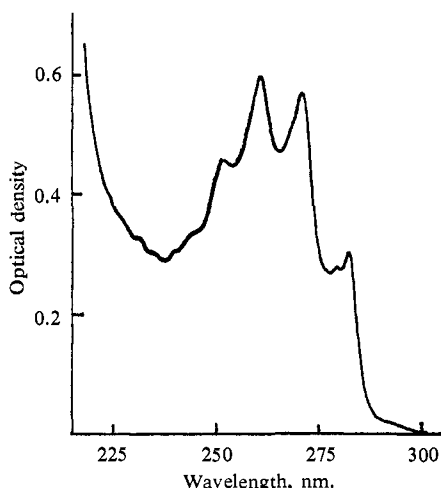
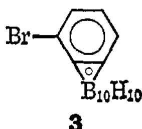


Figure 3. The ultraviolet spectrum of benzocarborane, 2.17 × 10^{-4} M in 2,2,4-trimethylpentane (recorded on a Cary Model 14).

When an excess of N-bromosuccinimide was used for the dehydrogenation of dihydrobenzocarborane (**1**), a less volatile by-product believed to be 1-bromobenzocarborane (**3**) was also obtained, mp 90–92°, nmr (CCl_4) τ 3.22 (t, J = 1.5 Hz, 1H) and τ 3.58 (m, 2H), m/e 273.



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Nalini K. Hota, Donald S. Matteson[8]
Department of Chemistry, Washington State University
Pullman, Washington 99163
Received April 1, 1968

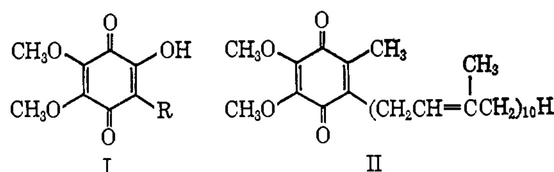
New Hydroxyquinones, Apparent Inhibitors of Coenzyme Q Enzyme Systems[1]

Sir:

New 2,3-dimethoxy-5-hydroxy-6-alkyl-1,4-benzoquinones (I)[2] have been synthesized by procedures that permit considerable structural variations. The alkyl groups are aliphatic, including isoprenoid. These 5-hydroxy analogs of coenzyme Q_{10} (II) are of particular interest for both *in vitro* and *in vivo* studies on the mechanism of the vitamin-like activity of coenzyme Q. The significant structural difference between these analogs and coenzyme Q is the presence of the 5-hydroxy group, instead of the 5-methyl group, which alters the redox characteristics of the quinone. Initial studies, *in vitro*, show that some of these 5-hydroxy analogs strongly inhibit succinoxidase and DPNH oxidase in an intact mitochondrial system or one which is extracted for removal of coenzyme Q_{10}.

The 5-hydroxy analogs were synthesized by complementary alkylation procedures. Where the 6-alkyl group is isoprenoid, the quinones were prepared by the

(1) Coenzyme Q. CIII.
(2) For R, see Table I.



acid-catalyzed alkylation[3] of 1,4,5-trihydroxy-2,3-dimethoxybenzene[4] with the appropriate allyl alcohol. The isoprenoid hydroquinones obtained were air oxidized during purification. Geranyl, farnesyl, tetraprenyl, solanesyl, decaprenyl, and phytyl derivatives were prepared in this manner. Catalytic reduction of the phytyl derivative (10% Pd–C, EtOH) gave the corresponding dihydrophytyl derivative.

Where the 6-alkyl group is nonisoprenoid, these compounds were prepared by the thermal decomposition of the appropriate diacyl peroxide[5] in the presence of 2,3-dimethoxy-5-hydroxy-1,4-benzoquinone. By this reaction, the pentadecyl, heptadecyl, nonadecyl, and 8′,11′,14′-heptadecatrienyl derivatives were prepared.

Difficulty was originally encountered in synthesizing the 2,3-dimethoxy-5-hydroxy-6-alkyl-1,4-benzoquinones due to low yields in preparing 2,3-dimethoxy-1,4-benzoquinone. It was found that this precursor of 1,4,5-trihydroxy-2,3-dimethoxybenzene can be prepared in good yield from 2,3-dimethoxyphenol by an improvement of the procedure of Smith, *et al.*[6] Also, the alkylation procedures gave very complex mixtures, and the desired products have only limited stability to the normal, tlc, purification procedures. It was found that these 5-hydroxy analogs have certain color characteristics which provided guidance for their isolation. On a silica gel G plate the quinone is violet, while it is yellow in organic solvents and red in solid form. Although reasonable stability was found upon thin-layer chromatography on silica gel G plates developed with 4:1 benzene–methanol, the hydroxyquinones do partially change to a second violet substance, with the same R_f, which is violet in organic solvents. Ultimately, it was observed that the hydroxyquinone can be selectively eluted from the silica gel with ether; the decomposition product is eluted with methanol. The hydroxyquinone can also be purified by chromatography on a silica gel column by eluting with hexane containing increasing increments of ether.

The structures of these new hydroxy analogs of coenzyme Q are appropriately assigned by their spectra (Table I).

Initial studies have been made on the activity, *in vitro*, of these new hydroxy analogs of coenzyme Q in mitochondrial succinoxidase and DPNH-oxidase systems which are either intact or extracted for removal of coenzyme Q_{10}.[7] These studies reveal that some of the compounds do have significant inhibitory activity.

The data in Table II show the *in vitro* activity in the succinoxidase system for enzyme preparations which have not been extracted for removal of coenzyme Q_{10}. The farnesyl, solanesyl, phytyl, dihydrophytyl, and

(3) G. D. Daves, Jr., H. W. Moore, D. E. Schwab, R. K. Olsen, J. J. Wilczynski, and K. Folkers, *J. Org. Chem.*, **32**, 1414 (1967).
(4) W. K. Anslow and H. Raistrick, *J. Chem. Soc.*, 1446 (1939).
(5) L. F. Fieser, J. P. Schirmer, S. Archer, R. Lorenz, and P. I. Pfaffenbach, *J. Med. Chem.*, **10**, 513 (1967).
(6) L. I. Smith, J. W. Opie, S. Wawzonek, and W. W. Prichard, *J. Org. Chem.*, **4**, 318 (1939).
(7) L. Szarkowska, *Arch. Biochem. Biophys.*, **113**, 519 (1966).

Table I. Spectral Data of 2,3-Dimethoxy-5-hydroxy-6-alkyl-1,4-benzoquinones

R	Uv $\lambda_{max}^{ethanol}$, mμ	Nmr[a] Vinyl	Methoxyls	Benzylic	Alkyl
Geranyl	298	5.0 (m)	6.00 (s), 6.17 (s)	7.02 (d)	8.0–9.0 (m)
Farnesyl	297	5.0 (m)	6.00 (s), 6.16 (s)	7.00 (d)	7.7–9.0 (m)
Tetraprenyl	297	5.0 (m)	6.00 (s), 6.16 (s)	7.00 (d)	7.7–9.0 (m)
Solanesyl	297	4.98 (m)	6.00 (s), 6.16 (s)	7.00 (d)	7.7–9.0 (m)
Decaprenyl	297	4.98 (m)	6.00 (s), 6.16 (s)	7.00 (d)	7.8–9.0 (m)
Phytyl	297	4.98 (t)	5.98 (s), 6.14 (s)	6.99 (d)	7.6–9.3 (m)
Dihydrophytyl	297		5.98 (s), 6.16 (s)	7.00 (d)	7.4–9.4 (m)
Pentadecyl	299		6.00 (s), 6.15 (s)	7.68 (m)	8.5–9.3 (m)
Heptadecyl	297		6.00 (s), 6.15 (s)	7.72 (m)	8.4–9.5 (m)
Nonadecyl	299		6.01 (s), 6.18 (s)	7.70 (m)	8.4–9.5 (m)
8′,11′,14′-Heptadecatrienyl	299	5.74 (m)	5.99 (s), 6.14 (s)	*b*	7.2–9.2 (m)
8′-(Cyclohexyl)octyl	297		6.01 (s), 6.17 (s)	7.70 (m)	8.2–9.5 (m)

[a] Spectra were obtained using carbon tetrachloride solutions with a Varian Associates HR-100 spectrometer. Values are in τ units. The letters in parentheses refer to peak shape: s = singlet, d = doublet, t = triplet, m = multiplet. [b] The absorption of the benzylic protons falls within the range of the absorption of the alkyl protons.

Table II. Hydroxyquinones in the DPNH-Oxidase and Succinoxidase Systems

	Intact mitochondrial systems							
	DPNH oxidase				Succinoxidase			
Addition[a]	Spec act.[b]	%	Spec act.[b]	%	Spec act.[b]	%	Spec act.[b]	%
None	0.396	65	0.406	50	0.452	85	0.366	80
Q_{10}	0.596	100	0.794	100	0.533	100	0.445	100
Q_{10} + 2,3-dimethoxy-5-hydroxy-6-farnesyl-1,4-benzoquinone			0.608	75	0.309	60		
Q_{10} + 2,3-dimethoxy-5-hydroxy-6-solanesyl-1,4-benzoquinone	0.364	60			0.164	30		
Q_{10} + 2,3-dimethoxy-5-hydroxy-6-phytyl-1,4-benzoquinone	See Table III						0.129	30
Q_{10} + 2,3-dimethoxy-5-hydroxy-6-dihydrophytyl-1,4-benzoquinone	0.302	50					0.096	20
Q_{10} + 2,3-dimethoxy-5-hydroxy-6-nonadecyl-1,4-benzoquinone	0.316	50			0.117	20		

[a] In each case, 100 mμmoles of the quinones was added. [b] Microatoms of oxygen per minute per milligram of protein.

Table III. 2,3-Dimethoxy-5-hydroxy-6-phytyl-1,4-benzoquinone (HPB) in the DPNH-Oxidase and Succinoxidase Systems

	Enzyme activity					
	DPNH oxidase				Succinoxidase	
	Extracted mitochondria		Intact mitochondria		Intact mitochondria	
Addn (mμmoles)	Spec act.[a]	%	Spec act.[a]	%	Spec act.[a]	%
None	0.056[b]	0	0.392	50	0.296	95
Q_{10} (100)	0.344	100	0.813	100	0.308	100
HPB (100)			0.168	20	0.0025	0
Q_{10} (100) + HPB (50)	0.256	70	0.569	70	0.098	30
Q_{10} (100) + HPB (100)	0.188	45	0.306	40	0.048	15
Q_{10} (100) + HPB (200)	0.041	0	0.066	10	0.035	10
Q_{10} (200) + HPB (50)			0.700	85	0.093	30

[a] Microatoms of oxygen per minute per milligram of protein. [b] When the enzyme is extracted, any residual respiration is assumed to be due to unextracted coenzyme Q. The blank is subtracted from each value obtained.

nonadecyl derivatives were tested under comparable conditions, each in the presence of an equimolar amount of coenzyme Q_{10}. Under these conditions the oxygen uptake was depressed, in every case, to below the value for the unsupplemented control. In the presence of the various hydroxyquinones, the enzyme activity ranged from 20 to 60% of that of the CoQ_{10}-supplemented control.

The 2,3-dimethoxy-5-hydroxy-6-phytyl-1,4-benzoquinone (HPB) completely inhibited succinoxidase in the absence of supplementary Q_{10} in the intact enzyme preparation (Table III). When this succinoxidase system was supplemented with 50, 100, or 200 mμmoles of HPB and 100 mμmoles of coenzyme Q_{10} the enzyme activity was depressed to 10–30% of that of the supplemented levels. This system exhibited the same specific activity when supplemented with 50 mμmoles of HPB and 100 or 200 mμmoles of Q_{10}.

In the DPNH-oxidase system of the intact enzyme (Table II), the farnesyl derivative depressed oxygen up-

take to 75% of the coenzyme Q_{10} supplemented control. The solanesyl, phytyl, dihydrophytyl, and nonadecyl analogs depressed enzyme activity to 40–60% of that of the supplemented control value; this was 5–15% below the activity observed in the unsupplemented controls.

HPB (100 mμmoles) also inhibited the intact DPNH-oxidase system in the absence of supplemental Q_{10} (Table III). In this case, the specific activity is intermediate between that observed when the system is treated with 100 and 200 mμmoles of HPB and 100 mμmoles of Q_{10}.

In both the extracted and intact DPNH-oxidase systems (Table III), an inverse relationship between enzyme activity and HPB concentration is observed. In the intact DPNH-oxidase system, the oxidase activity was depressed to 70, 40, and 10% of that of the supplemented control when 50, 100, and 200 mμmoles of HPB and 100 mμmoles of CoQ_{10} were added to the system. When the coenzyme Q_{10} content was increased from 100 to 200 mμmoles in the presence of 50 mμmoles of HPB, a partial reversal of the inhibition was observed. The oxygen uptake was elevated from 70 to 85% of the Q_{10}-supplemented control.

In the DPNH-oxidase system which had been extracted to remove Q_{10}, 200 mμmoles of HPB in the presence of 100 mμmoles of coenzyme Q_{10} completely inhibited the enzyme activity. As the concentration of the HPB was decreased to 100 and 50 mμmoles, keeping the supplement of CoQ_{10} constant, an increase in enzyme activity to 45 and 70% of the supplemented control was observed (Table III).

In both the succinoxidase and DPNH-oxidase systems of intact enzyme preparations, the 2,3-dimethoxy-5-hydroxy-6-phytyl-1,4-benzoquinone (HPB) was more active as an inhibitor than the other alkyl derivatives. Succinoxidase is more sensitive to HPB inhibition than DPNH oxidase. The structural nature of the aliphatic side chain in the 6 position seems to be less significant than the presence of the 5-hydroxy group for inhibitory activity. These exploratory data indicate that Q_{10} may reverse the inhibition by HPB of DPNH oxidase but may not reverse the inhibition of succinoxidase. This apparent difference is at least compatible with recent data showing two enzyme sites[8] for the participation of Q_{10} in electron transfer.

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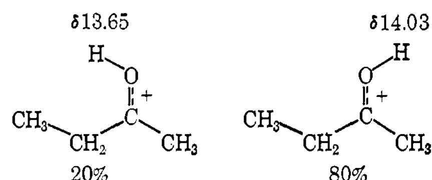
(8) G. Lenaz, G. D. Daves, Jr., and K. Folkers, *Arch. Biochem. Biophys.*, in press.
(9) Stanley G. Harris Postdoctoral Fellow for Biomedical Research.

Joseph C. Catlin, Ronald S. Pardini[9]
G. Doyle Daves, Jr., James C. Heidker, Karl Folkers
Stanford Research Institute
Menlo Park, California 94025
Received April 24, 1968

Protonated β-Phenyl Ketones. Intramolecular π Hydrogen Bonding[1]

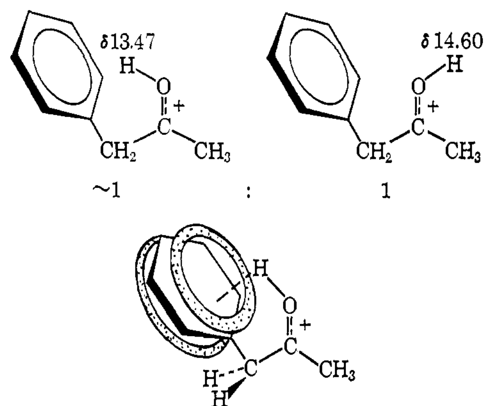
Sir:

In recent times protonated ketones have been observed by nmr in very strong acid media.[2] In such solvents under the right conditions, exchange of the C=O—H proton with the medium is sufficiently retarded that its nmr signal is observed. The chemical shift of the C=O—H proton can serve as a sensitive probe for inductive and conjugative electronic effects.[2] Further, with unsymmetrical ketones the C=O—H signals provide interesting information as to relative amounts of *syn* and *anti* isomers.[2] For example, in the case of protonated methyl ethyl ketone[2b] in SO_2–FSO_3H–SbF_5, the isomer ratio is 80:20, with the C=O—H proton *syn* to the smaller methyl group in the predominant isomer. In this connection protonated



β-phenyl ketones are of interest, and in this communication we report and discuss some of our observations on such species (Table I).

β-Phenyl ketones in an SO_2–FSO_3H–SbF_5 (9:7:2 mole ratio) medium at low temperatures typically show two C=O—H signals, one at unusually high field and the other at a much lower field. For example, phenyl acetone shows one signal at δ 13.47 ppm and the other at δ 14.60 ppm, for a Δδ of 1.13 ppm. As a result, the



chemical shift between *syn* and *anti* isomers is unusually high for β-phenyl ketones.[3] Further, the *syn*:*anti* ratios seem at first to be anomalous in view of the results obtained formerly in other cases. For example, with phenylacetone the isomer ratio is ~1:1, whereas on steric grounds it might have been expected to be much more lopsided than for methyl ethyl ketone.

From the available evidence we can assign the high-field C=O—H signal of the protonated β-phenyl ketones to the *syn* (S) isomer with the proton *syn* to the phenyl group and thus shielded due to the magnetic anisotropy of neighboring phenyl. The low-field C=O—H signal is thus assigned to the *anti* (A) isomer. In this isomer the proton *anti* to the phenyl group is somewhat deshielded due to the latter's inductive effect. This inductive effect may be illustrated with 2-indanone,

(1) Research supported by the National Science Foundation.

(2) (a) T. Birchall and R. J. Gillespie, *Can. J. Chem.*, **43**, 1045 (1965); (b) M. Brookhart, G. C. Levy, and S. Winstein, *J. Am. Chem. Soc.*, **89**, 1735 (1967); (c) G. A. Olah, M. Calin, and D. H. O'Brien, *ibid.*, **89**, 3586 (1967).

(3) Δδ is <0.4 ppm for all ketones not containing groups with large directional anisotropy.