New Epoxyubiquinones*

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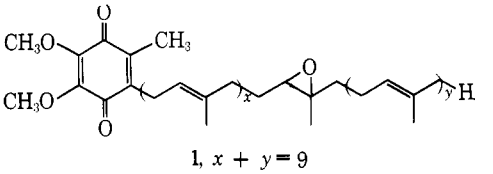
ABSTRACT: Five new epoxy derivatives of ubiquinone-10 have been obtained by the techniques used to fractionate the lipids from *Rhodospirillum rubrum*, and it was possible to characterize two of them rather extensively. The epoxy group is in the isoprenoid side chain, but not in the unit adjacent to the quinone nucleus or in the terminal unit. These epoxyubiquinones were obtained in relatively low amounts such as 1–2% of the concentration of ubiquinone-10. Synthetic mono- and diepoxy derivatives have been prepared from ubiquinone-10. A monoepoxy derivative of ubiquinone-2 and two monoepoxy derivatives of ubiquinone-3 have also been synthesized. For further characterization, the isolated and synthesized epoxides were compared by their reaction with acetic acid; the products were hydroxy acetates of comparable characteristics. The epoxides of ubiquinone-10 from the fractionation may be naturally occurring in *R. rubrum*, or just artifacts of the isolation procedure, or conceivably they are both naturally occurring and artifactual. It is not yet possible to differentiate between these possibilities. That five such epoxides of ubiquinone-10 were obtained from fractionation clearly shows a lack of reaction specificity of a single isoprenoid unit in the epoxidation.

Five new multiprenylbenzoquinones (**1**) have been obtained by the techniques of fractionation of lipids from *Rhodospirillum rubrum* and have been characterized as side-chain epoxy derivatives of ubiquinone-10 (Q-10, **2**, $n = 10$). The quantities of two of these epoxyubiquinones (**1**)[1] were about 1–2% of the concentration of ubiquinone-10. They exhibit indistinguishable ultraviolet, infrared, nuclear magnetic resonance, and mass spectral properties, but are separable chromatographically. Presumably, they differ only in regard to the position of the epoxide group in the side chain. Spectral evidence (nuclear magnetic resonance and mass spectra) shows that the isoprene unit adjacent to the quinone ring and the terminal isoprene unit are unsubstituted.

Three additional ubiquinone derivatives functionalized in the side chain have been obtained. These products were obtained in even smaller amounts and could not be as completely characterized; however, spectral evidence (nuclear magnetic resonance and mass spectra) indicate that these products are also epoxyubiquinones-10 (**1**).



1, $x + y = 9$

Prior to the characterization of these epoxyubiquinones (**1**), only two naturally occurring ubiquinone derivatives with "altered" side chains were known. Ubiquinone-10(H-10) (**3**) (Gale *et al.*, 1963), in which the terminal isoprene unit is saturated, has been isolated from several microbial sources (Lavate and Bentley, 1964). In these organisms, Q-10(H-10) (**3**) is the most abundant and in some cases the only Q found, and presumably is the normal, functional ubiquinone in these species. A second ubiquinone derivative with an altered side chain is present in rat urine, and appears to be a nonfunctional metabolic end product. This material which is presumed to be a

* From the Stanford Research Institute, Menlo Park, California. *Received July 18, 1967.* This research was partially supported by funds from the Merck Sharp and Dohme Research Laboratories, Rahway, N. J.

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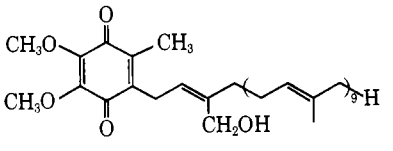
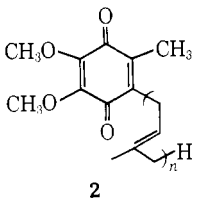
[1] Coenzyme Q. XCII. At the time nomenclature of quinones with isoprenoid side chains was considered by an IUPAC Subcommittee (*Biochim. Biophys. Acta 107*, 5 (1965)) no functional side-chain derivative of this class of natural products was known. Consequently, a systematic system of nomenclature for such side-chain derivatives was not considered. The discovery of epoxy derivatives of ubiquinones reported here, and hydroxy and side-chain ester derivatives of the plastoquinones, make necessary a systematic nomenclature for side-chain derivatives. Neither the nomenclature designation of **3** in general use [ubiquinone-10(H-10)] nor that recommended by the IUPAC Subcommittee (X-dihydroubiquinone-10) lends itself to generalization to include derivatives such as hydroxy. We propose a system that is straightforward and general. In this system, the designation of the groups of natural quinones and their abbreviations (IUPAC) are used together with the *Chemical Abstracts* side-chain numbering system. Thus, **8** is 6′,7′-epoxyubiquinone-3, or 6′,7′-epoxy-Q-3; **3** in this system would be named 38′,39′-dihydroubiquinone-10. Side-chain methyl groups are assumed to be unsubstituted unless specifically indicated, *e.g.*, 3′-hydroxymethylubiquinone-10 (A).



A

glucuronide yields the lactone **4** after acid hydrolysis (Gloor *et al.*, 1966).



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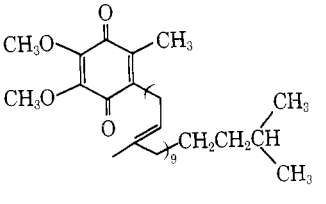

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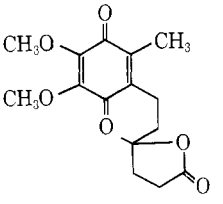


4

Until recently, very few naturally occurring epoxides were known. Epoxy fatty acids are now recognized to be widely distributed in plants (Wolff, 1966; Stumpf, 1963). Since such fatty acids have been shown (Gunstone, 1954; Hopkins and Chisholm, 1960) to be optically active, they appear to be established as enzymatic products. Several suggestions have been made (Wolff, 1966; Bloch, 1963) concerning possible biochemical roles of such compounds. Epoxycarotenoids have also been isolated, and their enzymatic deepoxidation has been studied, but little is known about their biological functions (Schimmer and Krinsky, 1966). Recent studies (Corey *et al.*, 1966; Corey and Russey, 1966; van Tamelen *et al.*, 1966) have shown that 2,3-epoxysqualene is an intermediate in the biosynthesis of sterols from squalene. Also, the structure of insect juvenile hormone has been elucidated as methyl 7-ethyl-3,11-dimethyl-10,11-epoxy-2,6-tridecadienoate (Röller *et al.*, 1967).

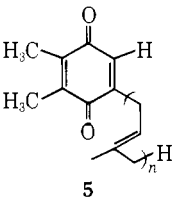
The origin of these epoxyubiquinones (**1**) has not been established. These epoxides may be artifacts of the isolation procedure or they may be naturally occurring in *R. rubrum*. Epoxide formation by radical oxidation of olefins is well known (Van Sickle *et al.*, 1967a,b) and formation of epoxyubiquinones appears possible by reaction of Q-10 with trace amounts of peroxides in the solvents used during the fractionation. Natural occurrence of such epoxyubiquinones could imply enzymic formation for a functional role or just in metabolism.

Control experiments in which pure ubiquinone-10 was treated with solvent systems including ether, which have been used in these studies and by other investigators, reveal that epoxides of Q-10 appear to be formed in trace amounts. In a related study, the quinone observed by Sottocasa and Crane (1965) in extracts from beef heart mitochondria is believed to be naturally occurring on the basis of control experiments to rule out artifact formation (Sottocasa and Crane, 1965). Recently, T. M. Farley, J. Blake, and K. Folkers (unpublished results) have isolated a quinone, which corresponds to the substance described by Sottocasa and Crane (1965), and have characterized it as an epoxyubiquinone-10.

Proof of natural occurrence of an epoxyubiquinone is analogous to proof of the natural occurrence of ubichromenol. In both cases, it is very difficult to prove natural occurrence of a substance which is formed artifactually by the normal procedures of fractionation. Both ubichromenol and epoxyubiquinones (**1**) appear to form readily from Q-10 under a variety of conditions; it is difficult to fractionate these natural source materials without utilizing procedures which could allow some formation of artifacts.

Plastoquinone-9 (PQ-A, **5**, $n = 9$) is found abundantly in the chloroplasts of green plants. Occurring with PQ-A are a series of related minor constituents originally designated (Kegel *et al.*, 1962; Henninger and Crane, 1963, 1964) as PQ-B, PQ-C, and PQ-D. Recently, Henninger and Crane (1966) reported that low concentrations (relative to PQ-A) of either PQ-B or PQ-C, in addition to PQ-A, are necessary for maximum activity in the Hill reaction in isolated spinach chloroplasts, and consider that this participation in activity might indicate a functional role. PQ-B is now recognized as consisting of a total of six separable compounds (Griffiths *et al.*, 1966) which appear to be fatty acid esters of side-chain hydroxyl-substituted derivatives of PQ-A (Griffiths, 1966; Das *et al.*, 1967); the products designated PQ-C and PQ-D have been shown to consist of a total of six side-chain hydroxyl-substituted derivatives of PQ-A (Griffiths *et al.*, 1966; Griffiths, 1966; Das *et al.*, 1967).

It is interesting to compare structurally the epoxyubiquinones (**1**) with PQ-B, PQ-C, and PQ-D. An epoxide could be a biosynthetic intermediate in the introduction of a hydroxyl function into an isoprenoid unit, as depicted in eq 1. This sequence is formally the addition of water to a double bond. A hydroxyubiquinone formed from an epoxide as in eq 1 would be a secondary or tertiary saturated alcohol. In contrast, hydroxyplastoquinone-9[1] (PQ-C) appears to have a hydroxyl group at carbons 1, 4, or 5, and is formally a product of oxygen insertion (eq 2). For that reason, PQ-C could not arise *via* an intermediate epoxy derivative similar to epoxyubiquinone (**1**).



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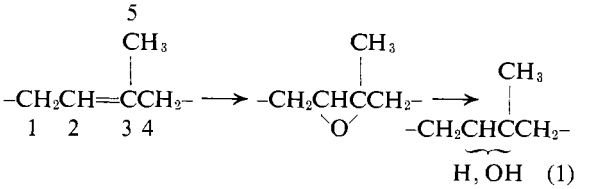
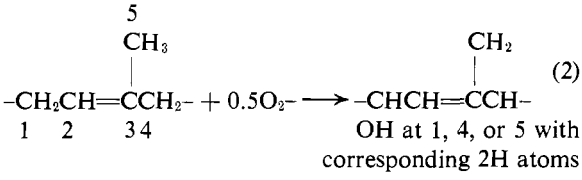


H, OH (1)

TABLE I: Spectral Data for Epoxyubiquinones.

Compound	Ultraviolet Spectra λ_{max}^{hexane} (mμ)	Mass Spectra *m/e* for M^+	Nuclear Magnetic Resonance Spectra[a]					
			Vinyl	Methoxyl	Benzyl	Methine	Side-Chain Alkyl	Epoxy Methyl
Product A		878						
Product B		878						
Epoxy-Q-10 (product C)	272		5.0 (m, 9)	6.12 (s, 6)	6.92 (d, 2)	7.52 (t, 1)	7.8–8.5	8.84 (s, 3)
Epoxy-Q-10 (product D)	271	878	5.0 (m, 9)	6.12 (s, 6)	6.92 (d, 2)	7.52 (t, 1)	7.8–8.5	8.82 (s, 3)
Product E		878						
Synthetic epoxy-Q-10	272		5.0 (m, 9)	6.12 (s, 6)	6.92 (d, 2)	7.52 (t, 1)	7.8–8.5	8.84 (s, 3)
Synthetic diepoxy-Q-10	272		5.0 (m, 9)	6.12 (s, 6)	6.92 (d, 2)	7.52 (t, 2)	7.8–8.5	8.83 (s, 6)
6′,7′-Epoxy-Q-3	271	402	5.06 (m, 2)	6.12 (s, 6)	6.91 (d, 2)	7.57 (t, 1)	7.8–8.6	8.85 (s, 3)
10′,11′-Epoxy-Q-3	271	402	5.08 (m, 2)	6.11 (s, 6)	6.92 (d, 2)	7.56 (t, 1)	7.8–8.6	8.82 (d, 6)
6′,7′-Epoxy-Q-2	271		5.09 (t, 1)	6.12 (s, 6)	6.90 (d, 2)	7.58 (t, 1)	7.8–8.6	8.82 (d, 6)

[a] s = singlet; d = doublet; t = triplet; m = multiplet. The numbers in parentheses are the number of protons.



(2)

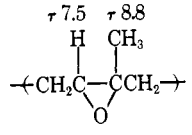
Epoxides from Fractionation of Lipids from R. rubrum. Column chromatography of the lipid extract obtained from cells of 1600 l. of medium containing *R. rubrum* yielded quinones (as detected by leucomethylene blue spray reagent) in numerous fractions more polar than those yielding ubiquinone (**2**). Fractions that appeared to contain the same quinone (or mixture of quinones) were pooled and further purified by thin layer chromatography. In this manner, five samples (A–E, Table I) were obtained which gave spectral evidence for the presence of epoxyubiquinones (**1**). The assignment of structures to components A, B, and E is based primarily on the appearance of appropriate parent ions in the mass spectra of these samples. Components C and D were obtained in larger quantities (11.8 and 12.0 mg, respectively) and have been fully characterized except for the positions of the epoxide functions in the multiprenyl side chain.

Quinones C and D were eluted from the original chromatographic column in widely separated fractions, but exhibited ultraviolet, infrared, nuclear magnetic resonance, and mass spectra which were essentially superimposable. Both exhibited ultraviolet spectra (λ_{max}^{hexane} 271 mμ) and infrared spectra indistinguishable from corresponding spectra of Q-10 (**2**, $n = 10$) (Linn *et al.*, 1959). The nuclear magnetic resonance spectra of C and D were very similar to that of Q-10 (**2**, $n = 10$) (Linn *et al.*, 1959; Trenner *et al.*, 1959), but possessed additional signals at τ 7.52 (one-proton triplet, $J = 6$ cps) and 8.82–8.84 (three-proton singlet). Mass spectra of C and D were indistinguishable, and exhibited intense peaks at *m/e* 235 and 197 and peaks at *m/e* 878 and 880 due to parent ions of the quinone and hydroquinone species, respectively (Muraca *et al.*, 1967).

These data (ultraviolet maxima, infrared carbonyl stretching bands, nuclear magnetic resonance methoxyl signals, and mass spectra peaks at 235 and 197) established that both quinones C and D possess an unaltered benzoquinone nucleus. Also, the presence in the nuclear magnetic resonance spectra of quinones C and D of characteristic two-proton doublets at τ 6.92 due to benzylic protons showed that the double bond in the isoprene unit nearest the quinone ring is intact. The presence in the mass spectra of intense peaks at *m/e* 235 is also evidence that the first isoprene unit in each compound is unaltered (Muraca *et al.*, 1967).

The spectral evidence that quinones C and D are derivatives of ubiquinone-10 (**2**, $n = 10$) in which one of the side-chain double bonds has been epoxidized is as follows. The parent ions in the mass spectra of both C and D at 878 correspond to Q-10 (mol wt 862)

plus an oxygen atom. This additional oxygen atom is located in the isoprenoid side chain, since the ring moiety is, in each case, identical with that of Q-10. Evidence that this oxygen atom is not present as a hydroxyl or a carbonyl group is the lack of appropriate absorption bands in the infrared spectra. Likewise, the mass spectra do not show peaks at M − 18 as would be expected for an alcohol (Biemann, 1962). The two key signals in the nuclear magnetic resonance spectra of C and D, a triplet (1 H) at τ 7.52 and a singlet (3 H) at τ 8.84 (8.82), are characteristic of isoprenoid epoxides (**6**). 10,11-Epoxy-3,7,11-trimethyl-2,6-dodecadienoic acid methyl ester (10,11-epoxyfarnesic acid methyl ester, **7**) shows corresponding signals at τ 7.3 (1 H, triplet) and 8.7 (6 H, doublet) (Bowers *et al.*, 1965).



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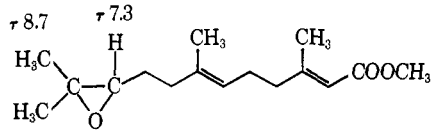

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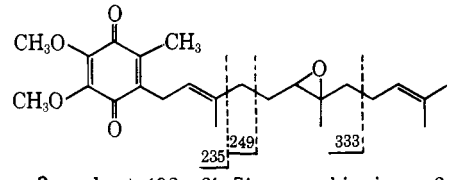
Since the nuclear magnetic resonance spectra of both C and D show a methyl signal at τ 8.8 as a singlet representing three protons, it is evident that the epoxidized isoprene unit is not terminal. A six-proton doublet, as in **7**, would be observed if the terminal unit were epoxidized.

Synthetic Epoxides. As an aid in characterizing the epoxides obtained from fractionation, a series of epoxyubiquinones were synthesized. Treatment of Q-10 (**2**, n = 10) with monoperphthalic acid in refluxing ether produced an epoxyubiquinone-10 (**1**) which possessed ultraviolet, infrared, and nuclear magnetic resonance spectra (Table I) indistinguishable from the corresponding spectra obtained from the isolated epoxides. Spectral evidence indicated that the first and last isoprene units of the synthetic epoxide were unsubstituted.

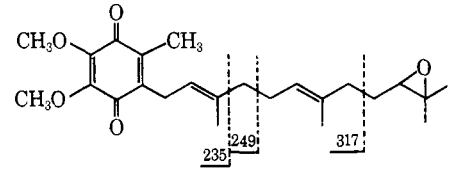
A synthetic diepoxyubiquinone-10 (**8**) was also obtained as evidenced by its nuclear magnetic resonance spectrum (Table I) which showed the signals at τ 7.5 and 8.8 to be equivalent to 2 H and 6 H, respectively. This ratio (2:6) and the presence of a typical benzyl doublet at τ 6.92 (Table I) indicate that neither the first nor the terminal double bond was epoxidized.

Epoxidation of the terminal isoprenoid unit was accomplished when Q-2 (**2**, n = 2) and Q-3 (**2**, n = 3) were treated with monoperphthalic acid. Thus, Q-2 upon treatment with monoperphthalic acid in refluxing ether yielded 6′,7′-epoxyubiquinone-2 as shown by the nuclear magnetic resonance spectrum (Table I) having a benzylic doublet at τ 6.90. Similarly, epoxidation of Q-3 produced both 6′,7′-epoxyubiquinone-3 (**9**) and 10′,11′-epoxyubiquinone-3 (**10**). No product has been observed in which the double bond nearest the quinone ring has been epoxidized.

Structure assignments of the products obtained by epoxidation of Q-3 (**2**, n = 3) were made on the basis of both nuclear magnetic resonance (Table I) and mass spectra. The appearance of prominent peaks in the mass spectra of both products due to cleavage at the allylic carbon–carbon bonds showed the position of the epoxide ring. The mass spectrum of 6′,7′-epoxyubiquinone-3 (**9**) showed prominent peaks at m/e 402 (M), 333 (M − 69), 249 [M − 69 − (68 + 16)], and 235 (base peak). Similarly, the mass spectrum of 10′,11′-epoxyubiquinone-3 (**10**) exhibited prominent peaks at m/e 402 (M), 317 [M − (69 + 16)], 249 [M − (69 + 16) − 68], and 235 (base peak).

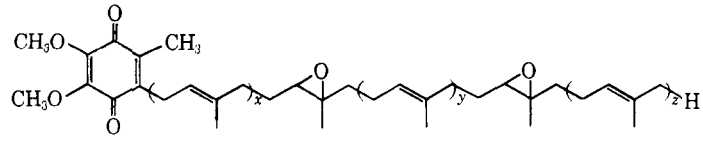


9, mol wt 402 6′, 7′-epoxyubiquinone-3



10, mol wt 402 10′, 11′-epoxyubiquinone-3

Finally, epoxyubiquinones C and D and synthetic epoxy-Q-10 (**1**) were converted into derivatives in which the epoxide ring has been cleaved. Treatment of synthetic epoxy-Q-10 (**1**) with glacial acetic acid at steam-bath temperature for 6 hr produced a hydroxy acetate as evidenced by the appearance of infrared absorption bands at 3500 (OH) and 1735 cm^{-1} (acetate). Similar treatment of the isolated epoxyubiquinones-10 (products C and D) also yielded hydroxy acetates; all three hydroxy acetates exhibited indistinguishable infrared spectra. Paper chromatographic R_F values of these hydroxy acetates and of the isolated and synthetic epoxyubiquinones (**1**) are recorded in Table II.

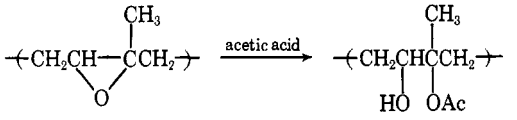


8, $x + y + z = 8$

TABLE II: Paper Chromatography of Epoxyubiquinones and Derivatives.[a]

Compound	R_F Value
Synthetic epoxy-Q-10	0.27
Hydroxy acetate	0.71
Epoxy-Q-10 (product C)	0.29
Hydroxy acetate	0.73
Epoxy-Q-10 (product D)	0.29
Hydroxy acetate	0.71
Synthetic diepoxy-Q-10	0.60
Q-10 (ref sample)	0.12

[a] Ascending, 1-propanol–water (7:3); Whatman No. 3MM impregnated with Dow-Corning No. 550 silicone oil. Sprayed with leucomethylene blue.



In an attempt to determine whether the isolated epoxyubiquinones (**1**) are of enzymatic origin, epoxyubiquinone C was examined by optical rotatory dispersion techniques;[2] no rotation was detected. However, it is possible that the rotation due to a single asymmetric center in this compound of mol wt 878 is below the level of detection using the limited quantities of material available.

Experimental Section

General Comments. The ultraviolet absorption spectra were measured with a Cary Model 14 M spectrophotometer. The infrared absorption spectra were determined with a Beckman IR-5 spectrophotometer. The nuclear magnetic resonance spectra were determined with a Varian HA-100 spectrometer using carbon tetrachloride solutions. Chemical shifts are expressed in τ values relative to tetramethylsilane as an internal standard.

The mass spectra were determined using a CEC Model 103-C mass spectrometer with increased magnetic field, electron multiplier–Wien filter detection system, and 1-mm slits to permit resolution at *m/e* values as high as 1000. The sampling system was modified to permit direct sample introduction by means of a heated probe.

Thin layer chromatography was carried out on silica gel G plates having a 0.3-mm layer of adsorbent; preparative layer chromatography was carried out using plates with a 1.0-mm layer of silica gel G as adsorbent. The plates were activated by heating at 130° for ~1.5 hr, and were stored in a dry cabinet after cooling. Freshly distilled hexane was always used.

Epoxyubiquinones (**1**) *from Fractionation of the Lipid Extract of R. rubrum.* The lipid extract obtained from 1600 l. of *R. rubrum* was chromatographed on silica gel as previously described (Olsen *et al.*, 1966). Following elution of the column with a total of 24 l. of solvent varying in small increments from pure *n*-hexane to 6% ether in hexane, fractions of 15–20 ml were obtained using a fraction collector. In fractions from 70 to 990, a number of quinones were present as shown by positive reactions with leucomethylene blue spray reagent.

PRODUCTS A AND B. Fractions 71–240 (7–8% ether in hexane) were pooled and the quinones present were further fractionated by preparative layer chromatography. The quinones were purified as follows: (1) layer chromatography on silica gel G, eluting with chloroform; (2) the desired fractions were further purified by layer chromatography on silica gel G, eluting twice with hexane–ether (3:1). In this manner, five purified samples containing quinones were obtained; the third (2.3 mg) and fourth (3.2 mg) of these (order determined by decreasing mobility in the last named chromatographic system) are designated products A and B in Table I.

PRODUCT C. Fractions 241–440 (8–10% ether in hexane) were pooled and the quinones present were fractionated by preparative layer chromatography (three separations using hexane–ether (3:1), chloroform, and hexane–ether (3:1) as developing solvents). Three samples were produced; the most polar of these (11.8 mg) is product C in Table I.

PRODUCT D. Fractions 441–560 (10—11% ether in hexane) were pooled and the quinones present were fractionated by five successive preparative layer chromatographic steps using hexane–ether (3:1) as the developing solvent system. Five quinone-containing samples were produced; the fourth of these (in order of increasing polarity) consisted of 12 mg and is designated product D (Table I).

PRODUCT E. Fractions 781–870 (15–17% ether in hexane) were pooled and the quinones present were fractionated by preparative layer chromatography using chloroform as the developing solvent. Three quinone-containing samples were obtained; the least polar sample (4.3 mg) is designated product E in Table I.

*Treatment of Ubiquinone-10 (Q-10, **2**, n = 10) with Monoperphthalic Acid* (Böhme, 1955). A mixture of 0.2 g of Q-10 in 20 ml of ether and 1 ml of an ether solution of monoperphthalic acid (60 mg/ml) was allowed to stand at room temperature for 2 hr and then heated under reflux for 30 min. The solution was then washed with aqueous sodium bicarbonate and dried. The solvent was removed and the crude product was subjected to preparative layer chromatography using hexane–ether (4:1) as the developing solvent system. Following

[2] These measurements were obtained through the courtesy of Dr. C. Djerassi, Dr. E. Bunnenberg, and Mrs. R. Records of Stanford University, using a Japan Spectroscopic Co., Ltd., recording spectropolarimeter.

development, the plates showed four yellow, leucomethylene blue positive bands (I–IV) plus a small amount of material remaining at the origin. Band I (least polar) consisted of unreacted Q-10. Bands II and IV were eluted, and the nuclear magnetic resonance spectra of the resulting samples were obtained.

The spectral data (Table I) on the product of band II corresponded to epoxy-Q-10 (**1**), and the data on the product from band IV corresponded to diepoxy-Q-10 (**8**). Insufficient product was obtained by elution of band III to permit characterization; the similarity of its thin-layer chromatographic behavior with that of the monoepoxy-Q-10 (band II) suggests that band III was a different monoepoxy-Q-10.

Treatment of Ubiquinone-3 with Monoperphthalic Acid (Böhme, 1955). A mixture of 75 mg of Q-3 in 30 ml of ether and 1 ml of an ether solution of monoperphthalic acid (60 mg/ml) was heated under reflux for 12 hr. The reaction mixture was washed with sodium bicarbonate solution and the dried ether solution was evaporated. Preparative chromatography of the residue on silica gel G plates developed in hexane–ether (3:1) showed three yellow bands above the origin. The band of highest mobility corresponded to unchanged Q-3. The material associated with the second band was eluted and rechromatographed in the above described system. By development of the plates five times, this band was separated into two components which were eluted separately. Spectral data (Table I) characterized the compound of higher R_F value as 6′,7′-epoxy-Q-3 (**9**). Similarly, the compound of lower R_F value was identified as 10′,11′-epoxy-Q-3 (**10**).

Treatment of Ubiquinone-2 with Monoperphthalic Acid. A mixture of 75 mg of Q-2 in 15 ml of ether and 1 ml of an ether solution of monoperphthalic acid (Böhme, 1955) (60 mg/ml) was heated under reflux for 2 hr. The reaction mixture was washed with aqueous sodium bicarbonate; the solution was then dried ($MgSO_4$) and the solvent was removed. Chromatography of the residue on silica gel G plates developed in hexane–ether (3:1) exhibited three yellow bands above the origin. The upper band was unchanged Q-2; elution of the middle band yielded a compound characterized as 6′,7′-epoxy-Q-2 by the spectral data recorded in Table I.

Preparation of Hydroxy Acetate by Treatment of Synthetic Epoxy-Q-10 (**1**) *with Acetic Acid.* A solution of 20 mg of synthetic epoxy-Q-10 (**1**) in 0.2 ml of glacial acetic acid was heated on a steam bath for 6 hr. The reaction mixture was then poured into a saturated sodium bicarbonate solution layered with hexane. The hexane layer was separated, dried, and the solvent was removed. The crude product was purified by preparative layer chromatography. A plate was developed halfway using benzene–ethyl acetate (3:2); the solvent was then changed to 15% ether in hexane, and the development was repeated. The product was observed as a yellow-orange band of mobility less than that of unchanged epoxy-Q-10 which was also observed. Elution from the adsorbent produced 15 mg of an orange viscous oil which exhibited spectral properties which characterize it as a side-chain hydroxy acetate derivative of Q-10: infrared spectra (smear): 3500 (OH), 1735 (acetate), and 1650 and 1610 cm^{-1} (quinone); nuclear magnetic resonance spectra (CCl_4): multiplet at τ 4.99 (9 H, vinyl), singlet at τ 6.12 (6 H, methoxyl), doublet at τ 6.92 (2 H, ring CH_2), singlet at τ 8.00 (3 H, acetate), 7.8–8.5 (alkyl), and 8.93 (3 H, methyl). Discrete signals due to the hydroxyl proton and the methine proton were not observed.

Preparation of Hydroxy Acetates of Isolated Epoxyubiquinones-10 (**1**). PRODUCTS C AND D. By the procedure described above for the preparation of a hydroxy acetate of synthetic epoxy-Q-10, hydroxy acetates of products C and D were prepared. These hydroxy acetates exhibited infrared spectra indistinguishable from that of the hydroxy acetate of synthetic epoxy-Q-10. A paper chromatographic comparison is recorded in Table II.

Control Experiments. The following experiments were performed in order to assess the possibility that the conditions used in the fractionation of lipids from *R. rubrum* allowed artifactual formation of epoxyubiquinones (**1**).

Pure ubiquinone-10 (**2**, $n = 10$) (100 mg) was divided into three portions and treated as follows. (1) Ubiquinone-10 was dissolved in 50 ml of diethyl ether and shaken for 18 hr. (2) Ubiquinone-10 was dissolved in 50 ml of diethyl ether and 25 ml of ethanol and shaken for 18 hr. (3) Ubiquinone-10 was dissolved in 50 ml of diisopropyl ether and shaken for 18 hr.

The ether used for control expt 1 and 2 was stock anhydrous ether from a container which had been opened for more than 1 week. Tests for peroxides [$Na_2Cr_2O_7 + H_2SO_4$ (Fieser, 1957) and starch iodide] were negative. The starch iodide procedure for peroxides showed that the diisopropyl ether used in expt 3 contained the equivalent of approximately 0.0125 mole of peroxide (Q-10 used = 0.035 mole).

Following the treatments indicated above, solvents were removed from test samples 1–3, and the residues were analyzed by thin layer chromatography on silica gel G plates developed in hexane–ether (8:2). The residues obtained from test samples 1 and 2 appeared to be pure Q-10; no other products were detected by leucomethylene blue or basic permanganate spray reagents. Analysis of sample 3 showed, in addition to Q-10, two faint leucomethylene blue positive spots below that due to Q-10. The more intense spot (lower R_F) corresponded to synthetic epoxy-Q-10 (**1**) used as a reference.

The Q-10 recovered from control tests 1 and 2 was combined and chromatographed on silica gel using 2% ether in hexane followed by 5% ether in hexane as eluting solvent systems. Total contact time of the Q-10 with the column was ~8 hr. The major Q-10 band and the following 150 ml of eluate were analyzed by thin layer chromatography as described above. No decomposition products were detected in the major Q-10 fraction. The residue from the 150 ml of eluate following the major Q-10 fraction was shown to consist of Q-10 plus several polar components appearing on thin layer chromatography as leucomethylene blue positive

spots at and slightly above the origin. In addition, a faint leucomethylene blue positive spot corresponding to synthetic epoxy-Q-10 was observed.

Acknowledgment

We are grateful to Dr. R. F. Muraca and Mrs. J. S. Whittick for the mass spectra which have significantly aided structural elucidation. We also express our sincere appreciation to Dr. Max Tishler.

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