Coenzyme Q. LVII. Synthesis of New Analogs of Coenzyme Q_4 for Biochemical Mechanism Studies*

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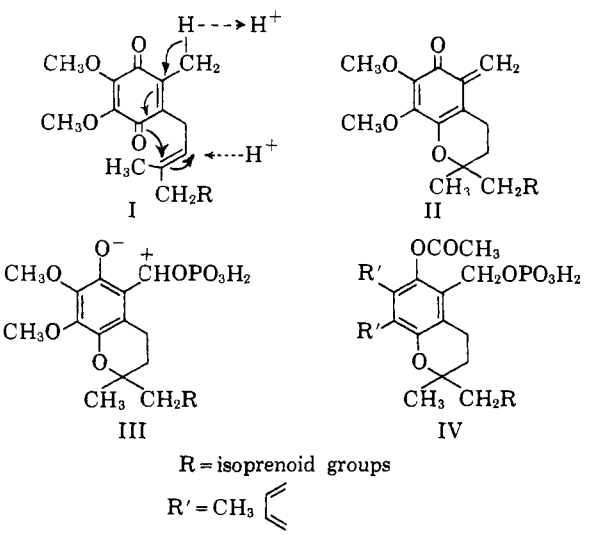
Coenzyme Q participates in mitochondrial electron transfer and has been proposed for a coupled role in oxidative phosphorylation. The mechanism of the latter, on the basis of an *o*-quinone methine species, requires the 5-methyl group. Analogs without the 5-methyl group may permit biological differentiation of electron transfer and the phosphorylation mechanism. Further, such analogs would contribute to the knowledge of the biosynthesis of coenzyme Q, and might also furnish one with biological inhibitors. Consequently, the following compounds in the hexahydrocoenzyme Q_4 series have been synthesized: 2,3-dimethoxy-5-phytyl-1,4-benzohydroquinone; 2,3-dimethoxy-5-phytyl-1,4-benzoquinone; 7,8-dimethoxy-3-ene-2-methyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromenol; 7,8-dimethoxy-2-methyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol; 2,3-dimethoxy-6-(3′-hydroxy-3′,7′,11′,15′-tetramethylhexadecyl)-1,4-benzoquinone; 2,3-dimethoxy-6-(3′-hydroxy-3′,7′,11′,15′-tetramethylhexadecyl)-1,4-benzohydroquinone.

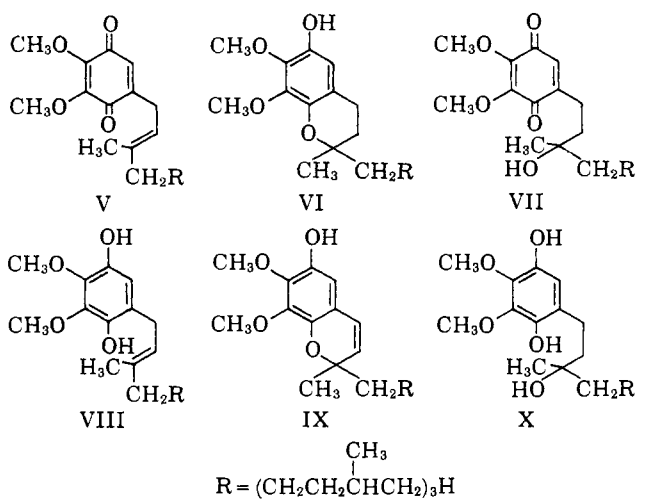
Coenzyme Q (I) has been assigned a position in the electron-transfer chain (Hatefi, 1963). Also, coenzyme Q has been proposed to function in a coupled step of oxidative phosphorylation. A mechanism which has been studied for coupling has involved the isomerization to the quinone methine (II), and then reaction of the quinone methine with P_i, which, after loss of two electrons, gives "active phosphate" (III) (Erickson *et al.*, 1963). Three examples of 5-phosphomethyl derivatives have been synthesized in the coenzyme Q and vitamin K groups (IV) (Wagner *et al.*, 1963). Such syntheses are also applicable in the vitamin E group (R′ = CH_3) (IV).



The presence of the 5-methyl group of coenzyme Q is essential for the isomerization to the *o*-quinone methine in this mechanism. Compounds without the 5-methyl group might dissociate the straight electron-transfer role in respiration from the possible coupling for ATP biosynthesis. Key "5-desmethyl" analogs have now been synthesized, and these new compounds are being biologically compared with their natural 5-methyl counterparts on the basis of these two biochemical mechanisms of coenzyme Q in the mitochondrion.

The biosynthesis of coenzyme Q appears to involve three steps of oxygen and carbon methylation (Olson, 1964), i.e., the two methoxy groups and the 5-methyl group. The order of these methylations is unknown. These new "5-desmethyl" analogs of coenzyme Q_4 will also contribute to the elucidation of the biosynthesis of coenzyme Q.

It has been believed that the reduced forms of the vitamin E and coenzyme Q groups, i.e., hydroquinones, chromanols, and chromenols, function as protective antioxidants. The ability of such compounds to function in these capacities of electron transfer and antioxidant depends directly upon the oxidation potential of the molecule, which is in turn dependent, in part, upon the nuclear substituents. For this reason, the absence of the methyl group in these new compounds may greatly affect these properties. These new compounds are also of interest to examine for possible inhibitory activities.



Synthesis of 2,3-dimethoxy-5-phytyl-1,4-benzoquinone (V) was accomplished by mild ferric chloride oxidation of the hydroquinone obtained by an acid-catalyzed condensation of phytol and 2,3-dimethoxy-1,4-benzohydroquinone. The condensation reaction was carried out in dioxane solution in the presence of boron trifluoride etherate. The reaction mixture was taken up in hexane and purified by chromatography through florisil to give a light-yellow oil which was shown by its physical and chemical properties to be 2,3-dimethoxy-5-phytyl-1,4-benzohydroquinone (VIII). The 60-megacycle NMR[1] spectrum of this product is the best

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[1] Abbreviation used in this work: NMR, nuclear magnetic resonance.

TABLE I
NMR DATA ON COENZYME Q_4 AND DERIVATIVES[a]

Compound	Aromatic	Vinyl	OH	Methoxy	Ring Methyl	Benzylic or Allylic	Saturated Alkyl
Hexahydro-CoQ_4		4.86 (m 10)		6.02 (s 6)	7.92 (s 3)	6.81 (d 2)	8.5–9.2 (m 38)
Hydroquinone of hexahydro-CoQ_4		4.86 (m 10)	4.86 (s 2)	6.12 (s 6)	7.85 (s 3)	6.66 (d 2)	8.5–9.2 (m 38)
Chromanol of hexahydro-CoQ_4			4.51 (s 1)	6.06 (s 3) 6.21 (s 3)	7.91 (s 3)	7.45 (t 2)	8.5–9.2 (m 38)
3′-Hydroxy derivative of hexahydro-CoQ_4			7.60 (s 1)	6.00 (s 6)	8.01 (s 3)	7.52 (t 2)	8.5–9.2 (m 38)
Quinone (V)		3.81 (t 1) 4.94 (t 1)		6.06 (s 3) 6.09 (s 3)		7.09 (m 2)	8.2–9.2 (m 38)
Hydroquinone (VIII)	3.70 (s 1)	4.96 (m 1)	4.96 (m 2)	6.18 (s 3) 6.26 (s 3)		6.48 (d 2)	8.2–9.2 (m 38)
Chromanol (VI)	3.77 (s 1)		4.94 (s 1)	6.16 (s 3) 6.26 (s 3)		7.38 (t 2)	8.2–9.2 (m 38)
3′-Hydroxyquinone (VII)[c]		3.63 (s 1)	7.66 (s 1)	5.96 (s 3) 6.01 (s 3)		7.45 (t 2)	8.2–9.2 (m 38)

[a] s = singlet, d = doublet, t = triplet, m = multiplet. The number in ( ) is the number of protons. [b] 2,3-Dimethoxy-5-methyl-6-(3′-hydroxy-3′,7′,11′,15′-tetramethylhexadecyl)-1,4-benzoquinone. [c] 2,3-Dimethoxy-6-(3′-hydroxy-3′,7′,11′,15′-tetramethylhexadecyl)-1,4-benzoquinone.

criterion of its identity (Table I). The ultraviolet-absorption spectrum showed λ_{max} at 262 mμ, and the infrared-absorption spectrum showed characteristic peaks at 3325 cm^{-1} (OH), and C–H deformation and C–O– stretching frequencies at 1420 cm^{-1} and 1190 cm^{-1}, respectively. Oxidation of the hydroquinone (VIII) with ferric chloride gave a good yield of the quinone (V). The quinone gave a characteristic NMR spectrum (Table I) and showed an ultraviolet-absorption maximum at 272 mμ. The ultraviolet-absorption maximum of hexahydrocoenzyme Q_4 is at 272 mμ (Shunk *et al.*, 1958). The quinone is converted into the chromenol, 7,8-dimethoxy-3-ene-2-methyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromenol (IX), by refluxing in anhydrous pyridine for several hours (McHale and Green, 1963).

Reaction of the quinone (V) with L-ascorbic acid–$CuCl_2$ in glacial acetic acid by the novel cyclization procedure readily resulted in nearly quantitative conversion to the chromanol (VI) (Moore and Folkers, 1964). The NMR spectrum of compound V is very similar (Table I) to the spectrum of the known compound, 7,8-dimethoxy-2,5-dimethyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol (chromanol of hexahydrocoenzyme Q_4), except that this latter chromanol shows a singlet at τ 7.91, corresponding to the 5-methyl group, and the new chromanol (VI) gives a singlet at τ 3.77 corresponding to the aromatic proton at C_5. The ultraviolet-absorption spectrum of compound VI in isooctane shows λ_{max} at 293 mμ. The λ_{max} for the chromanol of hexahydrocoenzyme Q_4 is 292 mμ. The infrared-absorption spectra of the two chromanols are also nearly identical.

Oxidation of the chromanol (VI) with ferric chloride gave an excellent yield of 2,3-dimethoxy-6-(3′-hydroxy-3′,7′,11′,15′-tetramethylhexadecyl)-1,4-benzoquinone (VII). The identity of this compound is based upon the comparison of its NMR, infrared-, and ultraviolet-absorption spectra, as well as the spectra of its hydroquinone (X) (obtained by sodium borohydride reduction) with the corresponding spectra of the known 5-methyl compounds in the coenzyme Q_4 series.

EXPERIMENTAL

2,3-Dimethoxy-5-phytyl-1,4-benzohydroquinone (VIII).—A solution of 9.8 g. (0.333 mole) of phytol, 40 ml of dioxane (freshly distilled from sodium), and 7.5 g (0.044 mole) of 2,3-dimethoxy-1,4-benzohydroquinone was stirred at room temperature in a 300-ml, 3-necked flask. The solution was purged with dry nitrogen, and a solution of 7 ml of freshly distilled BF_3-etherate in 10 ml of dioxane was added over a period of 1.25 hours. The reaction mixture was warmed on a water bath for 2 hours. During this time the reaction was followed by thin-layer chromatography on Silica Gel G in a solvent of 20% ether in *n*-hexane. At the end of the 2-hour period, no phytol could be detected on the thin-layer chromatograms by development with 2% aqueous potassium permanganate solution. Development of the plates with the Emmerie-Engel reagent (sensitive to easily oxidizable compounds) showed one major spot with an R_F value of 0.22. The reaction solution was cooled to room temperature, and two volumes of diethyl ether and one volume of distilled water were added. After extraction, the ether layer was separated and washed with distilled water, with 2% sodium hydroxide containing an excess of sodium hydrosulfite, and again with distilled water. The ether solution was dried over anhydrous sodium sulfate and then concentrated *in vacuo* to yield a viscous yellow-brown oil.

The crude product was dissolved in freshly distilled *n*-hexane and chromatographed through a column of Florisil. The column was first developed with 50% ether in *n*-hexane to elute a yellow oil showing no OH absorption in the infrared. The major product (8 g, 30% yield based upon phytol) was eluted with 10% methanol in ether. The NMR, infrared-, and ultraviolet absorption spectra of this compound are in agreement with the proposed structure (IV).

2,3-Dimethoxy-5-phytyl-1,4-benzoquinone (V).—The hydroquinone (IV) (3.76 g) was dissolved in 170 ml of diethyl ether. A solution of 40 g of $FeCl_3 \cdot 6\,H_2O$ in 35 ml of methanol and 85 ml of water was added. The two-phase mixture was stirred at room temperature for 20 minutes. Thin-layer chromatography on Silica Gel G (25% ether in *n*-hexane) showed that the oxidation reaction was complete in approximately 5 minutes. The chromatogram showed the disappearance of the spot corresponding to the hydroquinone (R_F = 0.22) and the appearance of a yellow spot at R_F = 0.33 corresponding to the quinone (V). To the reaction mixture was added 300 ml of low-boiling petroleum ether and 300 ml of distilled water. The organic phase was collected and washed several times with water; it was washed until the water no longer removed any colored

material. The organic phase was then dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* leaving a bright-yellow oil. The spectral and analytical data (Table I) on the quinone are in agreement with the structure.

Anal. Calcd for $C_{28}H_{46}O_4$; C, 75.1; H, 10.4. Found: C, 74.9; H, 10.4.

A sample of the quinone was readily converted back to the hydroquinone by reduction with sodium borohydride in absolute ethanol. The comparison of the reduced product and the original hydroquinone (IV) was made on thin-layer chromatograms of alumina (50% ether in *n*-hexane) and Silica Gel G (30% ether in *n*-hexane).

7,8-Dimethoxy-3-ene-2-methyl-2-(4',8',12'-trimethyltridecyl)-6-chromenol (IX).—A solution of 25 mg of 2,3-dimethoxy-5-phytyl-1,4-benzoquinone in 15 ml of anhydrous pyridine was refluxed for 20 hours. During this time the appearance of 7,8-dimethoxy-3-ene-2-methyl-2-(4',8',12'-trimethyltridecyl)-6-chromenol was followed by thin-layer chromatography on 0.3-mm Silica Gel G plates. As soon as the spot corresponding to the starting quinone had disappeared, the reaction was stopped. The product gave a positive Emmerie-Engel test, which would be expected for a chromenol. The R_F value of the product on a 0.3-mm Silica Gel G plate was 0.22. The plates were developed in 25% ether in *n*-hexane and detected by both the Emmerie-Engel reagent and a 2% aqueous potassium permanganate solution.

7,8-Dimethoxy-2-methyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol (VI).—A solution of 500 mg (1.04 mmoles) of 2,3-dimethoxy-5-phytyl-1,4-benzoquinone, 906 mg (5.15 mmoles) of L-ascorbic acid, and 300 mg of cupric chloride dihydrate in 50 ml of glacial acetic acid was heated slowly to the reflux temperature. By the time the reflux temperature was reached, the color of the the reaction mixture had changed from orange to brown. This solution was refluxed for approximately 2 hours. Thin-layer chromatograms (0.3-mm Silica Gel G—30% ether in *n*-hexane) showed a disappearance of the quinone and a simultaneous appearance of a spot corresponding to the chromanol (VI). The dark-brown reaction mixture was poured into two volumes of distilled water and then two volumes of redistilled *n*-hexane was added. After extraction, the hexane layer was collected and the aqueous layer was washed twice with *n*-hexane. The combined *n*-hexane solution was washed twice with distilled water, twice with 10% sodium bicarbonate, and again twice with distilled water. The *n*-hexane solution was then dried over anhydrous sodium sulfate and the solvent was removed *in vacuo* leaving a viscous yellow-brown oil, 500 mg (quantitative conversion). This material showed only one spot on silica gel and alumina thin-layer chromatographic plates. The spot was detected by Emmerie-Engel reagent. The spectral data, NMR, infrared, and ultraviolet, are all in agreement with the structure (VI).

An analytical sample was prepared by preparative thin-layer chromatography on Silica Gel G plates using 30% ether in *n*-hexane as solvent.

Anal. Calcd for $C_{28}H_{48}O_4$: C, 74.9; H, 10.8. Found: C, 74.5; H, 10.6.

2,3-Dimethoxy-6-(3'-hydroxy-3',7',11',15'-tetramethylhexadecyl)-1,4-benzoquinone (VII). 7,8-Dimethoxy-2-methyl-2-(4',8',12'-trimethyltridecyl)-6-chromanol (VI) was oxidized analogous to the procedure for the oxidation of the hydroquinone (VIII) to the quinone (V). The spectral data (Table I) on the quinone (VII) are in agreement with the structure. The product shows an R_F value of 0.49 by thin-layer chromatography on 0.3 mm Silica Gel G plates in a solvent of 80% ether in *n*-hexane. The product gives a positive test with leucomethylene blue as would be expected for a quinone. The quinone nucleus was confirmed by the strong carbonyl absorption at 1645 cm^{-1} in the infrared spectrum.

2,3-Dimethoxy-6-(3'-hydroxy-3',7',11',15'-tetramethylhexadecyl)-1,4-benzohydroquinone (X).—A solution of 1.5 mg of 2,3-dimethoxy-6-(3'-hydroxy-3',7,'11',15'-tetramethyl-hexadecyl)-1,4-benzoquinone (VII) in 10 ml of absolute ethanol was allowed to stand at room temperature while a few crystals of sodium borohydride were added. The characteristic color of the quinone immediately faded and the solution became colorless. A thin-layer chromatogram of the product on 0.3-mm Silica Gel G plates (80% ether in *n*-hexane) showed an R_F value of 0.52. The spot was detected by both 2% potassium permanganate and by the Emmerie-Engel reagent. An ultraviolet-absorption spectrum of the product in absolute ethanol showed a λ_{max} at 288 mμ.

References

Erickson, R. E., Wagner, A. F., and Folkers, K. (1963), *J. Am. Chem. Soc. 85*, 1535.

Hatefi, Y. (1963), *Advan. Enzymol. 25*, 275.

McHale, D., and Green, J. (1963), *Chem. Ind. (London)*, 1867.

Moore, H. W., and Folkers, K. (1964), *J. Am. Chem. Soc. 86*, 3393.

Olson, R. (1964), *Federation Proc. 23*, 3393.

Shunk, C. H., Linn, B. O., Wong, E. L., Wittreich, P. E., Robinson, F. M., and Folkers, K. (1958), *J. Am. Chem. Soc. 80*, 4753.

Wagner, A. F., Lusi, A., Erickson, R. E., Arison, B., Trenner, N. R., and Folkers, K. (1963). *J. Am. Chem. Soc. 85*, 3793.