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Menachromanol from a Photosynthetic Bacterium

By R. POWLS and E. R. REDFEARN

Department of Biochemistry, University of Leicester

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Chlorobium thiosulphatophilum has been shown to contain two naphthaquinones, menaquinone-7 and 'chlorobiumquinone' (Frydman & Rapoport, 1963). The possibility that other related compounds are also present has been investigated.

C. thiosulphatophilum (N.C.I.B. 8346) was grown anaerobically as described by Larsen (1953). The harvested organism was extracted with acetone, the acetone extract diluted with water and the lipid extracted into ether. After the ethereal extract had been evaporated to dryness *in vacuo* the lipid was adsorbed on a silicic acid–Celite (2:1, w/w) column. The less-polar lipid constituents were eluted with 25% (v/v) diethyl ether in light petroleum (b.p. 40–60°), leaving the chlorophyll and other polar lipids adsorbed on the column.

A small sample of the eluted lipid was chromatographed on thin layers of silica gel G with 10% (v/v) di-isopropyl ether in light petroleum. The developed chromatoplates were sprayed with 0.002% (w/v) fluorescein in ethanol and examined under ultraviolet light. Strongly ultraviolet-absorbing compounds detected were 'chlorobiumquinone' (R_f 0.30) and menaquinone-7 (R_f 0.74). However, a compound that showed a strong blue fluorescence in ultraviolet light (R_f 0.56) was also observed. This compound gave an immediate Emmerie–Engel positive reaction, suggesting that it may be phenolic.

To obtain more of this compound the lipid eluted from the first column was rechromatographed on a second silicic acid column. The fluorescent compound was eluted along with the menaquinone by 2.5% (v/v) diethyl ether in light petroleum, the γ -carotene having been previously eluted with 0.5% (v/v) diethyl ether in light petroleum. The

fluorescent compound was separated from the menaquinone by thin-layer chromatography as before. The compound in cyclohexane showed λ_{max} at 245 (intense) 320 and 338 m μ (weak), which are very similar to those shown by naphthachromanol (Mamont, Cohen, Azerad & Vilkas, 1965).

To test the possibility that this compound was menachromanol, phylochromanol was prepared from phyloquinone (Chen & Dallam, 1963). On comparison, the two compounds were extremely similar, showing identical spectra and fluorescence in ultraviolet light. However, they differed in their polarity; the *Chlorobium* compound had R_f 0.44 on thin-layer chromatography with 7% (v/v) di-isopropyl ether in light petroleum, whereas that of phylochromanol was 0.30.

Comparison of the infrared spectra of the two compounds showed many points of similarity, the differences being accounted for by the presence of an unsaturated side chain in the compound isolated from *Chlorobium* (Table 1).

From this evidence the compound appears to be a menachromanol, and, as we have confirmed that the menaquinone from *Chlorobium* is menaquinone-7 by nuclear-magnetic-resonance spectrometry, it is likely to be menachromanol-6. To prove this completely it would be necessary to synthesize the compound; unfortunately, published methods for cyclizing the corresponding quinone were found to lead to further cyclization in the side chain.

Russell & Brodie (1961) have shown exogenous phyloquinone to be cyclized to phylochromanol by extracts of *Mycobacterium phlei*, and, moreover, have demonstrated chromanol formation from the endogenous menaquinone-9(2H) in phosphorylating preparations. There have been many

Table 1. *Assignment of peaks in the infrared spectra*

Grouping (Bellamy, 1958)	Observed $\nu_{\max}$ (cm. ⁻¹)		
	<i>Chlorobium</i> compound	Phyllochromanol	Literature
Phenolic -OH deformation (3616-3585 cm. ⁻¹)	3520	3420	3440 (plastochromanol*)
Aromatic C=C (1650-1600 cm. ⁻¹)	1625	1620	1612 (plastochromanol*) 1618 (α -tocopherol†)
Aromatic enhanced by conjugated rings (1600-1560 cm. ⁻¹)	1599 (very strong)	1580 (very strong)	1587 (vitamins K†)
Aromatic ring (1504-1493 cm. ⁻¹)	1500 (weak)	1500 (weak)	
Aryl ethers (1270-1230 cm. ⁻¹ ; very strong)	1270 (strong)	1275 (strong)	1224 (plastochromanol*) 1250 (α -tocopherol†)
Alkyl ethers (O rings) (1140-1070 cm. ⁻¹)	1085	1090	1080 (plastochromanol*)
Four adjacent aromatic H atoms (770-735 cm. ⁻¹ ; very strong)	760 (very strong)	762 (very strong)	
Unconjugated C=C (1680-1620 cm. ⁻¹)	1660	—	1667 (isoprenoids†)
Skeletal vibrations	1115 (strong)	1110 (weak)	1100 (present in vitamin K ₁ , absent from vitamin K ₂ †)
Trisubstituted ethylene (840-800 cm. ⁻¹)	834, 805 (shoulder)	—	Isoprenoids [840, 805 (shoulder)†]
Skeletal vibrations (saturated units)	—	731	735 (phytol†)

* Whittle, Dunphy & Pennock (1965).

† Pennock (1965).

suggested mechanisms for oxidative phosphorylation involving quinone-chromanol conversions (see Vilkas & Lederer, 1963). Gutnick & Brodie (1965) reported a phosphate-dependent incorporation of tritium from tritiated water into added phylloquinone during oxidative phosphorylation in *M. phlei* extracts, suggesting that such a conversion was occurring.

With the finding of menachromanol in *Chlorobium*, after the discovery of plastochromanol in green leaves (Whittle *et al.* 1965), it is possible that similar quinone-chromanol conversions are involved in photosynthetic electron transport. However, the metabolic significance of these conversions and their relationship to the phosphorylation reactions remain to be investigated.

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