ISOLATION OF UBIQUINONE-5, NEW MEMBER OF UBIQUINONE GROUP[1]

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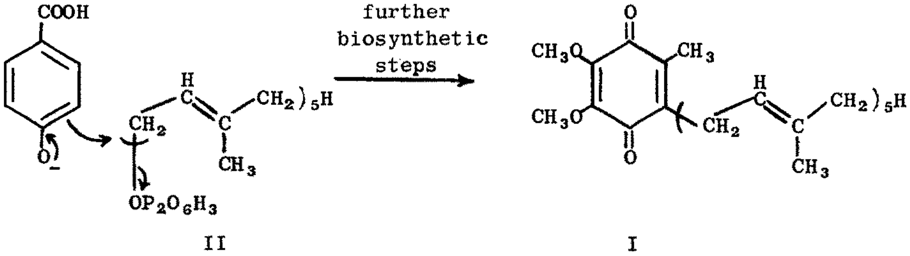
A new member of the ubiquinone group has been isolated from the cells of Escherichia coli 08, and its structure is established as ubiquinone-5, Q_5 (I). It was shown that this strain of E. coli contains four ubiquinones, i.e., Q_8, Q_7, Q_6, and Q_5; however, Q_8 is the major component. Ubiquinone-5 has been known previously by synthesis (Isler et al., 1961), but not as a natural product.



p-Hydroxybenzoic acid is now established as a precursor to the ubiquinones in living organisms (Parson and Rudney, 1964, 1965; Aiyar and Olson, 1964). It is apparent (Olsen et al., 1965) that the first reaction of p-hydroxybenzoic acid in the biosynthetic pathway to the Q's is alkylation, presumably by a multiprenyl pyrophosphate. Thus, pentaprenyl pyrophosphate (II) could participate in the alkylating step in the biosynthesis of ubiquinone-5 (I) by the mechanism depicted.

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Among the many terpenoid compounds found in nature there has been a striking lack of C_{25}-terpenoids. Recently Nozoe et al. (1965) reported the isolation and structure elucidation of ophiobolin ($C_{25}H_{36}O_4$), a fungal metabolite of complex structure. The authors believe this compound to be the first known example of a C_{25}-terpenoid in nature and formulate ophiobolin as arising biogenetically from a hypothetical five-unit terpenoid pyrophosphate (II). The isolation of ubiquinone-5, a compound with an intact pentaprenyl chain, provides strong evidence for the existence in nature of pentaprenyl pyrophosphate (II) or its biological equivalent.

Ubiquinone-5 was isolated by extensive fractionation of the Q-fraction obtained from column chromatography of E. coli lipids, and it constituted about 1% of the total amount of Q. The ultraviolet and infrared absorption spectra of this new compound are typical for ubiquinones (Langemann and Isler, 1965). Its position on reverse phase paper chromatography (Table I) (Lester and Ramasarma, 1959), and its NMR spectrum (Langemann and Isler, 1965) showing five vinylic protons, besides the other protons, indicated that the compound possessed a side chain of five isoprene units. Final proof of the molecular size of this analog was obtained from the mass spectrum which showed the parent mass to be 522.379 (calcd. for $C_{34}H_{50}O_4$: 522.3709). We are indebted to Dr. Raffaele F. Muraca and Mrs. Julia S. Whittick for this spectrum.

Although it has been a frequent point of view that most species of living organisms tend to have only one ubiquinone, there are some exceptions reported. Rat liver contains Q_9 and Q_{10} in considerable amounts (Linn et al., 1959), and others (Diplock et al., 1960) showed that rat tissue may contain Q_{10}, Q_9, Q_8, and Q_7. In Torula yeast (Candida utilis) are found Q_9 and Q_7 (Lester and Crane, 1959). The Q-components from 18 representative molds were isolated and characterized by UV, melting point, and paper chromatography (Lavate and Bentley, 1964). Of six organisms having Q_9 as the major component, two showed trace amounts of Q_8 and two others had 12 and 30% of Q_8. Two of the molds were shown only to contain Q_{10}, and among nine organisms containing dihydro-Q_{10} one had 26% Q_{10}, one had trace amounts of Q_{10}. Giberella fujckuroi had 8% Q_{10} and trace amounts of Q_9 and Q_8.

Only Q_8 has been reported earlier from E. coli (Lester and Crane, 1959; Bishop et al., 1962). In the present study, in addition to Q_8, the major component, and the new Q_5, the previously known Q_7 and Q_6 were also isolated.

On the basis of the cited examples and the results from E. coli, it is reasonable to believe that many microorganisms actually contain several ubiquinones as both major and minor constituents.

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Experimental

Isolation and Separation of Ubiquinones. - One kilogram of dry material of E. coli 08 was generously provided by Drs. Thomas H. Stoudt and H. Boyd Woodruff of Merck and Co., Inc., Rahway, New Jersey. The cells were extracted by shaking 15 hours with 5 liters of ether-ethanol (3:1). The filtrate was evaporated in vacuo, and the residue was dissolved in 300 ml of aqueous methanol (95%). After addition of 300 ml of hexane, the mixture was filtered, and the organic phase was separated. The aqueous phase was further shaken with 150 ml of hexane, and the combined extracts were dried over Na_2SO_4. The solvent was evaporated, and the residue (14 g) was dissolved in 80 ml of hexane. The orange-brown solution was chromatographed on a column of silica gel (5.2 x 90 cm). The column was developed by elution with 3 liters of hexane followed by increasing one-percent increments of ether in hexane in 3-liter portions, beginning with 1% ether in hexane.

The ubiquinones were eluted with 5% ether in hexane and were collected in two portions. The first of these fractions contained practically pure Q_8. The residue obtained from the second fraction was dissolved in a small volume of ether and streaked on silica gel G plates (0.5-1 mm). The plates were developed in chloroform, air dried, and again developed in chloroform. This procedure was repeated three times. The upper and lower halves of the yellow quinone band

(1-2 cm wide) were collected separately and eluted with ether. The material obtained from the lower half of the Q-band was subjected to the thin layer chromatographic procedure described above. Four successive separations were carried out in this manner; each time the upper half of the yellow quinone band was discarded. Finally, a sample which on chromatography on silicon-coated paper (Lester and Ramasarma, 1959) proved to be pure ubiquinone-5. Q_8, Q_7, Q_6, and Q_5 were present to different extents in the intervening fractions

Table I

PAPER CHROMATOGRAPHY OF UBIQUINONES

	R_f Values	
	Experiment No. 1	Experiment No. 2
Q_{10} (a)	0.21	0.22
Q_9 (a)	0.30	0.30
Q_8 (b)	0.38	0.40
Q_7 (b)	0.50	0.50
Q_6 (a,b)	0.61	0.62
Q_5 (b)	0.73	0.72

Descending, *n*-propanol:water (7:3);
Whatman No. 3MM impregnated with
Dow-Corning No. 550 silicon.
Sprayed with leucomethylene blue.

[a] Reference samples.
[b] From *E. coli*.

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