not identified. At the end of the run with the chloropropyl ethyl sulfone, the reaction mixture was yellow, while the chloroethyl propyl sulfone reaction mixture was colorless.

Acknowledgment. The author is deeply grateful to Professor F. G. Bordwell for initially calling attention to this interesting system and for his thoughtful comments and valuable counsel in preparation of the manuscript. Special thanks are due to N. F. Chamberlain of the Esso Research and Engineering Co. for his help with the n.m.r. determinations, and to Esso Research and Engineering Co. directly for permission to publish these results.

New Method for Structural Assignments of Hydroxy Analogs of Coenzyme Q[1]

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Contribution from the Stanford Research Institute, Menlo Park, California.
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Abstract: A new method is now available which permits a direct structural assignment of quinones, chromenols, and chromanols of the coenzyme Q group which are unsymmetrically substituted in the benzenoid nucleus. The new method is based upon assignment of the nuclear magnetic resonance absorption of each of the two methoxy groups to a position on the chromenol and chromanol nucleus of a typical coenzyme Q; in turn, such assignments may be related to the quinone. Certain hydroxyquinones related to both coenzyme Q_7 and Q_{10} have been assigned structures in the past on the basis of the understanding that such hydroxyquinones were single compounds. This new method for structural assignments has revealed that the previously described hydroxy analogs of coenzyme Q_7 and Q_{10} are in reality approximately 50:50 mixtures of the two possible hydroxy derivatives.

Several compounds in the coenzyme Q group have been described in which one of the two methoxy groups has been replaced by another substituent.[2-7] Until now, no technique has been reported that could distinguish which of the two methoxy groups had been replaced or modified. A procedure utilizing nuclear magnetic resonance (nmr) spectroscopy has now been developed which allows unambiguous structural assignments of such compounds. This procedure involves the conversion of a substituent on the quinone nucleus into a deuterated methoxy group followed by ring closure to the chromenol (V) or chromanol (VI) derivative. The separate absorptions of the two methoxy groups in the nmr spectra of the chromenol and chromanol derivatives have now been structurally assigned, and comparison of the spectrum of the deuterated derivative with that of its undeuterated analog allows unequivocal structural assignments.

The chemical shifts of the methoxy protons in hexahydrocoenzyme Q_4 are almost identical, differing by only *ca.* 0.1 cps. In contrast, the nmr spectra of the chromenol (V)[8] and the chromanol (VI)[9,10] show clearly distinguishable methoxy absorptions at τ 6.13, 6.25, 6.14, and 6.25, respectively. There has been unequivocal assignment of the peak at τ 6.13 to the C_7-methoxy and the peak at τ 6.25 to the C_8-methoxy in the chromenol (V), and the peak at τ 6.14 for the C_7-methoxy group and the peak at τ 6.25 for the C_8-methoxy group in the chromanol (VI) have been established from the nmr spectra of the corresponding compounds in which the C_7-methoxy group was labeled with deuterium.

These deuterated derivatives were synthesized as depicted in I → V and VI. Fumigatin, 2-hydroxy-3-methoxy-6-methyl-1,4-benzoquinone (I), was reduced to the hydroquinone (II) by aqueous sodium dithionite. The hydroquinone II was alkylated with phytol and the hydroxyquinone (III) was obtained in 41% yield after ferric chloride oxidation followed by purification by column chromatography on Florisil. The procedure used to prepare III was a modification of the one used previously to make isoprenoid derivatives of fumigatin.[11]

The spectral data (ultraviolet, infrared, and nmr) of III are in agreement with the structure. The infrared spectrum shows O–H stretching absorption at 3400 cm^{-1} and typical quinone carbonyl absorptions at 1640 and 1615 cm^{-1}. The ultraviolet absorption spectrum shows λ_{max} 271 mμ ($\epsilon^{1\%}_{1cm}$ 237), 278 mμ ($\epsilon^{1\%}_{1cm}$ 238), and 440 mμ ($\epsilon^{1\%}_{1cm}$ 5.3). The nmr spectrum shows absorptions at τ 5.12 (1) t, CH=; 6.00 (3) s, $-OCH_3$; 6.85 (2) d, ring $-CH_2$; 7.98 (3) s, ring $-CH_3$; 8.07 (2) t, allylic $-CH_2$; 8.23 (3) s, allylic $-CH_3$; 8.91 (19) m, $-CH_2CH_2CH-$; 9.15 (12) m, $-CH_3$.

Methylation of III with deuterated methyl iodide in acetone in the presence of anhydrous potassium carbonate gave an 83% yield of deuterated hexahydrocoenzyme Q_4 (IV). The nmr spectrum of IV differed only from that of undeuterated hexahydrocoenzyme Q_4

(1) Coenzyme Q. LXVI.
(2) I. Imada, *Chem. Pharm. Bull.* (Tokyo), **11**, 815 (1963).
(3) I. Imada and H. Morimoto, *ibid.*, **13**, 130 (1965).
(4) I. Imada, Y. Sanno, and H. Morimoto, *ibid.*, **12**, 1056 (1964).
(5) B. O. Linn, N. R. Trenner, C. H. Shunk, and K. Folkers, *J. Am. Chem. Soc.*, **81**, 1263 (1959); C. H. Shunk, D. E. Wolf, J. F. McPherson, B. O. Linn, and K. Folkers, *ibid.*, **82**, 5914 (1960).
(6) C. H. Shunk, J. F. McPherson, and K. Folkers, *J. Org. Chem.*, **25**, 1053 (1960).
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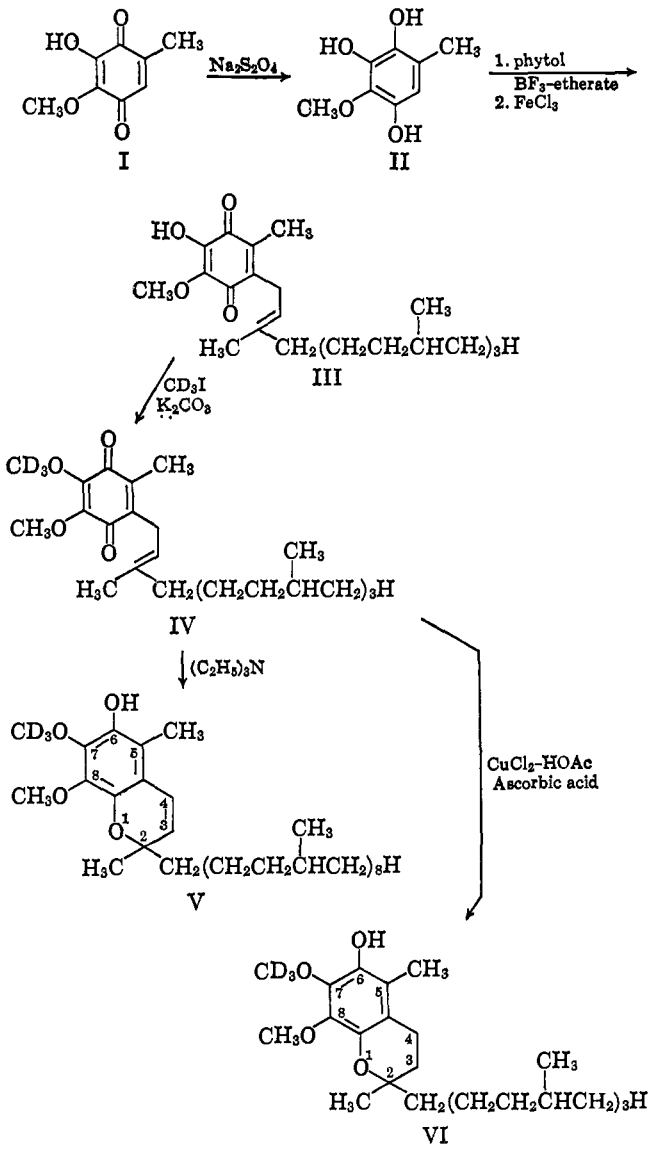
(9) N. R. Trenner, E. L. Wong, B. O. Linn, and K. Folkers, *J. Am. Chem. Soc.*, **85**, 239 (1963).
(10) H. W. Moore and K. Folkers, *ibid.*, **86**, 3393 (1964).
(11) C. H. Shunk, J. F. McPherson, and K. Folkers, *J. Org. Chem.*, in press.

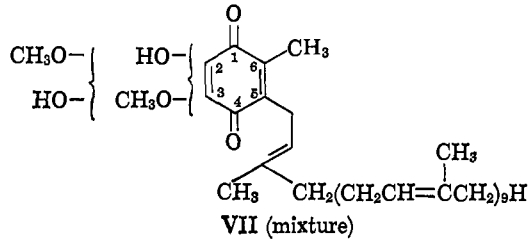


in that the methoxy absorption at τ 6.06 in the spectrum of IV corresponded to only three protons instead of six. A solution of IV in triethylamine was heated at 100° in an atmosphere of nitrogen in a sealed tube for 2 hr, and the chromenol (V) was obtained in a 60% yield.[12] Comparison of the nmr spectrum of V with that of the undeuterated chromenol of hexahydrocoenzyme Q_4 allows the unambiguous assignment of the peak at τ 6.13 to the C_7-methoxy group and the peak at 6.25 to the C_8-methoxy group (Figure 1). The spectrum (Figure 1) of the deuterated chromenol V shows only one methoxy absorption at τ 6.25 while the spectrum of its undeuterated analog shows methoxy absorptions at τ 6.13 and 6.25.

The absorption of the C_5-methyl group in V at τ 7.92 is unique as an internal standard and, thus, the difference between this absorption and those of the methoxy groups can be used as an additional check on the assignments of the methoxy groups. The spectrum of V shows a difference of 100.2 cps between the methoxy and the C_5-methyl absorptions while the spectrum of the undeuterated chromenol shows differences of 107.4 and 100.2 cps, respectively, for the low- and high-field methoxy absorptions and the C_5-methyl peak. Thus, the high-field methoxy absorption (τ 6.25) is due to the C_8-methoxy group and the low-field methoxy peak (τ 6.13) is due to the C_7-methoxy group.

The chromanol (VI) was prepared[7] from IV in 90% yield using $CuCl_2 \cdot 2H_2O$ and ascorbic acid in glacial acetic acid solvent. The nmr spectrum of VI (Figure 2) shows only one methoxy absorption at τ 6.25 corresponding to three protons while the spectrum of the undeuterated analog was identical with the above except for the presence of an additional methoxy peak at τ 6.14. On the basis of the C_5-methyl absorption at τ 8.00 as an internal standard, there is a difference of 105.0 cps between this peak and the methoxy peak in the spectrum of VI, and differences 111.0 and 105.0 cps, respectively, for the low- and high-field methoxy absorption in the spectrum of the undeuterated chromanol. These data, therefore, allow the assignment of the high-field methoxy absorption (τ 6.25) to the C_8-methoxy group and the low-field methoxy absorption (τ 6.14) to the C_7-methoxy substituent.

These data are conveniently applied to the structural elucidation of the hydroxyquinone (VII) which was obtained by the photolytic demethylation of coenzyme Q_{10}. Imada, *et al.*,[3,4] have previously photolyzed



coenzyme Q_7 in an ethanolic solution by sunlight. They considered that they had a single hydroxyl derivative and assigned the hydroxy to the 2 position and the methoxy to the 3 position on the basis of a color reaction of the chromenol, obtained by base-catalyzed ring closure of the hydroxyquinone. However, the data of Imada, *et al.*, do not eliminate the possibility that the photolytic "product" is a mixture of two isomeric hydroxyquinones, the 2- and 3-hydroxyquinones. A refluxing ethanolic solution of coenzyme Q_{10} was photolyzed with a 150-w sunlamp. The infrared, ultraviolet, and nmr spectral data, as well as thin layer chromatographic behavior of the "product" appeared to be consistent with a homogeneous compound; however, when VII was methylated with deuterated methyl iodide and then cyclized to the chromenol, the nmr spectrum showed that the deuterium was equally distributed between the 7- and the 8-methoxy groups. This spectrum showed methoxy absorptions at τ 6.07 and 6.18 in a 1:1 ratio corresponding to a total of three protons. For that reason, the photolytic "product," VII, must actually consist of a 50:50 mixture of the 2- and the 3-hydroxyquinones.

Another application of this new method solved the orientation of the amino and methoxy groups in rhodoquinone.[7]

Experimental Section

2-Hydroxy-3-methoxy-5-phytyl-6-methyl-1,4-benzoquinone (III). A solution of 600 mg (3.57 mmoles) of fumigatin in 60 ml of 1:1 ethanol–diethyl ether was vigorously stirred at room temperature under nitrogen while 30 ml of a 10% aqueous solution of sodium

(12) I. Imada and H. Morimoto, *Chem. Pharm. Bull.* (Tokyo), **12**, 1047 (1964).

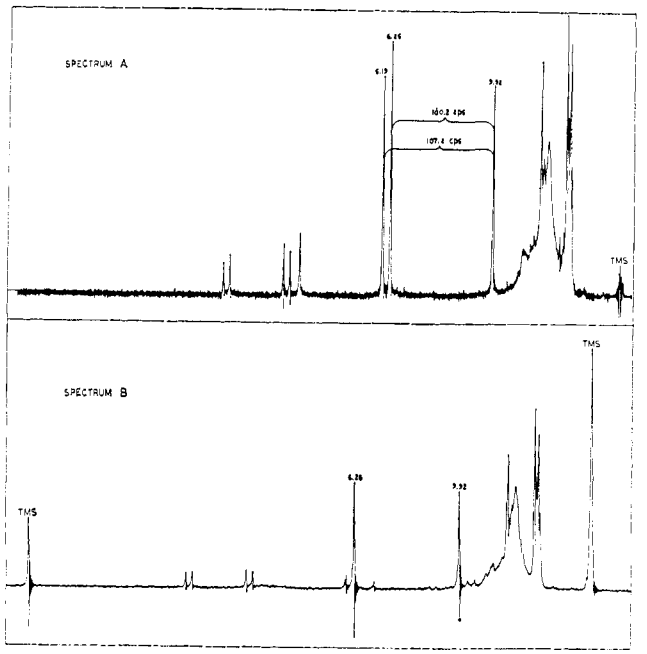


Figure 1. Nuclear magnetic resonance spectra of 2,5-dimethyl-7,8-dimethoxy-2-(4′,8′,12′-trimethyltridecyl)-6-chromenol (spectrum A) and 2,5-dimethyl-7-methoxy-d_3-8-methoxy-2-(4′,8′,12′-trimethyltridecyl)-6-chromenol (spectrum B).

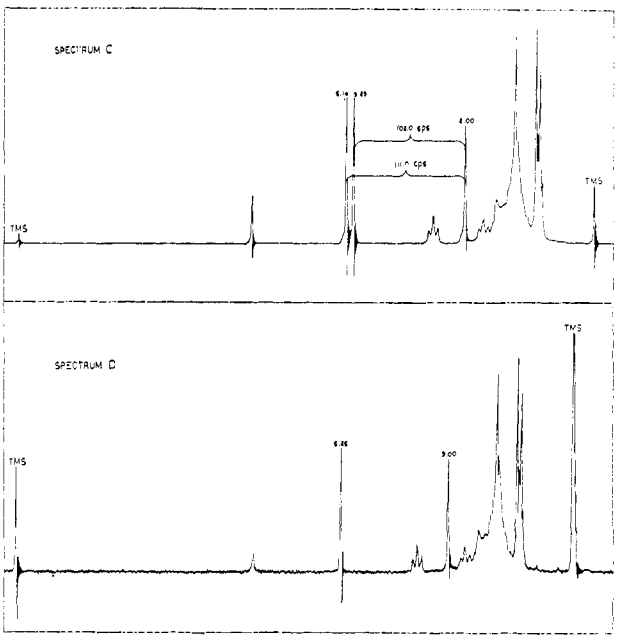


Figure 2. Nuclear magnetic resonance spectra of 2,5-dimethyl-7,8-dimethoxy-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol (spectrum C) and 2,5-dimethyl-7-methoxy-d_3-8-methoxy-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol (spectrum D).

dithionite was slowly added (30 min). Small pieces of Dry Ice were added to the reaction solution during the addition of the dithionite. The reaction solution then was extracted three times with ether and the combined ether extract was concentrated *in vacuo* to give 600 mg of the hydroquinone of fumigatin as a light orange oil.

The hydroquinone was dissolved in 25 ml of freshly distilled dioxane, and 3.5 g of phytol and 500 mg of anhydrous $MgSO_4$ were added. The reaction was carried out in a 100-ml, three-necked flask equipped with a condenser and protected with $CaCl_2$, a nitrogen inlet tube, and a dropping funnel. Freshly distilled BF_3–etherate (1.7 ml) was added over a period of 1 hr and the reaction mixture was allowed to continue to stir at room temperature for an additional 2.5 hr. The reaction mixture was then poured into 500 ml of water and the aqueous solution was extracted four times with 100-ml portions of ether. The combined ether extract was dried over anhydrous Na_2SO_4, and the solvent was then removed *in vacuo* to give a viscous red oil. This oil was then dissolved in 200 ml of diethyl ether and the ethereal solution was shaken in a separatory funnel with a solution of 4 g of $FeCl_3 \cdot 6H_2O$ in 1:1 H_2O–CH_3OH (100 ml). After approximately 2 min of shaking, the ether solution was collected, washed with water, dried, and concentrated to give a dark red oil. This oil was placed on a column containing 400 g of Florisil and eluted with 500 ml of ether, 500 ml of a 1:1 solution of ether–ethanol, and then with 500 ml of ethanol. The desired product (III) forms a purple complex with the Florisil and is not eluted from the column until a solvent containing acetic acid is used. The quinone (III) was eluted from the column with 5% acetic acid in ether. Removal of the solvent gave 650 mg. The spectral (infrared, ultraviolet, and nmr) data, described above, are consistent with structure III.

2-Methoxy-d_3-3-methoxy-5-phytyl-6-methyl-1,4-benzoquinone (IV). A solution of 100 mg of III in 5 ml of acetone, 100 mg of deuterated methyl iodide, and 25 mg of anhydrous K_2CO_3 was refluxed for 3 hr. During this time, the solution changed from purple to yellow. The solvent then was removed and the resulting yellow oil was subjected to preparative thin layer chromatography on silica gel G plates in a solvent of 1:1 $CHCl_3$–C_6H_6. The product (IV) was isolated in 83% yield. The spectral data (infrared, ultraviolet, and nmr) are all consistent with the assigned structure.

5-Methyl-7-methoxy-d_3-8-methoxy-2-methyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromenol (V). The preparation of the chromenol (V) was based on the method reported by Imada and Morimoto.[3] A solution of 25 mg of IV in 2 ml of triethylamine was purged with nitrogen and sealed in a glass ampoule. The ampoule was heated to 100° for 2 hr; during this time, the color of the solution changed from yellow to nearly colorless. The solvent then was removed *in vacuo* and the chromenol (V) was isolated by thin layer chromatography on alumina plates in a solvent of 1:1 $CHCl_3$–C_6H_6, yield 20 mg. (80%). The infrared, ultraviolet and nmr spectra were consistent with structure V.

5-Methyl-7-methoxy-d_3-8-methoxy-2-methyl-2-(4′,8′,12′-trimethyltridecyl)-6-chromanol (VI). A solution of 50 mg (0.103 mmole) of IV, 90.6 mg (0.515 mmole) of L-ascorbic acid, and 30 mg of cupric chloride dihydrate in 10 ml of glacial acetic acid was heated slowly to the reflux temperature. By the time the reflux temperature was reached, the color of the reaction mixture had changed from orange to brown. The solution then was refluxed for 2 hr and, during that time, the reaction was followed by thin layer chromatography on silica gel G plates developed in 18% ether in *n*-hexane. The chromatograms showed a complete disappearance of the quinone after about 45 min and the gradual appearance of two spots, one corresponding to the hydroquinone and the other to the 6-chromanol of hexahydrocoenzyme Q_4. After 2 hr the only detectable spot by the Emmerie–Engel reagent was that of 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 extracting three times with *n*-hexane, the combined extract was washed twice with water, twice with 10% sodium carbonate, and again twice with water. The *n*-hexane solution then was dried over anhydrous sodium sulfate. A thin layer chromatogram of the *n*-hexane solution was then compared against a standard sample of the 6-chromanol of hexahydrocoenzyme Q_4. The chromatogram plate was 0.3-mm silica gel G and the developing solvent was 30% ether in *n*-hexane. The *n*-hexane solution showed only one spot which corresponded exactly with that of the authentic sample when the plate was developed with either 2% aqueous potassium permanganate or the Emmerie–Emgel reagent. Concentration of the *n*-hexane solution gave 47 mg (90% yield) of the chromanol (VI). The infrared, ultraviolet, and nmr spectra of the chromanol are all consistent with structure VI.

7,8-Dimethoxy-2,5-dimethyl-2-[3′-methyl-2′-butenyloctakis(3′-methyl-2′-butenylene)methyl]-6-chromenol and 2-Hydroxy-3-methoxy- and 2-Methoxyl-3-hydroxy-5-methyl-6-[3′-methyl-2′-butenylenakis(3′-methyl-2′-butenylene)]-1,4-benzoquinone. Three grams of coenzyme Q_{10} was dissolved in 200 ml of absolute ethanol and the resulting solution was refluxed by means of a 150-w G.E. sunlamp for 24 hr. The solvent then was removed and the orange-red residue was chromatographed through a column of Florisil. Elution with 20% ether in *n*-hexane gave 1.2 g of unreacted quinone

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.

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(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).