NEW MULTIPRENYLQUINONES IN THE BIOSYNTHESIS OF UBIQUINONE*

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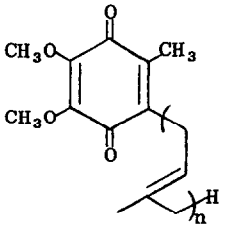
Evidence for another precursor in the sequence of biosynthesis of ubiquinone, and two other precursors representing nonspecific reactions or an alternative sequence, has been newly obtained.

The isolation and identification of new multiprenyl-phenols (Olsen et al., 1966) and -benzoquinones (Friis et al., 1966) from Rhodospirillum rubrum allowed the formulation of an apparent biosynthetic sequence from p-hydroxybenzoic acid (HBA) to ubiquinone (1) (Friis et al., 1966). In this sequence, 2-decaprenyl-5-hydroxy-6-methoxy-3-methyl-1,4-benzoquinone (2, n = 10), although not then known, was envisioned (Friis et al., 1966) as the precursor immediately preceding ubiquinone (1). This precursor has now been isolated from R. rubrum. Also, utilizing cells of R. rubrum incubated with HBA ($U^{14}C$), this precursor, 2, was shown to be derived from HBA. These findings are in accord with its predicted existence as a precursor and provide additional support for the sequence (Friis et al., 1966).



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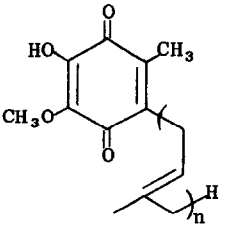
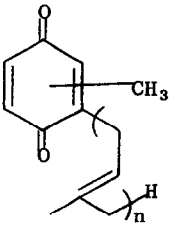


2



3

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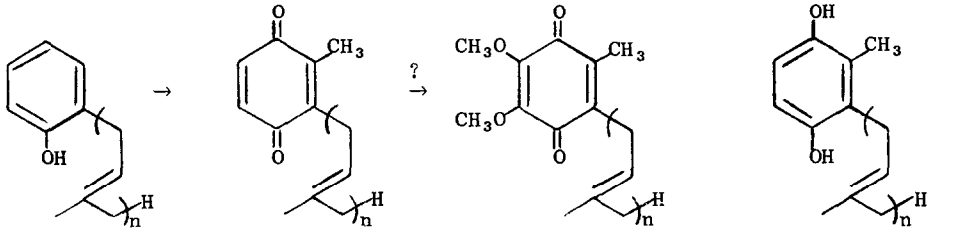
Two other multiprenylbenzoquinones, methylnonaprenylbenzoquinone (3, n = 9) and methyldecaprenylbenzoquinone (3, n = 10), from polar and purified fractions of the lipid extract (Olsen et al., 1966), have been newly identified by the presence of characteristic (Muraca et al., 1967) signals in mass spectra. Although the mass spectra establish the presence of compounds 3 (n = 9, 10), the position of the methyl group is not proven.

The presence of these methylmultiprenylquinones in R. rubrum is noteworthy, since the only multiprenylquinones previously recognized from R. rubrum are ubiquinone (1) and rhodoquinone (Moore and Folkers, 1966a) and their precursors; vitamin K (Dam, 1944); plastoquinones (Lester and Crane, 1959; Fuller et al., 1961) and precursors of the tocopherols have not been obtained from R. rubrum. Consequently, these methylmultiprenylbenzoquinones may be intermediates to ubiquinones-9 and -10 (1, n = 9, 10) in another or branch biosynthetic pathway. If these methylmultiprenylbenzoquinones (3, n = 9, 10) should have structure 5 (n = 9, 10), 2-methyl-3-multiprenyl-1,4-benzoquinone, by analogy with the structure of ubiquinone, an alternative sequence of hydroxylation and methylation steps (4 → 5 → 1) can be visualized which might utilize 2-methyl-3-multiprenyl-1,4-benzoquinone (5) as an intermediate in the conversion of 2-multiprenylphenol (4) (Olsen et al., 1966). Alternatively, the methylmultiprenylbenzoquinones may indicate only a lack of substrate specificity in the apparent conversion of precursor 4 to 5 and without continuation to ubiquinone:

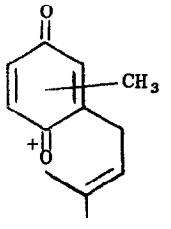


4 5 1 6

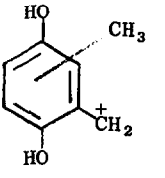
Olson (Olson, 1966) suggested that an unidentified intermediate in the biosynthesis of ubiquinone-9 (1, n = 9) in rat liver tissue (Olson and Aiyar, 1966) might possess structure 6 (n = 9), which is the hydroquinone corresponding to 5 (n = 9).

In the fractionation (Friis and Folkers, unpublished data) of the lipid extract (Olsen et al., 1966) from R. rubrum, quinonoid material (detected by its sensitivity to leucomethylene blue) was observed in fractions eluted with hexane/ether (3:1). Following further purification by thin layer chromatography, the material, which was leucomethylene blue sensitive, was separated into three fractions (A, B, and C) by thin layer chromatography on silica gel G plates developed in chloroform (fraction A highest R_f).

Each of these fractions was examined by the mass spectrometric procedures which have been described (Muraca et al., 1967). Each fraction consisted of complex mixtures of several quinones as evidenced by the appearance of typical M and M+2 parent ion clusters (Muraca et al., 1967). The major components (as judged by the relative intensities of parent ion peaks) in the two more polar quinone fractions (B and C) gave rise to parent ions at m/e 802, 804 (B), and m/e 734, 736 (C), respectively. The most intense peak in the spectrum of B and C appeared at m/e 175, and the second most intense peak, in each case, appeared at m/e 137. These peaks can be assigned to fragment ions 7 and 8, respectively, in analogy to the mass spectra of other multiprenylquinones (Muraca et al., 1967).



7 m/e 175



8 m/e 137

The hexane/ether (3:1) fraction, from cells of R. rubrum, which had been incubated with radioactive HBA (Parson and Rudney, 1964) ($2 \cdot 10^6$ dpm) for 7 hours in light and 14 hours in darkness as previously described (Olsen et al., 1966), was shown to possess radioactivity ($2 \cdot 10^4$ dpm). For comparison, the corresponding ubiquinone (1) possessed $5 \cdot 10^5$ dpm. Thin layer chromatography of the hexane/ether (3:1) fraction was carried out on silica gel G plates

which were developed 5 times in hexane/ether (4:1); radioactive counting on a Nuclear-Chicago strip counter revealed several bands associated with radioactivity. One of the radioactive zones, appearing as a purple band ($\sim$ 3 cm above the origin), was eluted and further purified by thin layer chromatography on a silica gel G plate developed in chloroform/methanol (9:1, $R_f \sim 0.3$). The product obtained was shown to be a quinone (positive reaction with leucomethylene blue) and exhibited ultraviolet spectral behavior which characterizes it as a 2-multiprenyl-5-hydroxy-6-methoxy-3-methyl-1,4-benzoquinone (2). A ten-unit multiprenyl side chain (i.e., 2, n = 10) is assigned by analogy with the major ubiquinone (1, n = 10) produced by R. rubrum (Olsen et al., 1966). This product (2, n = 10) exhibited λ_{max}^{hexane} 277 mμ; λ_{max}^{EtOH} 282 and 430 mμ; and $\lambda_{max}^{EtOH + NaOH}$ 283 and 535 mμ. 2-Solanesylfumigatin (2, n = 9), synthesized by Shunk et al. (Shunk et al., 1966), exhibited $\lambda_{max}^{isooctane}$ 270.5 and 277.5 mμ; 2-phytyl-5-hydroxy-6-methoxy-3-methyl-1,4-benzoquinone (Moore and Folkers, 1966b) (phytylfumigatin) exhibited λ_{max}^{EtOH} 277 and 428 mμ, and $\lambda_{max}^{EtOH + NaOH}$ 281 and 536 mμ.

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