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SHORT COMMUNICATION

THE OCCURRENCE OF 1-*O*-METHYL-2-DEMETHYL-PHYTYLPLASTOQUINOL IN *EUGLENA GRACILIS*

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Abstract—The isolation and characterization of 1-*O*-methyl-2-demethylphytylplastoquinol from photoheterotrophic cells of *Euglena gracilis* is described.

INTRODUCTION

THE STUDY of the biosynthesis of isoprenoid quinones and chromanols by higher plants and algae has led to the isolation and characterization of two new naturally occurring isoprenoid quinones, phytylplastoquinone (2,3-dimethyl-5-phytyl-1,4-benzoquinone) and 2-demethylphytylplastoquinone, and a novel isoprenoid quinol ether, phytylplastoquinol monomethyl ether (2,3-dimethyl-4-methoxy-5(or -6)phytylphenol),‡ from the alga *Euglena gracilis*.[1,2] In this communication we report the isolation of a second isoprenoid quinol monomethyl ether, 1-*O*-methyl-2-demethylphytylplastoquinol (I), from this organism.

RESULTS AND DISCUSSION

Ah Law, whilst isolating 2-demethylphytylplastoquinone from streptomycin-bleached cells of *E. gracilis* strain Z,[2] isolated small amounts of a compound whose UV spectrum and TLC properties were those expected of a 2-demethylphytylplastoquinol monomethyl ether. The new compound was also found to be present in photoautotrophic and photoheterotrophic cells of the alga. To characterize it more fully 1·16 mg was purified from a 20-l. batch of photoheterotrophically grown cells.

The UV spectrum of the new ether (λ_{max} 290 nm in cyclohexane) was of little diagnostic value; however, when it was considered in conjunction with the facts that the new ether was, (i) more polar than phytylplastoquinol monomethyl ether on reversed phase TLC, and (ii) co-occurred with 2-demethylphytylplastoquinone, it was apparent that it was probably a 2-demethylphytylplastoquinol monomethyl ether.

The mass spectrum of the new ether showed the molecular ion M^+ at *m/e* 416 ($C_{28}H_{48}O_2$), together with the major fragment ions at *m/e* 205, 191 and 151 (Scheme 1).[5,6]

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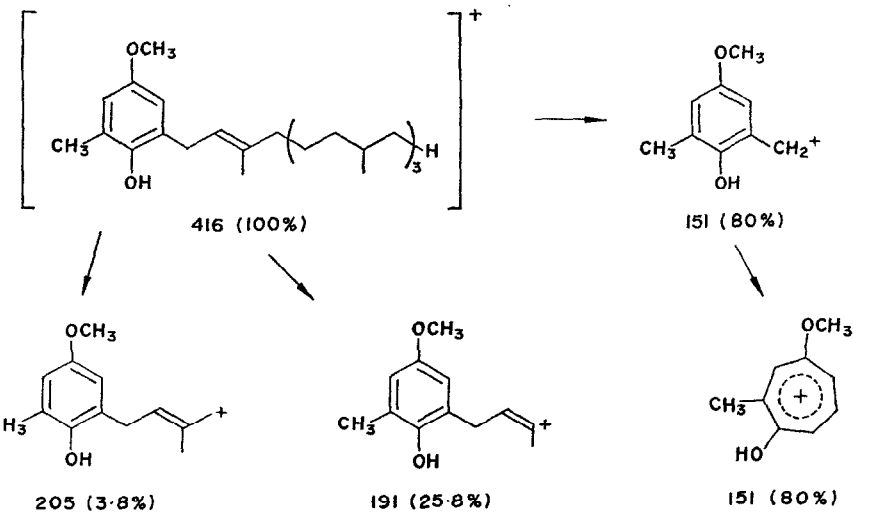
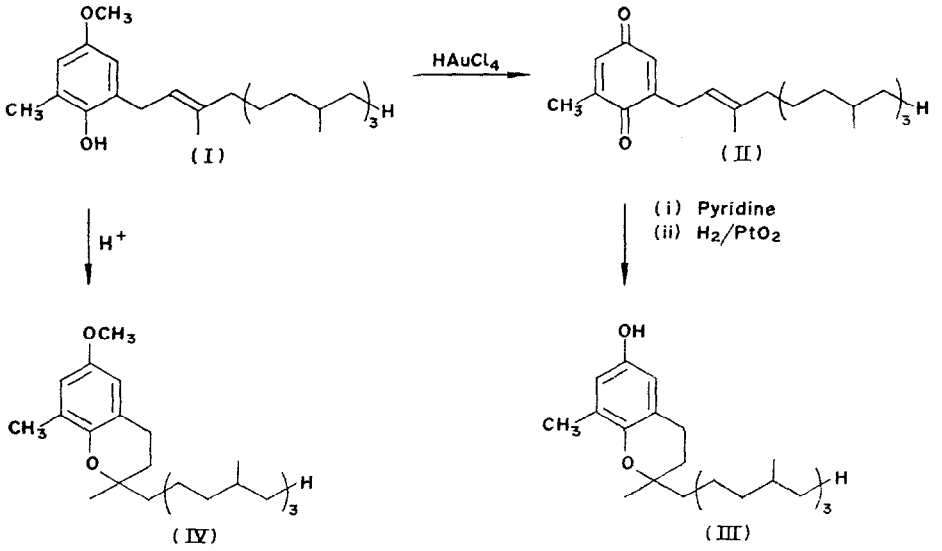
‡ There have been reports of the isolation of a positional isomer of this ether from *E. gracilis*.[3–5] However, we have evidence that suggests the isomer was mischaracterized and that it is in fact phytylplastoquinol monomethyl ether.

[1] G. R. WHISTANCE and D. R. THRELFALL, *Phytochem.* **9**, 213 (1970).
[2] D. R. THRELFALL, AH LAW and W. A. WHITE, *Proc. Biochem. Soc.*, Bangor (1971).
[3] JEAN E. VANCE and R. BENTLEY, *Fedn Proc. Fedn Am. Socs. Exp. Biol.* **28**, 905 (1969).
[4] JEAN E. VANCE and R. BENTLEY, *Fedn Proc. Fedn Am. Socs. Exp. Biol.* **29**, 936 (1970).
[5] JEAN E. VANCE, Ph.D Thesis, University of Pittsburgh (1969).
[6] AH LAW and D. R. THRELFALL, unpublished observations.



SCHEME 1. FRAGMENTATION OF 1-*O*-METHYL-2-DEMETHYLPHYTYLPLASTOQUINOL. THE FIGURES IN PARENTHESIS ARE THE OBSERVED PEAK INTENSITIES IN THE MASS SPECTRUM.

Oxidation of the ether with $HAuCl_4$ gave a quinone with UV properties qualitatively identical to those of natural and synthetic 2-demethylphytylplastoquinone (II). In contrast to the quantitative oxidation of phytylplastoquinol monomethyl ether to phytylplastoquinone,[1] 50% of the new ether was unoxidized under the conditions employed. However, when $FeCl_3$ was substituted for $HAuCl_4$ the oxidation went to completion. The quinone was characterized as 2-demethylphytylplastoquinone (II) by TLC (adsorptive, reversed-phase and Ag^+ ion), by UV spectroscopy (λ_{max} 252 nm in EtOH; changing to λ_{max} 289 nm after



SCHEME 2. CHEMICAL SYNTHESES OF 2-DEMETHYLPHYTYLPLASTOQUINONE (II), δ-TOCOPHEROL (III) AND δ-TOCOPHEROL MONOMETHYL ETHER (IV) FROM 1-*O*-METHYL-2-DEMETHYLPHYTYLPLASTOQUINOL (I).

$NaBH_4$ treatment), by mass spectrometry and by the UV and TLC properties of its chromanol (δ-tocopherol) (III).[2]

Under acid conditions the new ether cyclized quantitatively into a chromanol with spectral, GLC and TLC properties identical to those of δ-tocopherol monomethyl ether (IV) showing that it must be 1-*O*-methyl-2-demethylphytylplastoquinone. It is noteworthy that under the same conditions phytylplastoquinol monomethyl ether was converted to γ-tocopherol monomethyl ether, showing that it too is a 1-*O*-methyl ether.

Studies in which photoautotrophic and photoheterotrophic cultures of the alga were allowed to metabolize [α-^{14}C]homogentisic acid have indicated that 1-*O*-methyl-2-demethylphytylplastoquinol and its corresponding quinone, 2-demethylphytylplastoquinone, belong to the biogenetic group of compounds made up of the plastoquinones, tocopherols, tocotrienols, tocopherolquinones and tocotrienolquinones.[7] They also showed that just as the phytylplastoquinol monomethyl ether is probably a metabolite of phytylplastoquinone,[8] so too 1-*O*-methyl-2-demethylphytylplastoquinol is probably a metabolite of 2-demethylphytylplastoquinone.

Apart from making the obvious suggestion that the reduced forms of the phytyl quinones could act as precursors of tocopherols[1,2] it is premature at this stage to ascribe biological functions to these new phytyl quinones and ethers. What can be said, however, is that it is not unreasonable to expect the isolation of 6-methylphytylplastoquinone and the 1-*O*-methyl ethers of 6-methylphytylplastoquinol, plastoquinol-9 and α-tocopherolquinol from biological sources.

EXPERIMENTAL

Euglena gracilis strain Z was obtained from The Culture Collection of Algae and Protozoa, Botany School, Cambridge. Photoheterotrophic cells of the organism were obtained by growing it for 4 days at 28° with constant illumination and agitation [180 rev/min; Psycrotherm Incubator Shaker (New Brunswick Scientific Co.)] in a medium containing 0·5% NaOAc, 0·5% proteose peptone (Oxoid) and 0·2% yeast extract (Difco) in tap water. The medium (20 l.) was dispensed as 1-l. volumes in 2-l. conical flasks.

The lipids were extracted from the wet cell mass (equivalent to 40 g dry wt.) by a routine procedure[9] and then chromatographed on a column of Brockmann grade III acid-washed alumina (Woelm) developed with 0·25% and 1% Et_2O in light petroleum (b.p. 40–60°).[9] The 1-*O*-methyl-2-demethylphytylplastoquinol (1·16 mg) along with plastoquinone-9 (10·3 mg), 2-demethylphytylplastoquinone (0·31 mg), phytylplastoquinol monomethyl ether (6·12 mg) and ergosterol esters was eluted by 1% Et_2O in light petroleum. The quinones and ethers (R_f 0·45–0·56) were separated from ergosterol esters (R_f 0·67) and minor pigments by TLC on Rhodamine 6G-impregnated silica gel G developed with benzene, and the 1-*O*-methyl-2-demethylphytylplastoquinol purified by TLC on, (i) paraffin-impregnated silica gel G developed with aq. 98% acetone (1-*O*-methyl-2-demethylphytylplastoquinol, R_f 0·82; phytylplastoquinol monomethyl ether, R_f 0·58; 2-demethylphytylplastoquinone, R_f 0·40; plastoquinone-9, R_f 0·05) and, (ii) Rhodamine 6G-impregnated silica gel G developed with benzene–light petroleum (b.p. 40–60°) (1:1) (R_f 0·35).

Oxidation of 1-*O*-methyl-2-demethylphytylplastoquinol with $HAuCl_4$ or $FeCl_3$ was carried out under our standard conditions.[1] The 2-demethylphytylplastoquinone (II) obtained from oxidation of the ether was converted into its chromanol, δ-tocopherol (III), by the same sequence of reactions as those used to form γ-tocopherol from phytylplastoquinone (Scheme 2).[1]

The 1-*O*-methyl-2-demethylphytylplastoquinol was converted into its corresponding chromanol, δ-tocopherol monomethyl ether (IV), by refluxing it for 1·5 hr in 1·5 ml HOAc and 0·15 ml of conc. HCl.[10]

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[7] D. R. THRELFALL and G. R. WHISTANCE, in *Aspects of Terpenoid Chemistry and Biochemistry* (edited by T. W. GOODWIN), pp. 357–400, Academic Press, New York (1971).
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[9] D. R. THRELFALL and T. W. GOODWIN, *Biochem. J.* **103,** 573 (1967).
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Key Word Index—Euglena gracilis; Euglenophyta; 1-*O*-methyl-2-demethylphytylplastoquinol.