the formation of acetic acid from dethiogliotoxin by the Kuhn–Roth procedure. It was suggested that the acetic acid formed by this degradation arose from carbon atoms 3 and 3a of dethiogliotoxin. The dethiogliotoxin obtained from gliotoxin into which serine-3-C^{14} was incorporated, was degraded by the Kuhn–Roth procedure. The results are shown in Table IV. The distillate which should have contained carbon atoms 3 and 3a (as acetic acid) had comparatively little radioactivity, whereas the CO_2 collected during the period of reflux was more radioactive. Using serine as a model for carbon atoms 3, 3a and 4 of dethiogliotoxin, Johnson and Buchanan[12] reported the formation of acetic acid by the Barthel–LaForge procedure. To determine if serine gave rise to acetic acid, both serine-1-C^{14} and serine-3-C^{14} were degraded. Essentially all of the radioactivity was evolved as CO_2 during reflux.[17] On the basis of these data, it appears that the Kuhn–Roth degradation of dethiogliotoxin does not specifically yield acetic acid from carbon atoms 3 and 3a.

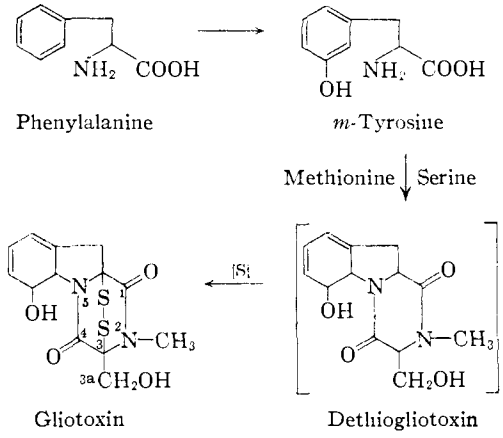
Alkaline degradation of the gliotoxin from the serine-3-C^{14} showed that 19% of the radioactivity was in the indole-2-carboxylic acid. Since 25% was found in the N-methyl group, 56% of the radioactivity is, therefore, located in carbon atoms 3, 3a and 4. The specific location of the radioactivity is being investigated further and will be reported later.

Results of experiments using serine-1-C^{14} showed that no radioactivity was present in the indole-2-carboxylic acid or the N-methyl group of gliotoxin. Thus, all of the radioactivity was in carbon atoms 3, 3a or 4. The fact that all of the radioactivity from serine-1-C^{14} is located in carbon atoms 3, 3a or 4 strongly suggests that this amino acid is a direct precursor for this portion of gliotoxin.

(17) To be published.

Since conversion of glycine to serine is known,[18] glycine-2-C^{14} was studied as the precursor for carbon atoms 3 and 3a of gliotoxin. Glycine was incorporated into gliotoxin to a smaller extent than serine. On the basis of the data obtained from the serine and glycine studies, it is postulate that glycine is converted to serine which, in turn, serves as the precursor for carbon atoms 3, 3a and 4 of gliotoxin.

Since the hydroxylation of phenylalanine can occur before cyclization of the side chain of phenylalanine, reduction of the aromatic ring probably occurs in a subsequent step. On the basis of this assumption and the data presented in this study, the following pathway for the biosynthesis of gliotoxin which accounts for all of the carbon atoms is proposed.



(18) J. S. Fruton and S. Simmonds, "General Biochemistry," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp. 771–778.

STILLWATER, OKLAHOMA

[CONTRIBUTION FROM THE MERCK SHARP AND DOHME RESEARCH LABORATORIES, DIVISION OF MERCK AND CO., INC.]

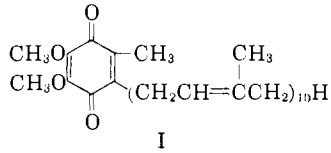
Coenzyme Q. XII. Ethoxy Homologs of Coenzyme Q_{10}. Artifact of Isolation

BY BRUCE O. LINN, NELSON R. TRENNER, BYRON H. ARISON, ROY G. WESTON, CLIFFORD H. SHUNK AND KARL FOLKERS

RECEIVED JULY 31, 1959

An artifact of coenzyme Q_{10} produced during the isolation from beef myocardial tissue has been discovered. Ethoxy and the diethoxy homologs of coenzyme Q_{10} have been characterized. Evidence for the probable mechanism of formation of these homologs indicates that Q_{10} had undergone alkali-catalyzed ethanolysis, a unique reaction of methoxy-1,4-benzoquinones. A method of isolation which avoids ethanolysis of Q_{10} is given. Comparison of our data on ethoxy homologs with those of early ubiquinone preparations shows close similarities and differentiates these compounds from coenzyme Q_{10}.

We have described[1] our initial experience with the isolation of coenzyme Q_{10} (I) from beef myocardial tissue. The product melted at 49.5–50.5°, and there was no evidence for the presence of other quinones of the same class. However, further processing did yield crystalline materials, m.p. *ca.* 45–48°, which were revealed to contain two quinones by paper chromatography. A critical examination of these lower-melting mixtures has revealed the presence of a homolog, which has been structurally elucidated. The mechanism of its origin has been determined; substantiating data were communicated.[2]



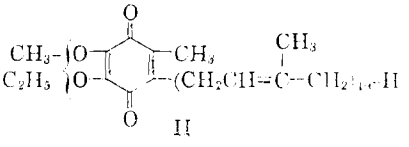
I

(1) B. O. Linn, A. C. Page, Jr., E. L. Wong, P. H. Gale, C. H. Shunk and K. Folkers, THIS JOURNAL, **81**, 4007 (1959).

(2) B. O. Linn, N. R. Trenner, C. H. Shunk and K. Folkers, *ibid.*, **81**, 1263 (1959).

The crystalline materials showing more than one component were further purified over additional Florisil columns. The second orange band which left the column yielded pure coenzyme Q_{10}. The concentrate from the first orange band was crystallized from ethanol. A pure product, m.p. 43–43.5°, was obtained. The ultraviolet spectra of this product and its reduced form (sodium borohydride) closely resembled those of coenzyme Q_{10} and coenzyme Q_{10} hydroquinone, respectively, indicating a quinone of similar structure. The infrared spectrum showed bands characteristic of the 2,3-dialkoxy-1,4-benzoquinone functionality at 6.02, 6.18 and 7.92 μ and appeared similar to that of coenzyme Q_{10}; but certain differences were observed. The analytical data are consistent with $C_{60}H_{92}O_4$ and show the presence of two alkoxyl groups; Q_{10} is $C_{59}H_{90}O_4$.

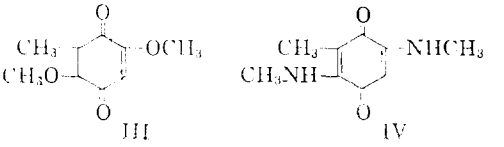
The nuclear magnetic resonance spectrum revealed the structural difference between this product and Q_{10}. The resonance spectrum at 40 mc. in carbon tetrachloride solution was essentially the same as that of coenzyme Q_{10}[3] except for the following differences. Additional proton resonances are found at 5.59, 5.78 and 5.95 τ[4] which correspond to three of the bands of the quartet expected from the methylene protons of an ethoxy group, CH_3CH_2O–, bonded to a benzoquinone ring. The fourth band of the quartet is obscured by the proton resonance of $\mathbf{CH_3}O$– at 6.11 τ. The total number of protons for one methoxyl group, $\mathbf{CH_3}O$–, and for the methylene protons of one ethoxy group, $CH_3\mathbf{CH_2}O$–, is 5, which is in good agreement with the value, 4.8, measured from the spectrum. Two bands of the resonance triplet expected for $\mathbf{CH_3}CH_2O$– are found at 8.81 and 8.57 τ. The third band of the triplet is obscured by the proton resonance of $\mathbf{CH_3}C$=(side chain) at 8.35 τ. Integration of the area of the two exposed bands × 1.5 indicates that three protons were involved. Thus, the n.m.r. analysis shows that this product is a coenzyme Q_{10} homolog in which one of the two methoxy groups has been replaced by an ethoxy group, as in II.



II

In one Q_{10} preparation, which melted at 46.5–47.5°, the ethoxy homolog content amounted to 25%. The relative amount of ethoxy homolog formed appeared to be proportional to the length of the reflux period. The total yield of products was greatly reduced when the reflux period was extended.

The reactivity of methoxy substituents on the 1,4-benzoquinone ring appears more similar to that of carboxylic methyl esters than to that of the typical aliphatic or aromatic methyl ethers. For example, 3,6-dimethoxytoluquinone (III) has been reported[5] to react rapidly with methylamine in ethanolic solution; crystals of 3,6-bismethylamino-2,5-toluquinone (IV) were formed almost immediately. Although we were unable to find



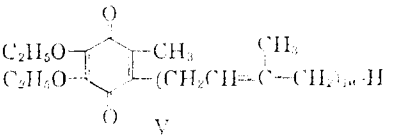
III IV

examples of alcoholysis of methoxy substituted 1,4-benzoquinones in the literature, it was reasonable to expect that coenzyme Q_{10} had undergone such an alcohol exchange during the isolation process which involved hydrolysis of the myocardial tissue in hot alkaline ethanol.[1] This interpretation was tested by subjecting pure coenzyme Q_{10} to similar hydrolysis conditions and by substituting methanol for ethanol in the hydrolysis step of the isolation process.

Pure coenzyme Q_{10} was dissolved in a dilute ethanolic solution of sodium hydroxide containing pyrogallol, and the mixture was refluxed for an hour. Spots characteristic of Q_{10} and the ethoxy homolog were found by paper chromatographic analysis of the extractives (see "reprocessed Q_{10}" in Table II). Purification over Florisil separated the quinones which were further identified by ultraviolet and infrared analysis.

When a batch of beef hearts was processed with substitution of methanol for ethanol in the saponification step, the resulting coenzyme Q_{10} was pure by available criteria, and the papergrams did not reveal any of the ethoxy homolog.

The reaction of coenzyme Q_{10} in absolute ethanol in the presence of a large excess of sodium ethoxide and pyrogallol was also examined. The mixture was refluxed for one hour. After purification of the extractives, a product was obtained which was identified as the diethoxy homolog of coenzyme Q_{10} (V), m.p. 34.5–35.5°. The n.m.r. spectrum in



V

carbon tetrachloride is the same as that of the ethoxy homolog except for the following differences. The absence of the proton resonance of $\mathbf{CH_3}O$– at 6.11 τ shows that both methoxy groups are absent. As a result, the fourth band at 6.09 τ of the resonance quartet, 5.59, 5.76, 5.92 and 6.09 τ, expected for the methylene protons of an ethoxy group, $CH_3\mathbf{CH_2}O$–, attached to the benzoquinone ring is now observed. Integration of the area assigned to the proton resonance bands of the $\mathbf{CH_3CH_2}$– group gives evidence that two ethoxy groups are present. The analytical data are consistent with $C_{61}H_{94}O_4$ and show the presence of two alkoxyl groups.

Radial paper chromatographic analysis using Vascline-treated paper and either water–dimethyl-

(3) D. E. Wolf, C. H. Hoffman, N. R. Trenner, B. H. Arison, C. H. Shunk, B. O. Linn, J. F. McPherson and K. Folkers, THIS JOURNAL, **80**, 4752 (1958).

(4) τ is calculated form the equation $\tau = \nu_0/40 + 3.50$ where ν_0 is the observed band position in c.p.s. relative to benzene protons as external standard; see G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

(5) W. K. Anslow and H. Raistrick, *J. Chem. Soc.*, 1446 (1939).

formamide–Vaseline as previously described[1] or water–dimethylformamide–isoöctane as the mobile phase has been very useful for detecting and identifying the ethoxy and diethoxy homologs. These products were readily distinguishable from each other and from coenzymes Q_9[6] and Q_{10}. R_f values are given in Table II.

During 1955 to 1958, Morton and co-workers[7-9] reported the designation, ubiquinone, "for a substance" which melted at 33–34, 36 and 41° "melting points sharp." These melting points are significantly lower than the melting point of coenzyme Q_{10} (m.p. 48–49°). A comparison of the infrared data reported for ubiquinone[7,8] and the data found for coenzyme Q_{10}, the ethoxy homolog and the diethoxy homolog has revealed that these early ubiquinone preparations[7-9] and coenzyme Q_{10} are not identical (see Table I).

TABLE I

WAVE LENGTHS OF THE MAJOR INFRARED ABSORPTION BANDS IN μ[a,b]

Coenzyme Q_{10} (I)	Ethoxy homolog (IV)	Diethoxy homolog (V)	Ubiquinone "best fraction"
3.45	3.45	3.42	3.43
6.03	6.02	6.01	6.04
6.18	6.18	6.18	6.22
7.24	7.23	7.20	7.25
7.50	7.50	7.50	7.5
7.76	7.76	7.75	7.76
7.90	7.92	7.94	7.95
8.30	**8.32**		
		8.51	**8.54**
8.67	**8.66**		
9.05	9.07	9.06	9.13
9.76	9.75	9.70	9.76
	10.10	**10.20**	**10.20**
10.55			
	11.18	**11.05**	**11.1**
11.97	11.92	11.90	11.91
13.45	13.44	13.44	13.5

[a] The spectra for coenzyme Q_{10} and the ethoxy homologs in carbon disulfide solution were obtained using a Baird infrared recording spectrophotometer, model B. [b] The bands for ubiquinone, also in carbon disulfide, were previously reported.[7,8]

The ultraviolet spectra as well as the infrared spectra in the "functional group" region from 2.5 to 8.0 μ are very similar. However, certain differences in the infrared spectra determined using carbon disulfide solutions are readily apparent at the longer wave lengths and serve for identification of these homologs. A unique band at 10.55 μ is observed for coenzyme Q_{10}.[10] This band is absent in the spectrum for the ethoxy homolog while new bands are found at 10.10 and 11.18 μ. The 8.30, 8.67 and 10.55 μ bands of coenzyme Q_{10} are missing from the spectrum of the diethoxy homolog while new bands are found at 8.51, 10.20 and 11.05 μ. The infrared spectrum reported[7] for the "best fraction" of ubiquinone appears identical to that of the diethoxy homolog. The spectrum of a second fraction of ubiquinone was reported[7] to differ from that of the "best fraction" only by the disappearance of the 8.54 μ band and the appearance of bands at 8.36 and 8.66 μ. The absence of the characteristic band at 10.55 μ shows that the minor component also is not coenzyme Q_{10}; its spectrum closely resembles that of the ethoxy homolog.

Confirmation of this interpretation has been sought in the following manner. Pure coenzyme Q_{10} was subjected to hot ethanolic potassium hydroxide solution, allowance being made for the water content of tissue, similar to those conditions originally used by Morton and co-workers[7] for the isolation of ubiquinone. Thus, neither pyrogallol nor a nitrogen atmosphere was employed. The extractives were analyzed on papergrams. None of the Q_{10} remained after the mixture had been refluxed for 0.5 hour. In a second experiment, the reaction mixture was stirred at 25° and portions removed at intervals for papergram analysis. After 0.5 hour, spots corresponding to both the ethoxy and diethoxy homologs were found; after one hour the Q_{10} spot had disappeared (see Table III).

It appears that exposure of coenzyme Q_{10} to the hot ethanolic alkali of the hydrolysis step in the isolation process strongly favors the formation of the ethoxy and diethoxy homologs. The use of pyrogallol and a nitrogen atmosphere for the hydrolysis step greatly reduces the loss of coenzyme Q_{10} and has given a pure crystalline product melting sharply in the range of 48–50.5°.[1,11-13] It has been shown that even when employing these conditions, the formation of and contamination with the ethoxy homologs may occur. However, the ethanolysis of Q_{10} has been avoided in isolation by substitution of methanol for ethanol as the solvent for the hydrolysis step.

It now appears that the melting points and infrared spectra of the early ubiquinone preparations of Morton and co-workers[7-9] are very similar to those properties of the ethoxy and diethoxy homologs. Crane, *et al.*,[11] in 1957, first reported a crystalline quinone melting at 48–49° of unambiguous properties. In 1958, Morton and co-workers[13] and Bouman, *et al.*,[14] described the same quinone and now have used the expression ubiquinone, not as originally defined[8,9] but synonymously with coenzyme Q_{10}.

Experimental

Physical Measurements.—Melting points were taken on a Kofler micro hot stage using a stage-calibrated thermometer. Ultraviolet spectra were determined using a Cary recording spectrophotometer, model 11 and absorption of chromatographic fractions at 275 mμ was measured using a

(6) R. L. Lester, F. L. Crane and Y. Hatefi, THIS JOURNAL, **80**, 4751 (1958).

(7) G. N. Festenstein, F. W. Heaton, J. S. Lowe and R. A. Morton, *Biochem. J.*, **59**, 558 (1955).

(8) R. A. Morton, G. M. Wilson, J. S. Lowe and W. M. F. Leat, *Chemistry & Industry*, 1649 (1957).

(9) R. A. Morton, G. M. Wilson, J. S. Lowe and W. M. F. Leat, *Biochem. J.*, **68**, 16P (1958).

(10) The infrared spectrum of Q_{10} in crystalline form does not show this band.

(11) F. L. Crane, Y. Hatefi, R. L. Lester and C. Widmer, *Biochem. Biophys. Acta*, **25**, 220 (1957).

(12) F. L. Crane, R. L. Lester, C. Widmer and Y. Hatefi, *ibid.*, **32**, 73 (1959).

(13) R. A. Morton, U. Gloor, O. Schindler, G. M. Wilson, L. H. Chopard-dit-Jean, F. W. Hemming, O. Isler, W. M. F. Leat, J. F. Pennock, R. Ruegg, U. Schwieter and O. Wiss, *Helv. Chim. Acta*, **41**, 2343 (1958).

(14) J. Bouman, E. C. Slater, H. Rudney and J. Links, *Biochim. et Biophys. Acta*, **29**, 456 (1958).

Beckman quartz spectrophotometer, model DU. Nuclear magnetic resonance spectra were determined using a Varian Associates high resolution spectrophotometer and 15–20 weight per cent. concentrations of solute in carbon tetrachloride. Infrared data are described in Table I. The quinones were reduced with sodium borohydride as previously described[1] in order to obtain spectral data of the corresponding hydroquinones.

Isolation of Coenzyme Q_{10} (I) and the Ethoxy Homolog of Coenzyme Q_{10} (II) from Beef Myocardial Tissue Using an Ethanolic Hydrolysis Step.[1]—The components in one coenzyme Q_{10} preparation (m.p. 46.5–47.5°, $\Delta E^{1\%}_{1cm}$ (quinone–hydroquinone) 139 at 275 mμ as compared to 142 for Q_{10}[1]) were separated as follows. A portion, 65.4 mg., of this preparation was dissolved in 50 ml. of *n*-hexane, and the solution was applied to a chromatographic column, 2.7 × 100 cm., containing 250 g. of magnesium silicate (Florisil) in *n*-hexane. The column was eluted with a 5% solution of ethyl ether in *n*-hexane; 112-ml. fractions were collected. The development of the column was followed by measuring the ultraviolet absorption at 275 mμ. The intensity of the yellow color of the eluates appeared proportional to the ultraviolet absorbance. Colorless eluates amounting to 4,920 ml. were discarded. The first component, the ethoxy homolog, left the column in 890 ml. of yellow solution. Colorless eluates amounting to 560 ml. were collected before the second component, coenzyme Q_{10}, began to emerge. The major portion of the coenzyme Q_{10} (I) was eluted with 1890 ml. of solution. The distribution curve plotted from the absorption data showed that two components had been separated. It was found that the original sample, m.p. 46.5–47.5°, had contained approximately 25% of the ethoxy homolog II.

Beef heart batches which were not cooled rapidly following the alkaline ethanolic hydrolysis step[1] yielded non-saponifiable fractions that gave low yields of coenzyme Q_{10} and relatively large amounts of the ethoxy homolog. For example, column chromatography of the non-saponifiable fraction from 136 kg. of beef heart[1] gave 38.6 g. of a red liquid which failed to crystallize from ethanol. This red liquid was recovered, dissolved in 100 ml. of *n*-hexane, and applied to a chromatographic column, 4.6 × 100 cm., containing 600 g. of 50/100 mesh sodium aluminum silicate (Decalso) in *n*-hexane. The column was eluted with a 5% solution of ethyl ether in *n*-hexane. Two orange bands developed. The eluates from these bands were collected separately and concentrated under reduced pressure. The residues were crystallized from absolute ethanol.

The slower moving band gave 0.98 g. of coenzyme Q_{10} (I), m.p. 49–50°; infrared data are given in Table I. The physical properties of this product are the same as previously reported for coenzyme Q_{10}.[1] The faster moving band gave 0.88 g. of the ethoxy homolog of coenzyme Q_{10} (II) which melted at 43.0–43.5°[15] and had the absorption characteristics; quinone in isoöctane, max. at 271 mμ, $E^{1\%}_{1cm}$ 165; min. at 235 mμ, $E^{1\%}_{1cm}$ 30; quinone in ethanol, max. at 275 mμ, $E^{1\%}_{1cm}$ 160; min. at 236 mμ, $E^{1\%}_{1cm}$ 29; hydroquinone in ethanol, max. at 290 mμ, $E^{1\%}_{1cm}$ 47; min. at 250 mμ, $E^{1\%}_{1cm}$ 9.3; $\Delta E^{1\%}_{1cm}$ at 275 mμ (quinone–hydroquinone) 137; infrared data are given in Table I and n.m.r. analysis in the Discussion.

Anal. Calcd. for $C_{60}H_{92}O_4$ (877.34): C, 82.13; H, 10.57; alkoxyl, 2.00 moles per mole of sample. Found: C, 82.19, 81.90; H, 10.34, 10.50; alkoxyl, 2.02 moles.

Isolation of Coenzyme Q_{10} (I) from Beef Myocardial Tissue Using a Methanolic Hydrolysis Step.—The fat and connective tissue was removed from fresh beef hearts and the myocardial tissue was ground in a meat grinder using a 1/8 in. screen. A methanolic 14.5% potassium hydroxide solution was prepared by dissolving 25.6 kg. of potassium hydroxide pellets in 180 liters of methanol. To this stirred solution was added 9.0 kg. of pyrogallol and 136 kg. of ground beef myocardial tissue. The system was flushed with nitrogen, and the mixture was heated at reflux for 90 minutes[16] under a nitrogen atmosphere. The resulting black solution was rapidly cooled by pumping onto 60 kg. of crushed ice in a suitable extraction vessel. A 130-liter portion of *n*-hexane was vigorously agitated with the hydrolysis mixture for 5 minutes. The mixture was allowed to settle until at least one-half of the *n*-hexane separated. The *n*-hexane layer was washed with warm water until all the dark brown color and alkali were removed. The resulting *n*-hexane solution was yellow-orange and clear. When emulsions formed on water washing, ammonium or potassium chloride was added to assist the separation.

A second and third extraction of the hydrolysis mixture using 60-liter portions of *n*-hexane was performed, using the same wash technique. The combined hexane extract was washed with water and then dried over anhydrous sodium sulfate. The solution was concentrated to *ca.* 20 liters under reduced pressure with warming to 50° and then stored at 0–5°.

The concentrates from three such batches were combined and adsorbed on a chromatographic column containing 4 kg. of 60/100 mesh Florisil in hexane. The column was washed with about 30 liters of hexane and then eluted with 10% ether in *n*-hexane. A yellow-green colored band presumably containing the carotenes was eluted usually in about 40 liters of the solution and was discarded. Next, an orange band containing coenzyme Q_{10} and cholesterol was eluted in about 12 liters of solution. This fraction was concentrated under reduced pressure at 50° to *ca.* 1000 ml. and then cooled to 5°. Cholesterol crystals formed and were removed by filtration. These crystals were washed until colorless with portions of cold *n*-hexane, usually three 100- to 300-ml. portions depending upon the quantity of cholesterol obtained. The filtrate and *n*-hexane washings were combined and concentrated under reduced pressure to *ca.* 500 ml. A second crop of cholesterol was removed as before. The filtrate and washings were combined and the solvent removed by concentration under reduced pressure at 40°. About 100 ml. of absolute ethanol was added and then evaporated under reduced pressure in order to remove the residual *n*-hexane. The residue was dissolved in 1000 ml. of absolute ethanol with warming to 60°. The hot solution was decanted from a dark insoluble oil. The oil was leached with 100 ml. of hot absolute ethanol. The ethanol solutions were combined, placed in the dark, and allowed to stand overnight at room temperature (25°). Orange crystals formed and were collected. These crystals were dissolved in 500 ml. of absolute ethanol and allowed to crystallize as before. The orange crystals were collected, washed with three portions of cold ethanol and dried in high vacuum at 25°.

In this manner 27 g. (66 mg./kg. of moist myocardial tissue) of coenzyme Q_{10} (I) was generally obtained. These preparations melted over a one degree range from 48–50.5° and were found to be 95–100% pure based upon spectrophotometric analysis.[1] Chromatographic analysis on paper of this product and the mother liquors did not reveal any of the ethoxy homolog. The physical properties of this product were identical with those of coenzyme Q_{10}[1] prepared using the ethanolic hydrolysis step.

Conversion of Coenzyme Q_{10} (I) to the Ethoxy Homolog II Using Hydrolysis Conditions Similar to those for the Isolation Procedure[1] for Coenzyme Q_{10}.—To a 10% sodium hydroxide solution containing 30 g. of sodium hydroxide pellets in 300 ml. of 90% ethanol was added 15 g. pyrogallol and 199 mg. of coenzyme Q_{10}. The system was flushed out with nitrogen, and then the mixture was stirred and heated at reflux for 15 minutes. More water, 90 ml., was added to give the mixture a concentration of 31% water which is approximately the concentration found in the beef heart saponification mixtures.[1] The reaction solution was refluxed for 45 minutes longer, then cooled and diluted by the addition of 50 ml. of water. The resulting solution was extracted with three 200-ml. portions of petroleum ether. The combined petroleum ether extracts were washed with water until free of alkali, dried over anhydrous sodium sulfate and evaporated under reduced pressure.

In order to ensure complete conversion to the quinone form, the residue was subjected to mild oxidation as follows: The orange liquid residue was taken up in 25 ml. of ethyl ether. Anhydrous magnesium sulfate, *ca.* 1 g., and powdered silver oxide, 400 mg., were added. The mixture was stirred for one hour. The solids were removed, and the solvent evaporated under reduced pressure giving 107 mg. of a red liquid residue. This material was shown to contain two benzoquinones, coenzyme Q_{10} (I) and the ethoxy homolog (II), by paper chromatographic analysis (see "reprocessed Q_{10}" in Table II).

(15) Other preparations have melted over a 2° range from 37–43.

(16) The reflux period is critical. The tissue is not completely hydrolyzed in a shorter time and the yield of Q_{10} decreases if heating is continued.

The liquid residue was dissolved in *n*-hexane and applied to a chromatographic column, 1.7 × 75 cm., containing 50 g. of Florisil in *n*-hexane. Two orange bands were developed and eluted with 5% ethyl ether in *n*-hexane as described above. The faster moving band gave 15 mg. (7%) of the ethoxy homolog II and the slower moving band gave 31 mg. (16%) of coenzyme Q_{10} (I). These products were identified by infrared and ultraviolet absorption analyses and paper chromatographic analysis.

Conversion of Coenzyme Q_{10} (I) to the Diethoxy Homolog V Using Sodium Ethoxide.—To a solution containing 65 mmoles of sodium ethoxide in 50 ml. of absolute ethanol was added 1.00 g. (7.90 mmoles) of pyrogallol and 200 mg. (0.23 mmole) of coenzyme Q_{10}. The mixture was stirred and heated at reflux for one hour, cooled in an ice-bath and acidified with 28 ml. of 2.5 *N* hydrochloric acid. Water was added, and the resulting solution extracted with three portions, of ether. The combined ethereal solution was extracted with five portions of water and dried over anhydrous magnesium sulfate. To the ethereal solution was added 1 g. of anhydrous magnesium sulfate and 0.40 g. of powdered silver oxide. This mixture was stirred for one hour. The solids were removed, and the solvent evaporated under reduced pressure leaving 342 mg. of an orange solid residue. The residue was leached with 10-ml. portions of warm isooctane until the solution was colorless. The orange colored isoöctane solutions were combined and applied to a chromatographic column (1.1 × 25 cm.) containing 9.0 g. of 60/100 mesh magnesium silicate (Florisil) in isoöctane. The orange band which formed was washed with 500 ml. of isoöctane and then eluted with a 2% ethyl ether–isoöctane solution. The yellow colored eluates were concentrated under reduced pressure leaving 84 mg. (40%) of orange crystals, the diethoxy homolog V, which was characterized by papergram, ultraviolet and infrared analyses. Further elution of the column with 20% ether–isoöctane yielded 47 mg. of an orange liquid which was found by papergram analysis to contain Q_{10} (I) and the ethoxy homolog II.

An analytical sample was prepared by recrystallization of the diethoxy homolog V from ethanol–water giving yellow crystals which melted at 34.5–35.5° and had the absorption characteristics: quinone in isoöctane, max. at 271 mμ, $E^{1\%}_{1cm}$ 145; min. at 236 mμ, $E^{1\%}_{1cm}$ 33; quinone in ethanol, max. at 276 mμ, $E^{1\%}_{1cm}$ 149; min. at 239 mμ, $E^{1\%}_{1cm}$ 39; hydroquinone in ethanol, max. at 292 mμ, $E^{1\%}_{1cm}$ 47; min. at 255 mμ, $E^{1\%}_{1cm}$ 13; $\Delta E^{1\%}_{1cm}$ at 275 mμ (quinone minus hydroquinone) 119; infrared data are given in Table I and n.m.r. analysis in the Discussion.

Anal. Calcd. for $C_{61}H_{94}O_4$ (891.36): C, 82.19; H, 10.63; alkoxyl, 2.00 moles per mole of sample. Found: C, 82.02; H, 10.44; alkoxyl, 1.85 moles.

Paper Chromatographic Analysis.—A reverse-phase, radial type of papergram analysis similar to that previously described[1] was employed. Circular Whatman No. 1 paper was impregnated with Vaseline by passing the paper through a 5% (w./v.) solution of Vaseline in isoöctane. The paper was air-dried. The mobile phases were prepared as follows: for experiment 1, a water–dimethylformamide (3:97) solution was equilibrated with Vaseline; for experiment 2, dimethylformamide was equilibrated with isoöctane. Another system which was used frequently for identification of these products is water–dimethylformamide (1:99) equilibrated with isoöctane (see Table III). The spots were detected by ultraviolet light. A summary of the results obtained from two typical papergrams is given in Table II.

By this technique the ethoxy homolog and the diethoxy homolog were readily distinguished from coenzymes Q_9[6] and Q_{10}. Mixtures of the ethoxy homologs with each other or with Q_{10} were resolved into two characteristic spots.

The "reprocessed coenzyme Q_{10}" gave two spots which corresponded to Q_{10} and the ethoxy homolog. Mixtures of the reprocessed Q_{10} with either Q_{10} or the ethoxy homolog gave only two spots while mixture with the diethoxy homolog gave three characteristic spots.

TABLE II

PAPERGRAM ANALYSIS OF COENZYME Q_{10} AND HOMOLOGS

Sample	Standard added	*Rf* values Expt. 1	Expt. 2
Coenzyme Q_9[a]		0.49	0.79
Coenzyme Q_{10}[b]		.40	.70
Ethoxy homolog[c]		.32	.63
Diethoxy homolog[d]		.29	.53
Ethoxy homolog	Coenzyme Q_{10}	.30, 0.37	.63, 0.69
Ethoxy homolog	Diethoxy homolog	.25, .31	.53, .60
Diethoxy homolog	Coenzyme Q_{10}	.25, .36	.53, .69
"Reprocessed Q_{10}"[e]		.32, .39	
"Reprocessed Q_{10}"	Coenzyme Q_{10}	.35, .39	
"Reprocessed Q_{10}"	Ethoxy homolog	.33, .41	
"Reprocessed Q_{10}"	Diethoxy homolog	.27, .35, 0.41	

[a] See ref. 6. [b] Structure I. [c] Structure II. [d] Structure V. [e] Products obtained by subjecting coenzyme Q_{10} to hydrolysis conditions.

Conversion of Coenzyme Q_{10} I to the Ethoxy II and the Diethoxy V Homologs Using Hydrolysis Conditions Similar to the Isolation Procedure for Ubiquinone.[7]—To 100 ml. of a solution which contained 8% (w./v.) potassium hydroxide and 33% (v./v.) water in ethanol and which had been equilibrated with *n*-hexane was added 50.0 mg. of coenzyme Q_{10}. The resulting solution was stirred at 25° for 22 hours, and 4.0-ml. portions were taken at regular intervals for papergram analysis. Each portion was acidified immediately with 8 ml. of 1 *N* hydrochloric acid and then extracted with three 2-ml. portions of ether. The ether solutions were washed with four 2-ml. portions of water and dried over anhydrous magnesium sulfate. After evaporation of the solvent, the residue was taken for papergram analysis which is summarized in Table III.

TABLE III

PAPERGRAM ANALYSIS OF RESIDUES

Reaction time	*Rf* values[a]
10 min.	0.54
30 min.	.39, 0.46, 0.55
60 min.	.39 0.45
2 hr.	.39
4 hr.	.00
22 hr.	.00

[a] The papergram system described above was used. The mobile phase was water–dimethylformamide (1:99) equilibrated with isoöctane. Spots were detected with ultraviolet light and a leucomethylene blue indicator spray.[1]

The standards, the ethoxy homolog, the diethoxy homolog and coenzyme Q_{10}, gave R_f values of 0.39, 0.45 and 0.55, respectively.

When a reaction solution prepared as described above was heated at reflux for 30 minutes and then subjected to papergram analysis as above, only a single spot, R_f 0.00, was found. Spectrophotometric analysis[1] of the residue gave a $\Delta E^{1\%}_{1cm}$ (quinone–hydroquinone) of −7 at 275 mμ showing that the quinone nucleus of Q_{10} had been destroyed.

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