A DESMETHYLMENOQUINOL DERIVATIVE ISOLATED FROM GREEN PHOTOSYNTHETIC BACTERIA

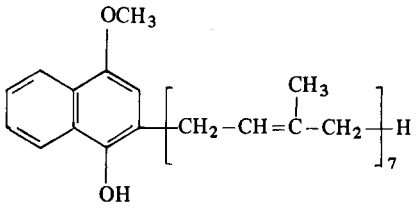
R.POWLS

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, England

Received 5 December 1969

1. Introduction

A compound which fluoresces blue in ultraviolet light was previously isolated from the green photosynthetic bacteria, *Chlorobium thiosulphatophilum* and *Chloropseudomonas ethylicum*, and tentatively identified as menachromanol-6 [1]. A more detailed study of its properties has indicated that such an identification is not justified. Nuclear magnetic resonance and mass spectra have now shown the compound to be 4-*O*-methyl-2-heptaprenyl naphthaquinol (I).



(I)

2. Materials and methods

The fluorescent compound was isolated from *C. thiosulphatophilum* (1 kg) as previously described [2], except that acetone instead of methanol was used for the initial extraction.

3. Results and discussion

Mass spectroscopy data were consistent with the compound being menachromanol-6, molecular formula $C_{46}H_{66}O_2$ (mass peak at *m/e* 650). The absorption spectrum of the compound showed λ_{max} 245, 321 and 334 nm in cyclohexane which was essentially the same as that of synthetic phyllochromanol and phylloquinol, except that it was displaced 4 nm towards lower wavelengths (fig. 1). This indicated that the compound was a reduced naphthoquinone derivative. Apart from the contribution of the isoprenoid side-chain the infra red spectrum is very similar to that of phyllochromanol. Ether bands are very prominent and these were originally attributed to the chromanol ring [1].

However the isolated compound is surprisingly different from phyllochromanol in its polarity; R_f 0.55 compared with the 0.21 of phyllochromanol on thin layer chromatography on silica gel with 10% isopropyl ether/light petroleum. This difference is unlikely to be due to the degree of saturation of the side chain. The non-polar nature of the compound was puzzling since it was Emmerie-Engel positive and the infrared spectrum indicated the presence of a hydroxyl group. However non-polar phenols are known, decaprenyl phenol is a case in point. No change in polarity occurred on acetylating the bacterial compound (the acetate showed λ_{max} 242 with a shoulder at 238 nm), whereas the R_f of phyllochromanol changed from 0.21 to 0.44 on acetylation.

The chromanol ring can be cleaved by oxidizing agents, such as ferrice chloride and chlorauric acid, to give the γ-hydroxyquinone. Oxidation of the bacterial compound gave desmethylmenaquinone-7 [2] and not the expected γ-hydroxymenaquinone-7. Hence it is unlikely that the bacterial compound has either a nuclear methyl group or a chromanol ring.

North-Holland Publishing Company – Amsterdam

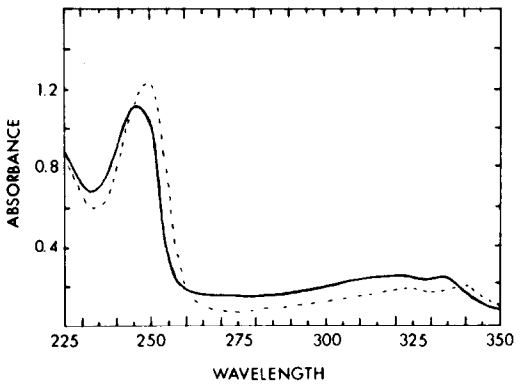


Fig. 1. Absorption spectra of cyclohexane solution of 4-*O*-methyl-2-heptaprenyl naphthaquinol (——) and phyllochromanol (- - - - -).

Nuclear magnetic resonance spectroscopy (table 1) showed the bands expected for structure (I), in particular a strong singlet due to the three protons of the methoxyl group at 6.07 τ. Oxidation of (I) would be expected to cleave the methoxyl group and yield desmethylmenaquinone-7 [3].

Compound (I) on storage slowly gave rise to a non-fluorescent, non-reducing compound which showed selective absorption, $\lambda_{max.}$ 267, 275, 350 with shoulders at 345 and 375 nm. This absorption spectrum was qualitatively indistinguishable from that of synthetic desmethylphyllochromenol (fig. 2). The breakdown product did however differ from desmethylphyllochromenol in many ways, particularly its lack of polarity, R_f 0.81 compared with 0.16 on TLC on Silica gel with 10% isopropyl ether/light petroleum. Moreover the infrared spectra of the two compounds differed considerably. The breakdown product lacked any absorption at 3390 cm^{-1} (phenolic hydroxyl group), but it had stronger absorption bands than did desmethylphyllochromenol at 1275 cm^{-1} (aryl ether) and 1104 cm^{-1} (alkyl ether, methoxyl) [4]. This evidence together with that from mass spectroscopy, which gave a molecular formula of $C_{46}H_{64}O_2$ (mass peak at *m/e* 648), indicated that the breakdown product must be 1-*O*-methyl-2-desmethyl menachromenol-6 (II). The breakdown of (I) to (II) occurs by oxidative cyclization.

The possibility that (I) was an artifact, being derived from another menaquinone derivative, seems unlikely. The concentration of (I) is the same order in the two

Table 1

Nuclear magnetic resonance spectrum of the fluorescent compound isolated from *Chlorobium thiosulphatophilum*.

τ Value	Structural element	Proton number
1.87 2.50	Four adjacent hydrogens in benzenoid ring	4
3.45	Free hydrogen in phenolic ring	1
4.91	Olefinic hydrogens of the chain	7
6.07	Methoxyl hydrogens	3
6.50	Allylic methylene of the ring-terminal unit of side chain	2
7.98	Methylenes of the side chain	24
8.13	*Trans* methyl in ring-terminal unit of the side chain	3
8.31	*Cis* methyl in terminal unit of side chain	3
8.38	*Trans* methyls in the side chain	18

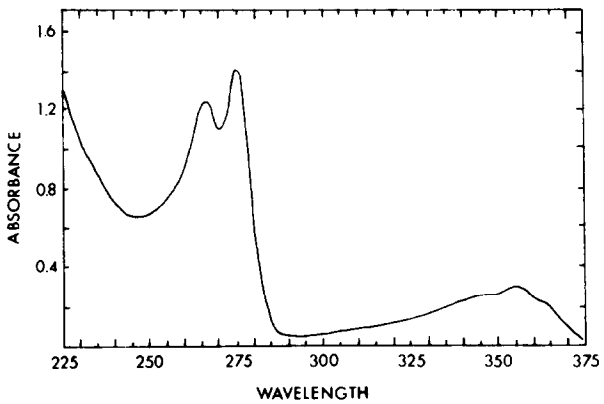
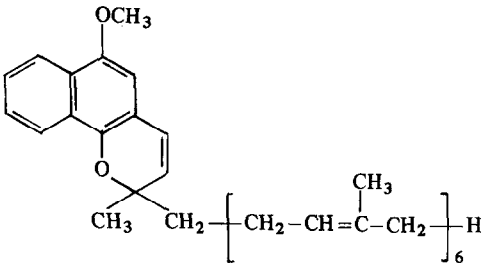


Fig. 2. Absorption spectra of cyclohexane solution of 10-methyl-2-desmethyl menachromenol-6.

bacteria, as in that of menaquinone-7, whereas chlorobiumquinone is hardly detectable in *Cps. ethylicum* [2]. This compound is unlikely to be derived from menaquinone-7 since it is not found in lipid extracts of *Chromatium* prepared in the same way, although these extracts do contain menaquinone-7.

The biological function of (I) is not clear, it may be acting as a precursor of the menaquinone. Methylation of one of the hydroxyl groups results in the



(II)

stabilization of the naphthoquinol nucleus to oxidation. This stabilization may be necessary to enable further nuclear substitution whilst still enabling the product to be oxidised to menaquinone. This is in contrast to a stabilization of the nucleus by the formation of a chromanol ring, oxidation of which would yield not menaquinone but the hydroxyquinone being formed instead. However the concentration of (I) did not change when *Cps. ethylicum*, grown on ethanol and bicarbonate was transferred to a sulphide medium [2]. Under these conditions a vast synthesis of chlorobiumquinone occurs at the expense of the other menaquinones. This finding suggests that (I) is unlikely to be a menaquinone precursor in this organism.

Acknowledgements

I wish to thank Dr. J.F.Pennock for many helpful discussions and Dr. M.C.W.Evans for the gift of bacterial cells.

References

[1] R.Powls and E.R.Redfearn, Biochem. J. 102 (1967) 3C.
[2] R.Powls and E.R.Redfearn, Biochem. Biophys. Acta 172 (1969) 429.
[3] O.C.Musgrave, Chem. Rev. 69 (1969) 499.
[4] L.J.Bellamy, The infrared spectra of complex molecules (Methuen, London, 1958) p. 114.