Phytochemistry, 1970, Vol. 9, pp. 355 to 359. Pergamon Press. Printed in England.

BIOSYNTHESIS OF UBIQUINONE—A SEARCH FOR POLYPRENYL PHENOL AND QUINONE PRECURSORS

D. R. THRELFALL and G. R. WHISTANCE

Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth

(*Received* 26 *June* 1969)

Abstract—Nine higher plant tissues and an alga, *Euglena gracilis*, have been examined by radiochemical and chemical means for the presence of possible polyprenyl phenol and quinone precursors of ubiquinone. Only in the case of the alga was any such compound detected: this was 2-nonaprenyl-6-methoxy-3-methyl-1,4-benzoquinone (5-demethoxyubiquinone-9). Compound W-9, a compound formerly believed to be a polyprenylphenol (or quinol) precursor of ubiquinone-9 in maize shoots, has been shown to be a mixture of ubiquinone-8 and plastochromanol. In addition it has been shown that, contrary to previous findings, higher plants and *E. gracilis* contain a series of ubiquinones.

INTRODUCTION

NUMEROUS investigations have shown that *p*-hydroxybenzoic acid is an extremely effective precursor of the *p*-benzoquinone nucleus of ubiquinone in a wide range of organisms.[1-4] However, with the exception of photosynthetic bacteria,[5] some non-photosynthetic Gram-negative bacteria[4, 6] and yeasts,[6] little is known of the identities of the intermediates involved in the conversion of this acid to ubiquinone.

Studies with *p*-hydroxy[U-^{14}C]benzoic acid have failed to demonstrate the presence in the green algae, *Euglena gracilis*[7] and *Ochromonas danica*,[3] and the higher plants, *Zea mays* (maize) and *Phaseolus vulgaris* (french bean),[3] of 2-polyprenylphenols and 6-methoxy-2-polyprenylphenols, compounds believed to be precursors of ubiquinone in bacteria. However, in the experiment with maize a highly radioactive isoprenoid quinol or phenol, compound W-9 (where the numeral indicates the number of prenyl units in the side chain), was detected, which was shown to undergo an apparent conversion to ubiquinone-9. In addition, a radioactive compound corresponding to compound W-10 was detected in *O. danica* and french bean, two organisms which contain ubiquinone-10.

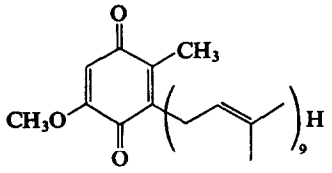
The investigations described in this paper were concerned with the detection and characterization in plants of polyprenyl phenol and quinone (or hydroquinone) precursors of ubiquinone of the type found in bacteria, and obtaining further information about the identity of compound W-9 in maize. Whilst carrying out these investigations results were obtained to show that, contrary to previous findings,[8] higher plants and *E. gracilis* contain a series of ubiquinones.

[1] H. RUDNEY and T. S. RAMAN, *Vitamins and Hormones* **24**, 531 (1966).
[2] R. E. OLSON, *Vitamins and Hormones* **24**, 551 (1966).
[3] G. R. WHISTANCE, D. R. THRELFALL and T. W. GOODWIN, *Biochem. J.* **105**, 145 (1967).
[4] G. R. WHISTANCE, J. F. DILLON and D. R. THRELFALL, *Biochem. J.* **111**, 461 (1969).
[5] G. D. DAVES, P. FRIIS, R. K. OLSEN and K. FOLKERS, *Vitamins and Hormones* **24**, 427 (1966).
[6] S. IMAMOTO and S. SENOH, *J. Chem. Soc. Japan* **89**, 316 (1968).
[7] R. POWLS and F. W. HEMMING, *Phytochem.* **5**, 1235 (1966).
[8] F. L. CRANE, in *Biochemistry of Quinones* (edited by R. A. MORTON), p. 183, Academic Press, London (1965).

RESULTS

Studies with Euglena gracilis

Light-grown 4-day-old cells (equivalent of 1·7 g dry wt.) of *E. gracilis* strain *Z* were incubated for 4 hr with 2 μc of *p*-hydroxy[U-^{14}C]benzoic acid. On column chromatography of the lipid extract radioactivity was present only in the 3 per cent- (7,660 dpm), 5 per cent- (80,700 dpm) and 12 per cent-diethyl ether in light petroleum (b. p. 40–60°) (E/P) (20,250 dpm) fractions. Examination of these fractions by routine TLC procedures showed that all the radioactivity in the 3 per cent- and 5 per cent-E/P fractions was associated with ubiquinones-9 and -8, whilst the radioactivity in the 12 per cent-E/P fraction was distributed between two compounds having the TLC properties expected of rhodoquinone-9 and 2-nonaprenyl-6-methoxy-3-methyl-1,4-benzoquinone [(5-demethoxyubiquinone-9)] [].



(I) 5-Demethoxyubiquinone-9

To obtain further proof of the presence of 5-demethoxyubiquinone-9 in *E. gracilis*, the lipids from the cells (equivalent to 1·1 g dry wt.) from a large-scale culture (50 l.) of the alga was examined, when 30 μg of a quinone was isolated which had u.v. spectral (λ_{max} 267 nm in *cyclohexane*; λ_{max} 271 nm *in ethanol changing to* λ_{max} 293 nm *after* $NaBH_4$ *treatment*) and TLC (adsorptive and reversed phase) properties identical to those of 5-demethoxyubiquinone-9 isolated from *Pseudomonas ovalis* Chester.[5,9] It is noteworthy that in this analysis (*a*) rhodoquinone-9 (145 μg/g dry wt.) and small amounts of ubiquinone-8 (2 μg/g dry wt., cf. ubiquinone-9 150 μg/g dry wt.) were also isolated, and (*b*) in agreement with the radiochemical experiment, 2-polyprenylphenols, 6-methoxy-2-polyprenylphenols, and 2-polyprenyl-6-methoxy-1,4-benzoquinones were not detected.

Studies with Maize—The Identity of Compound W-9

Six hundred 7-day-old etiolated maize shoots were incubated in the dark for 24 hr with 4 μc of *p*-hydroxy[U-^{14}C]benzoic acid. As in previous experiments,[3] the majority of the radioactivity recovered from column chromatography of the lipid extract was present in the fractions containing ubiquinone, i.e. 3 per cent- (118,000 dpm) and 5 per cent-E/P (136,500 dpm). Compound W-9 (37,130 dpm) was purified and resolved from ubiquinone-9 (1,950 μg; 211,890 dpm) by TLC. On further examination of the "purified" compound W-9 by the procedures outlined in the Experimental section, it was found to consist of two components, namely, plastochromanol (371 μg) and [^{14}C]ubiquinone-8 (40 μg; 32,280 dpm). Again as, in previous experiments,[3] no radiochemical or chemical evidence could be obtained for the presence of polyprenyl phenol or quinone precursors of ubiquinone.

Studies with Other Plant Tissues

In view of the results obtained with maize and *E. gracilis* it was decided to examine a range of higher plant species for prenylated precursors of ubiquinone and to determine the identities of the ubiquinone homologues present.

[9] G. R. WHISTANCE, B. S. BROWN and D. R. THRELFALL, *Biochim. Biophys. Acta* **176**, 895 (1969).

In no tissue examined could evidence be found for the presence of 2-polyprenylphenols, 6-methoxy-2-polyprenylphenols, 6-methoxy-2-polyprenyl-1,4-benzoquinones or 5-demethoxyubiquinones. However, all tissues were found to contain a series of ubiquinones (Table 1).

TABLE 1. UBIQUINONE CONTENT OF HIGHER PLANT TISSUES

Plant tissue	Wet wt. of tissue analysed (kg)	Ubiquinone content (μg/kg fresh wt.)					
		−7	−8	−9	−10	−11	−12
Etiolated maize shoots (*Zea mays*)	0·8	—	50	2420	Detected by radioautography		
Young lettuce (head of *Latuca sativa*)	0·4	—	13	331	—	—	—
Young chicory (*Chicorium intybus*)	1·0	—	3	260	—	—	—
Green melon (fruit of *Cucumis melo*)*	0·7	12	31	1786	121	—	—
Cucumber (fruit of *Cucumis sativus*)*	1·0	—	Trace	310	23	—	—
Leek (*Allium parrum*)	1·0	—	22	341	636	—	—
Brussels sprouts (leaves of *Brassica oleracea*)	1·0	—	—	24	2230	—	—
Green Peppers (fruit of *Capsicum annuum*)*	2·0	—	3	47	506	137†	8†

* Seeds removed before analysis.

† The characterization of these two quinones has been reported previously by Threlfall and Whistance.[11]

DISCUSSION

Of the higher plants and an alga (*Euglena gracilis*) examined for the presence of polyprenyl phenols and quinones of the type believed to be precursors of ubiquinone in bacteria, only in the case of the alga was such a compound detected. This compound was identified as 5-demethoxyubiquinone-9 (I), a quinone not previously known to occur in the plant kingdom but one which has recently been established unequivocally as a precursor of ubiquinone-9 in the bacterium *Pseudomonas ovalis* Chester.[10] Compound W-9, a compound originally believed to be a polyprenyl phenol or quinol precursor of ubiquinone-9 in maize shoots,[3] was found to consist, in fact, of a mixture of two compounds, ubiquinone-8 (the ^{14}C-labelled component in tracer experiments using *p*-hydroxy[U-^{14}C]benzoic acid) and plastochromanol (the phenolic component).

The apparent absence from the plant species examined of ubiquinone precursors of the 2-polyprenylphenol, 6-methoxy-2-polyprenylphenol, 6-methoxy-2-polyprenyl-1,4-benzoquinone type, and in the case of the higher plants the 5-demethoxyubiquinone type also, can probably be explained in one of two ways. Firstly, the compounds may be present in amounts too small to detect using our analytical methods. Secondly, they may not be involved in ubiquinone biosynthesis in plants, i.e. prenylation is the penultimate if not the ultimate step in this synthesis. In view of our ability to detect extremely small amounts of 2-polyprenylphenols and 6-methoxy-2-polyprenylphenols in biological materials,[4,9] the second of these explanations would at present appear the more likely. However, before any categorical statement to this effect can be made further experimental evidence is clearly required.

As stated above, compound W-9 was shown to consist in maize shoots of a mixture of ubiquinone-8 and plastochromanol, with ubiquinone-8 being the component labelled from

[10] G. R. WHISTANCE, B. S. BROWN and D. R. THRELFALL, in preparation.

p-hydroxy[U-^{14}C]benzoic acid in tracer experiments. The apparent precursor activity of ubiquinone-8 with respect to ubiquinone-9 observed previously in maize shoots[3] can be explained if the assumption is made that ubiquinone-8 turns over more rapidly than ubiquinone-9. Experimental support for this assumption comes from the finding that in this tissue the specific activity of ubiquinone-8 (588,000 dpm/μmole) labelled from *p*-hydroxy-[U-^{14}C]benzoic acid is some seven times greater than that of ubiquinone-9 (86,400 dpm/μmole) labelled from the same substrate.

In the course of our studies it became apparent that plants, contrary to previous findings that they contain only ubiquinone-9 or ubiquinone-10,[7] also contain lesser amounts of lower and in some instances higher ubiquinone homologues (Table 1). Perhaps the most striking result was the isolation of ubiquinone-11 and ubiquinone-12 from green peppers[11] and their detection in the ubiquinone fraction from maize shoots, for this was the first time that ubiquinones possessing side-chains greater than ten isoprene units in length had been found in nature. More recently, however, we have also detected these quinones in the photosynthetic bacteria, *Rhodospirillum rubrum*, *Rhodopseudomonas spheroides* and *Chromatium* strain D (G. R. Whistance and D. R. Threlfall, unpublished observations), and in the rat,[12] which suggests that they may, in fact, be of widespread distribution. The biosynthetic implications of the occurrence in any one organism of a series of ubiquinones have been discussed elsewhere.[4]

EXPERIMENTAL

Biological Material

Etiolated-maize-seedlings (*Zea mays* var. Rhodesian White Horse Tooth) and autotrophic cells of the alga, *Euglena gracilis* strain Z, were grown as described previously.[3,13] All other plant tissues were purchased locally.

Incubation of E. gracilis *and Maize Shoots with* p-*Hydroxy*[U-^{14}C]*benzoic Acid*

Etiolated 7-day-old maize shoots and cells (equivalent to 1·7 g dry wt.) of *E. gracilis* strain Z were incubated with *p*-hydroxy[U-^{14}C]benzoic acid (7·78 mc/mmole), prepared by alkaline fusion of L-[U-^{14}C]tyrosine, under conditions similar to those described previously.[3,14] In the experiment with maize shoots one modification made was that the tissues were exposed to the ^{14}C-substrate in the absence of light; this procedure prevents the synthesis of pigments which interfere with the analytical steps.

Isolation and Examination of Lipid Extracts

The lipids were extracted from the algal cells and higher plant tissues by routine procedures,[3,13] and then chromatographed on columns of Brockmann grade III acid-washed alumina (Woelm, anionotropic) developed by stepwise elution with 0·25%-, 1%-, 3%-, 5%-, 8%-, 12%- and 20%- ether–petroleum (40–60°) (E/P).[13]

The 1%-, 3%- and 8%-E/P fractions were examined for the presence of 6-methoxy-2-polyprenylphenols, 2-polyprenylphenols and 6-methoxy-2-polyprenyl-1,4-benzoquinones plus 5-demethoxyubiquinones, respectively, by u.v. spectroscopic and TLC methods similar to those used for the isolation of these compounds from the Gram-negative bacteria, *Pseudomonas ovalis* Chester and *Proteus mirabilis*.[4,9] The limit of detection by these methods is 0·5 μg of polyprenylphenol and 3 μg of polyprenylquinone.[4]

Ubiquinones, present in the 3%- and 5%-E/P fractions, were purified by adsorptive TLC[3] and the total amounts present estimated by u.v. spectroscopy (see below). They were then separated into their various homologues by reversed-phase TLC[15] and, after removal of the paraffin, re-estimated. Finally, the identities of the ubiquinone-homologues were confirmed by silver ion TLC.[4]

In the experiment with maize, compound W-9 and ubiquinone-9, present in the 5%-E/P fraction, were purified and separated from each other by adsorptive and reversed-phase TLC.[3] On further TLC (using low

[11] D. R. THRELFALL and G. R. WHISTANCE, *Biochem. J.* **113**, 38 (1969).
[12] F. E. FIELD, B.Sc. Thesis, University College of Wales, Aberystwyth (1969).
[13] D. R. THRELFALL and T. W. GOODWIN, *Biochem. J.* **103**, 573 (1967).
[14] G. R. WHISTANCE and D. R. THRELFALL, *Biochem. J.* **109**, 577 (1968).
[15] G. H. SPILLER, D. R. THRELFALL and G. R. WHISTANCE, *Arch. Biochem. Biophys.* **125**, 786 (1968).

plate loadings) on Kieselgel G (impregnated with Rhodamine 6G) developed with benzene, the "purified" compound W was separated into two u.v.-adsorbing bands (R_f 0·26 and 0·32). The compound with R_f 0·26 was identified as ubiquinone-8 by u.v. analysis and reversed phase and silver ion TLC. The compound with R_f 0·32 was found to have u.v. ($\lambda\lambda_{max}$ 294 and 300·5 nm in cyclohexane) and TLC properties identical to those of authentic plastochromanol.[16]

Spectroscopic Determinations

Ubiquinones were estimated as described previously.[14] Rhodoquinone-9, plastochromanol and 5-demethoxyubiquinone-9 were estimated in cyclohexane by taking $E^{1\%}_{1cm}$ values of 140 (Ref. 7), 56 (Ref. 16) and 145 (Ref. 6) at λ_{max} 280 nm, λ_{max} 294 nm and λ_{max} 266·5 nm respectively.

Radioassay

The procedures used have been described elsewhere.[3] All counts were corrected for background and instrument efficiency.

Acknowledgements This work was supported by the Science Research Council. We wish to thank Dr. J. F. Pennock (University of Liverpool) for supplying a sample of plastochromanol, and Miss Marian E. Williams for technical assistance.

[16] K. J. Whittle, P. J. Dunphy and J. F. Pennock, *Biochem. J.* **96**, 17c (1965).