and 1.1 g (70% yield based upon reacted quinone) of the chromenol of coenzyme Q_{10}.[13] A second product was eluted from the column with 5% acetic acid in ether and further purified by thin layer chromatography on silica gel G plates developed in acetic acid–ether–*n*-hexane (1:20:79) to give 100 mg (5% yield based upon reacted quinone) of a mixture of the hydroxyquinones (VII). The ultraviolet and visible absorption spectra of VII (mixture) show typical hydroxyquinone absorptions: in ethanol, λ_{max} 277 mμ ($\epsilon^{1\%}_{1cm}$ 44), 428 mμ ($\epsilon^{1\%}_{1cm}$ 5.7); in ethanolic KOH, λ_{max} 281 mμ ($\epsilon^{1\%}_{1cm}$ 28), 536 mμ ($\epsilon^{1\%}_{1cm}$ 15.4). The infrared absorption spectrum of a carbon tetrachloride solution showed O–H stretching at 3350 cm^{-1}, and the nmr spectrum showed absorptions at τ 4.96 (10) m, CH=; 6.04 (3) s, $-OCH_3$; 6.88 (2) d, ring $-CH_2-$; 8.07 (38) m, $CH_2CH{=}C(CH_3)CH_2-$ and ring $-CH_3$; 8.45 (32) m, $-CH_2CH{=}C(CH_3)CH_2$.

7-Methoxy-d_3-8-methoxy- and 7-Methoxy-8-methoxy-d_3-2,5-dimethyl-2-[3′-methyl-2′-butenyloctakis(3′-methyl-2′-butenylene)-methyl]-6-chromenol. A sample of the hydroxyquinone (VII, mixture) was methylated with deuterated methyl iodide and the resulting coenzyme Q_{10}-d_3 was isomerized to the chromenol by the methods described above.

Acknowledgment. This research was partially supported by funds from the Merck Sharp and Dohme Research Laboratories, and we express our appreciation to Dr. Max Tishler. We are grateful to Mr. William R. Anderson for the nmr spectra which were recorded with a Varian Associates HR-100 spectrometer. The spectra were obtained on carbon tetrachloride solutions with tetramethylsilane as an internal standard. The chemical shifts are expressed as shielding values, τ.[14] Dr. Clifford H. Shunk of the Merck Sharp and Dohme Research Laboratories, Rahway, N. J., kindly provided fumigatin.

(13) H. W. Moore and K. Folkers, *Ann. Chem.*, **684**, 212 (1965).

(14) G. V. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

Structure of Rhodoquinone[1]

Harold W. Moore and Karl Folkers

Contribution from the Stanford Research Institute, Menlo Park, California.
Received November 1, 1965

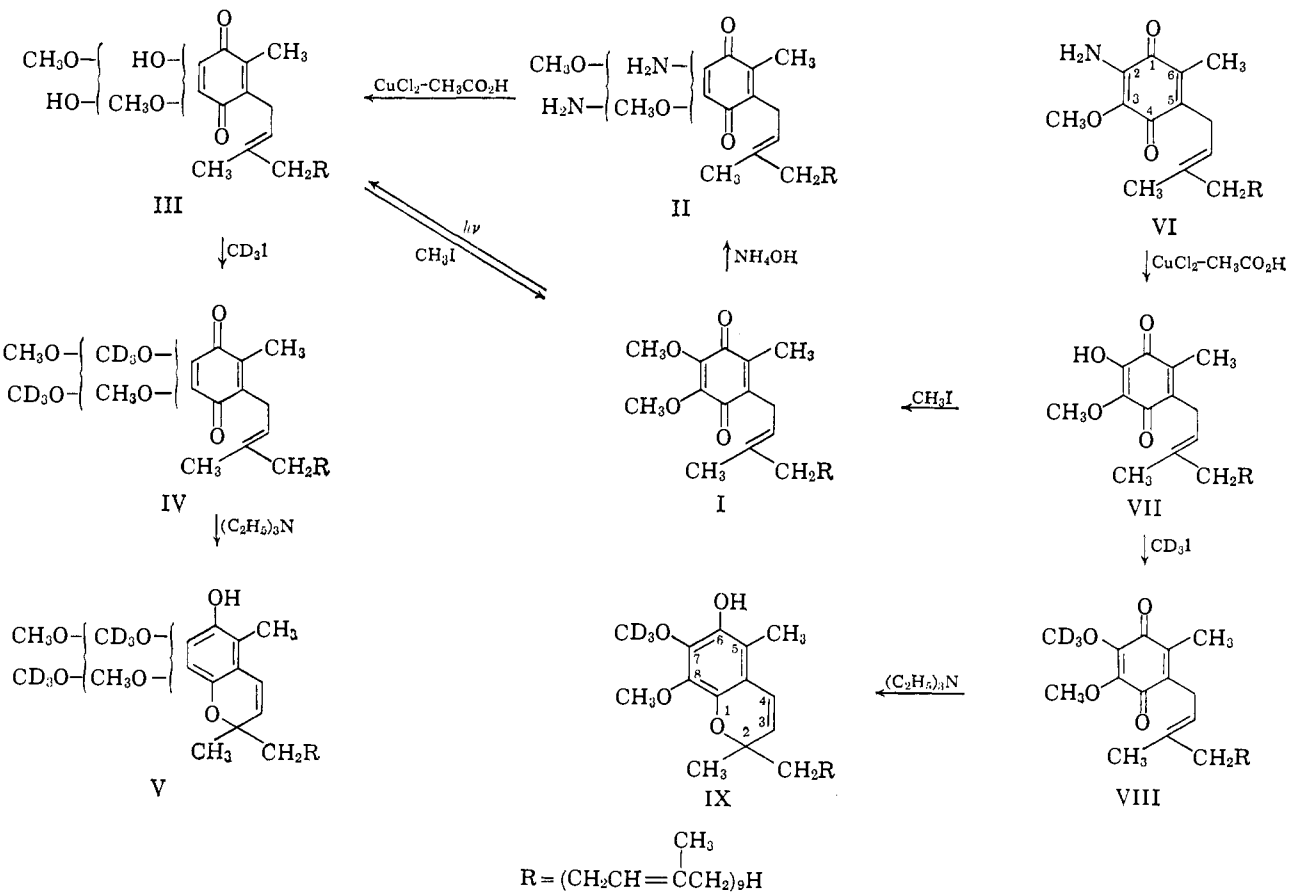
Abstract: When rhodoquinone was discovered from *Rhodospirillum rubrum*, it was assigned a hydroxyquinone structure analogous to coenzyme Q_{10}. This structure was shown to be incorrect, particularly by the properties of the synthetically produced hydroxyquinones. The infrared and nmr data on rhodoquinone surprisingly revealed the presence of an amino group, confirmed by the preparation of the amide with acetic anhydride. Rhodoquinone was converted to coenzyme Q_{10} by replacement of the amino group with a methoxy group, proving the nature and orientation of all substituents except the relative positions of the amino and methoxy groups. Natural rhodoquinone was converted to the 6-chromenol of coenzyme Q_{10}-d_3 (IX) which resulted in the over-all replacement of the amino group by a deuterated methoxyl function. Comparison of the nmr spectrum of this compound with that obtained from its undeuterated analog allowed unequivocal assignment of the deuterated methoxy in IX to the C_7 position which corresponds to the C_2 position in rhodoquinone. For that reason, rhodoquinone is 2-amino-3-methoxy-6-methyl-5-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butenylene)]-1,4-benzoquinone. The reaction of coenzyme Q_{10} with ammonia gives a mixture of two isomeric aminoquinones consisting of about 40% of the 2-amino and 60% of the 3-amino isomers.

In a communication[2] it was shown that rhodoquinone (VI), a naturally occurring quinone from *Rhodospirillum rubrum* and *Athiorhodaceae*, is an aminoquinone, but the orientation of the amino and methoxy groups was unsolved. It has now been established that the amino group is in the 2 position and that rhodoquinone, therefore, has structure VI, 2-amino-3-methoxy-6-methyl-5-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butylene)]-1,4-benzoquinone.

Rhodoquinone was discovered as a natural product from *R. rubrum* by Glover and Threlfall.[3] These workers assigned structure III, a hydroxyquinone, to the compound based upon C–H analysis, molecular weight determinations, microhydrogenation data, and ultraviolet and infrared spectroscopy.[4]

The hydroxyquinone structure for rhodoquinone was unambiguously eliminated by synthesis[5,6] of III and comparison of spectral and chromatographic data of III with natural rhodoquinone. The product (III) showed a color change characteristic of hydroxyquinones; an ethanolic solution was red-orange while an ethanolic potassium hydroxide solution of III was purple. No such change was observed with rhodoquinone. The ultraviolet and visible spectra of the above respective solutions of III showed λ_{max} 277 mμ ($\epsilon^{1\%}_{1cm}$ 44), 428 mμ ($\epsilon^{1\%}_{1cm}$ 5.7) and λ_{max} 281 mμ ($\epsilon^{1\%}_{1cm}$ 28), 536 mμ ($\epsilon^{1\%}_{1cm}$ 15.4). On the other hand, rhodoquinone showed no change when the spectrum of an ethanolic solution was compared with that taken of an ethanolic potassium hydroxide solution, λ_{max} 283 mμ ($\epsilon^{1\%}_{1cm}$ 121), 512 mμ ($\epsilon^{1\%}_{1cm}$ 14). An infrared spectrum of a carbon tetrachloride solution of III showed only one peak in the O–H stretching region at 3350 cm^{-1} and no absorption between 1500 and 1600 cm^{-1}, while rhodoquinone showed N–H stretching at 3495

(1) Coenzyme Q. LXVIII.
(2) H. W. Moore and K. Folkers, *J. Am. Chem. Soc.*, **87**, 1409 (1965).
(3) J. Glover and D. R. Threlfall, *Biochem. J.*, **85**, 14P (1962).
(4) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1959, p 248.
(5) I. Imada, *Chem. Pharm. Bull.* (Tokyo), **11**, 815 (1963).
(6) The hydroxyquinone (III) obtained by photolysis of coenzyme Q_{10} has recently been shown to be a 50:50 mixture of the 2- and 3-hydroxy isomers: H. W. Moore and K. Folkers, *J. Am. Chem. Soc.*, **88**, 564 (1966).



and 3370 and N–H deformation at 1560 cm^{-1}. The nuclear magnetic resonance (nmr) spectrum of III showed absorptions at τ 4.96 (10) m, CH=; 6.04 (3) s, $-OCH_3$; 6.88 (2) d, ring $-CH_2-$; 8.07 (38) m, $-CH_2-CH=CCH_3CH_2-$ and ring $-CH_3$; 8.45 (32) m, $-CH_2-CH=C(CH_3)CH_2-$. Rhodoquinone gave an nmr spectrum which was consistent with the interpretation that amino, methoxy, methyl, and *trans*-polyisoprenoid[7] groups are attached to the *p*-quinoid nucleus: τ 4.94 (10) m, CH=; 5.50 (2) b, $-NH_2$; 6.13 (3) s, $-OCH_3$; 6.88 (2) d, ring $-CH_2-$; 8.04 (38) m, $-CH_2CH=C(CH_3)CH_2-$ and ring $-CH_3$; 8.31 (3) s, *cis* $-CH_2-CH=C(CH_3)_2$; 8.38 (3) s, *trans* ring $-CH_2CH=C(CH_3)CH_2-$; 8.46 (26) m, *trans* $-CH_2CH=C(CH_3)-CH_2-$.

The assignment to the two amino protons to the τ-5.50 peak was substantiated by taking the spectrum of a carbon tetrachloride solution of rhodoquinone containing a catalytic amount of formic acid. Under these conditions, the only change in the spectrum was the disappearance of the peak due to the exchangeable amino protons.

Final confirmation that rhodoquinone is not the hydroxyquinone (III) comes from thin layer chromatographic comparison. Rhodoquinone shows an R_f value of 0.33 and III gives a value of 0.1 on silica gel G plates in a solvent of 40% ether in *n*-hexane.

The presence of an amino group in rhodoquinone was established by reacting the quinone with acetic anhydride to give a monoacetate (amide) derivative. The infrared spectrum of this derivative showed typical amide absorptions, 3250 (N–H stretching) and 1655 and 1618 cm^{-1} (amide I band and quinone carbonyl). The ultraviolet and visible spectra taken in ethanol showed λ_{max} 275 mμ ($\epsilon^{1\%}_{1cm}$ 178) and 400 mμ (sh) ($\epsilon^{1\%}_{1cm}$ 8). The nuclear magnetic resonance spectrum established the presence of an amide linkage: τ 3.15 (1) s, –NH–; 4.96 (10) m, CH=; 5.96 (3) s, $-OCH_3$; 6.88 (2) d, ring $-CH_2-$; 7.94 (3) s, CH_3CO-; 8.08 (38) m, $-CH_2CH=C(CH_3)CH_2-$ and ring $-CH_3$; 8.46 (32) m, $-CH_2CH=C(CH_3)CH_2-$.

Two experimental facts established the adjacent orientation of the amino and methoxy groups and also allowed the assignment of the methyl group to the 6 position and the polyisoprenoid function to position 5. These facts are (1) rhodoquinone can be converted to coenzyme Q_{10} (I), a compound of known structure, by a sequence of reactions which result in replacement of the amino group by a methoxy; and (2) coenzyme Q_{10} can be converted to synthetic rhodoquinone by replacement of one of the methoxy functions by an amino group. Aminoquinones can be deaminated readily to hydroxyquinones under strong acid conditions.[8] These conditions (strong, refluxing aqueous mineral acid) would be too severe for rhodoquinone owing to the facile cyclization of its polyisoprenoid side chain.[9] However, it was found that under mild conditions, employing cupric chloride dihydrate in hot, glacial acetic acid, rhodoquinone was converted to the hydroxyquinone (VII) in a few minutes. Methylation of the hydroxyquinone (VII) with methyl iodide in refluxing acetone in the presence of anhydrous potassium carbonate gave coenzyme Q_{10} (I). The identity of this product as

(7) R. B. Bates, R. H. Carnighan, R. O. Rakutis, and J. H. Schauble, *Chem. Ind.* (London), 1020 (1962).

(8) See, for example, W. K. Anslow and H. Raistrick, *J. Chem. Soc.*, 1446 (1939).

(9) C. H. Shunk, N. R. Trenner, C. H. Hoffman, D. E. Wolf, and K. Folkers, *Biochem. Biophys. Res. Commun.*, 2, 427 (1960).

coenzyme Q_{10} was established through comparison of its spectral data (infrared, ultraviolet, and nmr) and thin layer chromatographic R_f values with those obtained from authentic coenzyme Q_{10}. The reverse process, *i.e.*, conversion of coenzyme Q_{10} to rhodoquinone was also accomplished. The methoxy groups in coenzyme Q_{10} readily undergo alcoholysis in basic media.[10] The two methoxy groups appear to have comparable reactivity in such displacements since the monoethoxy homolog was shown to be a mixture of the two possible isomers.[10a] On the basis of the readily solvolyzable methoxy groups of coenzyme Q_{10}, it appeared that ammonolysis would give a satisfactory synthesis of rhodoquinone. This was accomplished by allowing coenzyme Q_{10} to react with concentrated ammonium hydroxide in a solvent of 1:1 ether–ethanol. The reaction gave a mixture of several unidentified products in addition to a purple quinone, mp 39–45°, which gave the same R_f values as natural rhodoquinone on alumina (0.37) and silica gel G (0.33) thin layer plates in 40% ether in *n*-hexane and in reverse-phase paper chromatography on silicon-impregnated paper (0.75) in a solvent of water–1-propanol (1:4).

The infrared, ultraviolet, and nmr spectra of synthetic and natural rhodoquinone are indistinguishable. Comparison of the melting points of the synthetic and natural rhodoquinone (39–45° *vs.* 69–70°) suggested that the synthetic product is actually a mixture of the two isomeric aminoquinones and that the natural product is formed enzymatically within the bacteria rather than artifactually by the ammonium ion in the growth medium. That the synthetic material (II) was in fact a mixture was shown as follows. The synthetic rhodoquinone was deaminated to the hydroxyquinone (III) as described above. This product was then methylated with deuterated methyl iodide to give coenzyme Q_{10}-d_3 (IV) which was ring closed to the 6-chromenol-d_3 (V) by heating a triethylamine solution of the quinone at 100° for 2 hr in a sealed ampoule. In a previous communication[6] it was shown that the nmr spectrum of the undeuterated 6-chromenol of coenzyme Q_{10} shows absorption of the C_7-methoxy at τ 6.13 and the C_8-methoxy absorbs at 6.25. The nmr spectrum of the 6-chromenol-d_3 (V) obtained above showed a total of three methoxy protons as two peaks at τ 6.13 and 6.25 in a ratio of approximately 1.8:1.2. For that reason, the synthetic aminoquinone (II) must be an isomeric mixture comprised of about 40% of the 2-amino and 60% of the 3-amino isomers.

The remaining question with regard to the structure of rhodoquinone concerns the orientation of the amino and methoxy groups. This problem was resolved by conversion of natural rhodoquinone to the 6-chromenol of coenzyme Q_{10}-d_3 (IX) through the above sequence, VI–VII–VIII–IX. The nmr spectrum of IX showed only C_8-methoxy absorption as a singlet corresponding to three protons at τ 6.25. For that reason, the deuterated methoxy group is at C_7 and, thus, the hydroxy group in VII and the amino group in rhodoquinone (VI) must be at position 2. The structure of rhodoquinone is then 2-amino-3-methoxy-6-methyl-5-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butenylene)]-1,4-benzoquinone (VI).

The structure of rhodoquinone may be of biological significance since it has been pointed out[11] that many naturally occurring aminoquinones, streptonigrin,[11] mitomycin C,[12] porfiromycin,[12] and the actinomycins,[13] possess marked anticancer activity.[14] The activity of these compounds, which have only the aminoquinone moiety in common, is lost by replacement of the primary amine function. Also, the apparent enzymatic formation of rhodoquinone from coenzyme Q_{10} in *R. rubrum*[15] may indicate that the aminoquinone has a photosynthetic function.

Experimental Section

Isolation of Rhodoquinone. Rhodoquinone was isolated from *R. rubrum* according to the published methods of Rudney and Parson.[16–18]

2-Hydroxy-3-methoxy- and 2-Methoxy-3-hydroxy-6-methyl-5-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butenylene)]-1,4-benzoquinone (III) from the Photolysis of Coenzyme Q_{10}. The experimental procedure employed for the synthesis of the hydroxyquinone mixture (III) has been described in earlier publications.[6,19]

2-Methoxy-3-acetamido-6-methyl-5-[3′-methyl-2′-butenylenakis-(3′-methyl-2′-butenylene)]-1,4-benzoquinone (VIII). A solution of 5 mg (0.006 mmole) of rhodoquinone (VI) in 5 ml of acetic anhydride was allowed to stand at room temperature for 24 hr. During this time, the color of the reaction solution changed from purple to yellow. The solvent was removed *in vacuo* and the resulting yellow oil was purified by preparative thin layer chromatography on silica gel G plates developed in 40% ether in *n*-hexane to give 4.7 mg of the amide derivative of rhodoquinone. The ultraviolet, infrared, and nuclear magnetic resonance spectra of this compound are all consistent with 2-methoxy-3-acetamido-6-methyl-5-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butenylene)]-1,4-benzoquinone (*vida supra*).

Preparation of Rhodoquinone (II). Two grams (0.0022 mmole) of coenzyme Q_{10} (I) was dissolved in 50 ml of a solution of diethyl ether–ethanol (1:1). Two milliliters of concentrated ammonium hydroxide was added and the reaction solution was allowed to stand at room temperature for 5 days. During this time, the color of the solution changed from yellow to deep orange-red. The solvent was removed *in vacuo* and the resulting red-orange oil was chromatographed through a column of 300 g of Florisil. Unreacted coenzyme Q_{10} (997 mg) was collected in the 10% ether–hexane eluate. A purple band which just followed the unreacted coenzyme Q_{10} down the column was eluted with 10% ether in *n*-hexane. This purple quinone (sensitive to leucomethylene blue reagent) was further purified by preparative thin layer chromatography on silica gel G plates developed in 40% ether in *n*-hexane. This material was recrystallized three times from 95% ethanol to give 118 mg, mp 39–45°. Thin layer and paper chromatographic comparison of II with natural rhodoquinone showed no difference in R_f values. The infrared, ultraviolet, and nuclear magnetic resonance spectra of II were indistinguishable from the corresponding data obtained from natural rhodoquinone.

2-Hydroxy-3-methoxy- and 2-Methoxy-3-hydroxy-6-methyl-5-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butenylene)]-1,4-benzoquinone (III) from Synthetic Rhodoquinone. A solution of 39 mg of synthetic rhodoquinone (II) in 2 ml of glacial acetic acid was treated with 20 mg of $CuCl_2 \cdot 2H_2O$ and 1 drop of water. The resulting purple mixture was heated to reflux. Upon obtaining the reflux tempera-

(10) (a) B. O. Linn, N. R. Trenner, B. H. Arison, R. G. Weston, C. H. Shunk, and K. Folkers, *J. Am. Chem. Soc.*, **82**, 1647 (1960); (b) C. H. Shunk, D. E. Wolf, J. F. McPherson, B. O. Linn, and K. Folkers, *ibid.*, **82**, 5914 (1960).

(11) K. V. Rao, K. Biemann, and R. B. Woodward, *ibid.*, **85**, 2532 (1963).

(12) J. S. Webb, D. B. Cosulick, J. H. Mowat, J. B. Patrick, R. W. Boschhard, W. E. Meyer, R. P. Williams, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, *ibid.*, **84**, 3185, 3187 (1962).

(13) H. Brockman, *Fortschr. Chem. Org. Naturstoff*, **18**, 1 (1960).

(14) L. D. Hamilton, W. Fuller, and E. Reich, *Nature*, **198**, 538 (1963).

(15) H. Rudney and W. W. Parson, *J. Biol. Chem.*, **238**, PC3137 (1963).

(16) W. W. Parson and H. Rudney, *ibid.*, **240**, 1855 (1965).

(17) W. W. Parson and H. Rudney, *Biochemistry*, **53**, 599 (1965).

(18) W. W. Parson and H. Rudney, *Proc. Natl. Acad. Sci. U. S.*, **51**, 444 (1964).

(19) H. W. Moore and K. Folkers, *Ann. Chem.*, **684**, 212 (1965).

ture, the color of the reaction mixture had changed from purple to yellow. The reaction mixture was cooled immediately and poured into 20 ml of water. The aqueous mixture was extracted three times with 10-ml portions of ether. The combined ether extract was dried over anhydrous sodium sulfate and then concentrated *in vacuo*. The red residue was subjected to preparative thin layer chromatography on silica gel G plates in a solvent of 1:1 $CHCl_3$–C_6H_6. The hydroxyquinone (III) which appeared as a deep purple spot was collected to give 15 mg of a mixture of 2-hydroxy-3-methoxy- and 2-methoxy-3-hydroxy-6-methyl-5-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butenylene)]-1,4-benzoquinone (III). The infrared, ultraviolet, and nuclear magnetic resonance spectra are all consistent with those of a mixture of the hydroxyquinones and indistinguishable from the spectral data obtained from the hydroxyquinone mixture synthesized by the photolytic O-demethylation of coenzyme Q_{10}.

2-Methoxy-d_3-3-methoxy- and 2-Methoxy-3-methoxy-d_3-6-methyl-5-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butenylene)]-1,4-benzoquinone (IV). A solution of 15 mg of the hydroxyquinone mixture (III) in 5 ml of acetone, 100 μl. of 99.9% deuterated methyl iodide, and 10 mg of anhydrous potassium carbonate was refluxed under nitrogen for 3 hr. During this time, the color of the reaction mixture changed from purple to yellow. Two volumes of water was added and the aqueous mixture was extracted with ether. The ether extract was dried over anhydrous sodium sulfate and the solvent then was removed *in vacuo*. The yellow residue was subjected to preparative thin layer chromatography on silica gel G plates in a solvent of 1:1 chloroform–benzene to give 9 mg of coenzyme Q_{10}-d_3 in which the deuterium was distributed between the 2- and 3-methoxy groups. The nmr spectrum of this material was identical with that obtained from an authentic sample of coenzyme Q_{10} except the methoxy absorption at τ 6.06 corresponded to only *three protons instead of six*. The infrared and ultraviolet absorption spectra were also consistent with the structure. Thin layer chromatographic comparison of IV with authentic coenzyme Q_{10} under a variety of conditions showed no difference.

7-Methoxy-d_3-8-methoxy- and 7-Methoxy-8-methoxy-d_3-2,5-dimethyl-2-[3′-methyl-2′-butenyloctakis(3′-methyl-2′-butenylene)-methyl)]-6-chromenol (V). A solution of 9 mg of IV in 3 ml of triethylamine was purged with nitrogen and sealed in a glass ampoule. The ampoule was heated at 100° for 2 hr; during this time, the color of the solution changed from yellow to nearly colorless. The solvent was then removed *in vacuo* and the 6-chromenol mixture (V) was isolated by thin layer chromatography on alumina plates in a solvent of 1:1 chloroform–benzene, yield 7 mg (66%). The 6-chromenol structure was confirmed by infrared, ultraviolet, and nmr spectral data and thin layer chromatographic comparison of V with authentic undeuterated 6-chromenol of coenzyme Q_{10}.

2-Hydroxy-3-methoxy-6-methyl-5-[3′-methyl-2′-butenylenakis-(3′-methyl-2′-butenylene)]-1,4-benzoquinone (VII) from Natural Rhodoquinone. The hydroxyquinone (VII) was prepared from natural rhodoquinone by treatment of the aminoquinone (VI) with $CuCl_2 \cdot 2H_2O$–CH_3CO_2H. The experimental procedure was completely analogous to the procedure described above which reported the conversion of II to III.

2-Methoxy-d_3-3-methoxy-6-methyl-5-[3′-methyl-2′-butenylenakis-(3′-methyl-2′-butenylene)]-1,4-benzoquinone (VIII). Coenzyme Q_{10}-d_3 (VIII) was prepared by methylation of VII with 99.9% deuterated methyl iodide. The experimental procedure was completely analogous to the procedure described above which reported the conversion of III to IV. The infrared, ultraviolet, and nmr spectra were consistent with structure VIII. Thin layer chromatographic comparison of VIII with an authentic sample of coenzyme Q_{10} showed no difference.

7-Methoxy-d_3-8-methoxy-2,5-dimethyl-2-[3′-methyl-2′-butenyl-octakis(3′-methyl-2′-butenylene)methyl]-6-chromenol (IX). The deuterated 6-chromenol (IX) was made by treatment of VIII with triethylamine. The procedure was analogous to the method described above which reported the conversion of IV to V. The infrared, ultraviolet, and nmr spectra were all consistent with structure IX. Thin layer chromatographic comparison of IV with an authentic undeuterated sample of the 6-chromenol of coenzyme Q_{10} showed no difference.

Acknowledgment. This research was partially supported by funds from the Merck Sharp and Dohme Research Laboratories and we express our appreciation to Dr. Max Tishler. The nmr spectra were recorded by Mr. William R. Anderson with a Varian Associates HR-100 spectrometer. Spectra were obtained in carbon tetrachloride solutions with tetramethylsilane as an internal standard. Chemical shifts are expressed as shielding values (τ) as defined by Tiers.[20]

(20) G. V. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).