaOH solution was added, and the mixture was stirred while 25 g of $Na_2S_2O_4$ was added in portions. The solution was extracted with 3 × 200 ml of $CHCl_3$, and the solvent solution was shaken for 5 min with 220 ml of 10% $Na_2Cr_2O_7$ containing 28 ml of 12 *N* H_2SO_4. Removal of the solvent and crystallization from hexane gave 6.8 g of product, mp 65–67°, lit.[20,21] mp 66–67°.

General Procedure for Radical Alkylation. To a solution of 0.1 mole of 2,3-dimethoxy-5-hydroxy-1,4-benzoquinone (I) in 200 ml of dry toluene was added in portions 0.3 mole of the appropriate diacyl peroxide with stirring at 95–100° for 1 hr. The mixture was heated for another hour, cooled in ice, and filtered. The solution was concentrated *in vacuo* to dryness and the residue subjected to dry-column chromatography[19] with hexane–Et_2O. The collected purple fraction was dissolved in 300 ml of hexane and extracted with 15 × 15 ml of MeCN. The solvent was removed, the residue was dissolved in 50 ml of benzene, and 5 ml of MeOH and 5 drops of H_2SO_4 were added. The solution was refluxed for 4 hr and extracted with H_2O. The solvent layer was extracted with 0.001 *N* NaOH, and the aqueous layer was washed with hexane.

After acidification of the H_2O extract with HCl, the quinone was separated with extraction by Et_2O. After removal of solvent, the product was recrystallized several times from hexane to give the first six alkylated quinones in Table I. The yields varied between 7 and 15%.

2,3-Dimethoxy-5-hydroxy-1,4-benzoquinone (II). 2,3-Dimethoxy-5-hydroxy-1,4-benzohydroquinone (II) was prepared from 2,3-dimethoxy-1,4-benzoquinone (I) by the Thiele–Winter reaction, acid hydrolysis (HCl) of the triacetate, and oxidation with Ag_2O in Et_2O, yield, 90%, mp 125°, lit.[22] mp 125°.

General Procedure for Acid-Catalyzed Alkylation. The 2,3-dimethoxy-5-hydroxy-1,4-benzohydroquinone (II) was alkylated with the appropriate isoprenoid alcohol using BF_3-etherate as catalyst.[17,18] The purification was performed by chromatography twice over deactivated silica gel[19] with hexane–Et_2O. The purple band was separated and dissolved in the required amount of 0.001 *N* NaOH; the H_2O solution was then extracted with hexane. Acidification of the H_2O solution afforded the product in 15–20% yield.

Results on Inhibition of CoQ_{10} Enzymes and Discussion. For several years, it has been common to assay the inhibition of analogs on the activity of the CoQ enzymes, succinoxidase and NADH-oxidase, on the basis of the following calculations. The concentration of the analog was expressed in nmoles/mg of protein. The specific activity of the enzyme was determined spectrophotometrically in the absence and then in the presence of the analog by measuring the change in optical density of dichlorophenolindophenol for succinoxidase or cytochrome *c* for DPNH-oxidase. The specific activities were expressed in μmoles of dichlorophenolindophenol or cytochrome *c* reduced per min per mg of mitochondrial protein. These *in vitro* tests were carried out both on mitochondrial enzyme preparations which had been solvent extracted for prior removal of coenzyme Q as well as on intact preparations.

To facilitate the comparison of the inhibitory activities of various analogs and derivatives, we now define an antimetabolite CoQ_{10} index which is calculated on the basis of the nmoles of analog per nmoles of CoQ_{10} in the actual mitochondrial preparation which causes approximately 50% inhibition of enzyme activity.[14]

In order to project antimetabolite or therapeutic activity *in vivo*, in a given disease state, we use nonextracted and nonsonicated mitochondria in order to simulate as closely as possible the natural molecular environment of the enzyme. The solvent extraction of mitochondrial preparations and other manipulations presumably alter the nature of the mitochondrial enzymes other than just remove CoQ.

The inhibitory activities of these analogs were measured by their effect on the oxygen uptake of the CoQ enzymes, which was determined by the Warburg manometric method and by oxygen polarography. Both methods gave similar results. The data on inhibition are in Table I and the antimetabolite CoQ indices are in Table II.

For most of the analogs, inhibition of the CoQ enzymes was determined at several concentrations so that the concentration which caused about 50% inhibition could be estimated.

For the CoQ–DPNH-oxidase system, antimetabolite indices of 5–32 were obtained for the decyl, cyclohexylpentyl, tetradecyl, pentadecyl, nonadecyl, and heneicosyl analogs. The cyclohexylpentyl analog was the more effective antimetabolite. The farnesyl and phytyl analogs had indices of 17 and 26, respectively.

The differences in the indices for these 8 analogs are small (6-fold) and it might well be that the differences would be even less if the compounds were tested at additional concentrate and with even greater precision of all parameters. However, it is evident by these data that the nature of the 6 substituent is not particularly specific for antimetabolite activity.

The antimetabolite CoQ indices for the CoQ–succinoxidase system varied from 5.5 to 14 for all 8 analogs, and no difference was evident for the farnesyl and phytyl analogs.

In general, these eight analogs were more effective antimetabolites of CoQ in succinoxidase than in NADH-oxidase, but the difference in sensitivity between the two systems was small.

These results are in general agreement with those of Catlin, *et al.*,[16] who reported that succinoxidase was more effected by the farnesyl, phytyl, and nonadecyl analogs than was NADH-oxidase. The data obtained for the phytyl compound without addition of exogenous CoQ_{10} show values which are comparable to our exper-

Table II. Antimetabolite CoQ Indices of 6-Alkyl-2,3-dimethoxy-5-hydroxy-1,4-benzoquinones

	DPNH-oxidase		Succinoxidase	
	Antimetabolite CoQ index	Inhibition,[a] %	Antimetabolite CoQ index	Inhibition,[a] %
6-*n*-Decyl	9	50	7.7	50
6-*n*-(5-Cyclohexyl)pentyl	5	57	5.5	50
6-*n*-Tetradecyl	10	48	8	50
6-*n*-Pentadecyl	10	50	7	50
6-*n*-Nonadecyl	32	50	8	50
6-*n*-Heneicosyl	11	48	14	50
6-Farnesyl	17	50	10	50
6-Phytyl	26	50	10	50

[a] Actual or approximately 50% inhibitions from extrapolation of the values in Table I.

iments. Their other tests were carried out in the presence of 100 nm of exogenous CoQ_{10} and exhibit a higher antimetabolite activity for the phytyl compared to the farnesyl compound. Our data, however, obtained without adding CoQ_{10}, show a higher antimetabolite activity for the farnesyl than for the phytyl group. This suggests that the improvements in synthesis and purification gave a purer product than that of Catlin, *et al.*,[16] and/or that CoQ_{10} is probably more active in reversing the inhibition caused by the farnesyl than by the phytyl compound.

Our data reveal that NADH-oxidase is more sensitive to structural changes in the 6 substituent than is succinoxidase on the basis of the analogs. This relationship is compatible to the greater sensitivity for coenzymatic activity of NADH-oxidase to members of the coenzyme Q group.[1,23] When we added CoQ_{10} to NADH-oxidase which had been treated with the decyl or the heneicosyl analog, complete reversal of the inhibition was observed. Under the conditions, CoQ_{10} caused 0.33–0.5 reversal of the inhibition of the nonadecyl, farnesyl, and phytyl analogs. In the succinoxidase, the addition of the CoQ_{10} caused only a small reversal of the inhibition due to the decyl, pentadecyl, nonadecyl, and farnesyl analogs.

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Folate Antagonists. 9. 2,4-Diamino-6-[(aralkyl)alkylamino]quinazolines, a Potent Class of Antimetabolites with Prodigious Antimalarial Effects†,‡

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Eighteen 2,4-diamino-6-[(aralkyl)alkylamino]quinazolines (VII) were prepared for antiparasitic and antimetabolite studies. The condensation of 5-chloro-2-nitrobenzonitrile with an *N*-alkylbenzylamine gave 5-[(benzyl)alkylamino]-2-nitrobenzonitriles (V) (25–78%), which were reduced to the corresponding 2-amino-5-[(benzyl)alkylamino]benzonitriles (VI) (48–98%). Cyclization of VI utilizing chloroformamidine·HCl or cyanoguanidine afforded the 2,4-diamino-6-[(benzyl)alkylamino]quinazolines (6–53%). 2,4-Diamino-6-{[ethyl(1-naphthylmethyl)]amino}quinazoline was prepared similarly (14%). Alternatively, reductive alkylation of the requisite 2,4,6-triaminoquinazoline (VIII) with an aliphatic aldehyde over Pt/C yielded various 2,4-diamino-6-(alkylamino)quinazolines (IX) (19–42%), which upon treatment with an α-chlorotoluene derivative afforded VII (21–61%). When administered orally to mice by drug-diet for 6 days, eight of the 2,4-diamino-6-[(aralkyl)alkylamino]quinazolines (VII) were 200–1160 times as potent as quinine·HCl against *Plasmodium berghei* and thus showed suppressive activity comparable with or superior to pyrimethamine (Q = 270), 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]quinazoline (II) (Q = 270), and 2,4-diamino-6-(2-phenyl-1-pyrrolidinyl)quinazoline (III) (Q = 210). Six quinazolines also displayed moderate repository antimalarial effects in mice, and several substances exhibited marked activity orally or parenterally against *P. gallinaceum* in chicks, *P. cynomolgi* and *P. knowlesi* in rhesus monkeys, and *Trypanosoma cruzi* in chick embryo cell culture and in mice. The triaminoquinazolines VII are potent inhibitors of *Streptococcus faecalis* R (*Strep. faecium* var. *durans*) (50% inhibition at 0.2–6 ng/ml), *Strep. faecalis* A (aminopterin-, methotrexate-resistant), and *Lactobacillus plantarum*. Overall structure–activity relationships are discussed.

2,4-Diamino-6-[(3,4-dichlorobenzyl)amino]quinazoline (I) and other 2,4-diamino-6-{[aralkyl and (heterocyclic)-methyl]amino}quinazoline antifolates[3,4] display strong antimalarial effects against sensitive and drug-resistant lines of *Plasmodium berghei* in mice, *P. gallinaceum* in chicks, and *P. cynomolgi* and *P. knowlesi* in rhesus monkeys.[3,4] These substances also possess an encouraging degree of activity against *Trypanosoma cruzi* in chick embryo cell cultures and in mice.[3,4] The corresponding 2,4-diamino-6-{[aralkyl

†This is paper 31 of a series on antimalarial drugs. For paper 30, see ref 1.

‡For the previous paper on folate antagonists see ref 2.