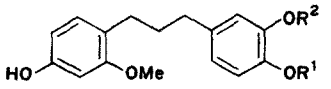
due to *o*-quinonemethide ions characterize 1,3-diarylpropanes with *o*-hydroxyl [1,3]. In the present case such a peak would appear at *m/e* 136. Its absence locates the methoxyl at the *o*-position. Since thus the OH in the ring under scrutiny must occupy the *p*-position, a positive Gibbs test of the compound locates the OH of the other ring at the *m*-position, as in 1a. This is the isomer of the Gibbs test negative metabolite 1b of *Iryanthera coriacea* Ducke for which very similar ^{1}HMR and MS were reported [3].



(1a) R^1 = Me, R^2 = H
(1b) R^1 = H, R^2 = Me

1-(4′-Hydroxy-2′-methoxyphenyl)-3-(3″-hydroxy-4″-methoxyphenyl)-propane (1a), oil. M found 288·1358, $C_{17}H_{20}O_4$ requires 288·1362, ν_{max}^{film} (cm^{-1}): 3400, 1620, 1600, 1525, 1470, 1240, 1200, 1160, 1035. λ_{max}^{EtOH} (nm): 225 inf., 280 (ε 19500, 7800). $\lambda_{max}^{EtOH+NaOH}$ (nm): 242, 294 (ε 19050, 10950). ^{1}HMR (100 MHz, $CDCl_3$, τ): 3·00 (*d*, *J* 8·0 Hz, H-6′), 3·15–3·35 (*m*, H-2″, 5″, 6″), 3·58 (*dd*, *J* 8·0, 2·0 Hz, H-5′), 3·62 (*d*, *J* 2·0 Hz, H-3′), 6·10 (*s*, OMe), 6·21 (*s*, OMe), 7·25–7·50 (*m*, $2ArCH_2$), 7·9–8·3 (*m*, $CH_2C\underline{H}_2CH_2$). MS (*m/e*): 288 (64%) M, 151 (43), 138 (81), 137 (100), 107 (16). *Diacetate*, oil. ν_{max}^{film} (cm^{-1}): 1760, 1618, 1580, 1500, 1465, 1260, 1200, 1035. ^{1}HMR (100 MHz, $CHCl_3$, τ): 2·88 (*d*, *J* 8·0 Hz, H-6′), 3·05 (*dd*, *J* 8·0, 2·0, H-5′ or 6″), 3·24 (*dd*, *J* 8·0, 2·0 Hz, H-6″ or 5′), 3·12 (*d*, *J* 2·0 Hz, H-2″), 3·15 (*d*, *J* 8·0 Hz, H-5″), 3·40 (*d*, *J* 2·0 Hz, H-3′), 6·19 (*s*, OMe), 6·21 (*s*, OMe), 7·25–7·60 (*m*, $2ArCH_2$), 7·9–8·3 (*m*, $CH_2C\underline{H}_2CH_2$), 7·80 (*s*, 2COMe).

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FORMATION OF UBIQUINONE BY TOBACCO PLANT CELLS IN SUSPENSION CULTURE*

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INTRODUCTION

Since their discovery by Lester [1] and Morton [2], ubiquinones have received much attention from many workers in various fields of research. In the case of cultured plant cells, Threlfall and Goodwin [3] reported the occurrence of ubiquinone-10 in Paul's scarlet rose while Thomas and Stobart examined the time-course of ubiquinone and α-tocopherol formation in *Kalanchoë crenata* callus [4].

According to our investigations [5], tobacco cells in suspension culture appear to contain much more ubiquinone-10 than the parent plants and so might be a suitable source for the large scale production of this compound. In order to obtain basic information on the synthesis of ubiquinone-10 by cultured plant cells, some observations on the variation in the ubiquinone content during the growth of the cultured cells have been made and are detailed in this paper.

RESULTS AND DISCUSSION

The time-course of ubiquinone formation during cell growth was examined using three kinds of cultured cells which have a high growth rate in suspension culture (Fig 1.) The clone BY-2 reached a maximum dry weight 6 days after inoculation, while their ubiquinone content decreased during the early period of the logarithmic phase of growth and then increased reaching the highest level (360 μg per g-dry weight) on the 10th day (Fig. 1). Changes in the ubiquinone content of Xanthi cells were

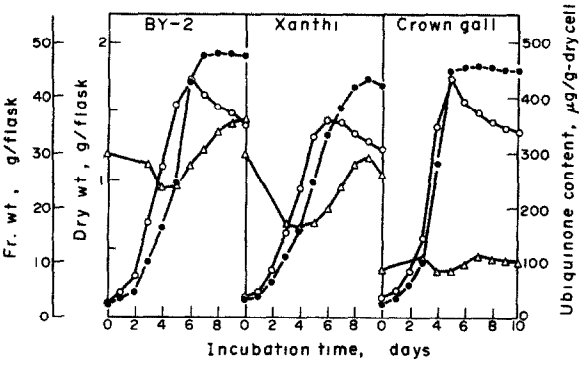

Fig. 1. Cell growth and ubiquinone content in cultured tobacco cells. For the culture media and conditions, see text. Cell growth: ●—● fr. weight; ○—○ dry weight; ubiquinone content: △—△.

*Studies on the Culture Conditions of Higher Plant Cells in Suspension Culture. Part 7.

Table 1. Ubiquinone contents of cultured higher plant cells

Cultured cells	Incubation time (days)	Ubiquinone content μg/g dry cell
Daucus carota	8	160
Lycopersicum esculentum	8	60
Lactuca scariola	14	133
Datura tatula	14	213

Cultured cells were harvested at 8 or 14 days after inoculation in the stationary phase. As for the culture media and cultural condition, see text.

similar to those of BY-2 cells, but those of crown gall cells were less clear cut. The pattern of the time-course of the ubiquinone formation in our experiments is similar to those described on *Candida* [6] and *Kalanchoë crenata* callus cells [4]. This seems to be a common phenomenon for ubiquinone formation among cultured cells, callus and microorganisms under aerobic conditions. The maximal yield with BY-2, Xanthi and crown gall cells were 522, 375 and 168 μg per flask respectively. The ubiquinone productivity of the cultured BY-2 cells is thus similar to that of microorganisms [7, 8]. Since the maximum yield of ubiquinone per flask was obtained at the stationary phase, a batch-culture rather than a continuous culture may be suitable for production.

The ubiquinone content of other cultured plant cells harvested at the stationary phase were also investigated (Table 1). Of all the cells investigated, BY-2 showed the highest ubiquinone productivity and *Lycopersicum esculentum* showed the lowest. It is obvious that there are remarkable differences in the ubiquinone content among the species of cultured cells.

According to Griffiths [9], the ubiquinone contents of tobacco leaves are 0.016–0.026 μmol per 10 g fr. wt, which is less than one-tenth of that in our BY-2 cells. It is apparent that cultured cells, especially BY-2 cells accumulate ubiquinone-10 much more than whole leaves. Further investigations are required to elucidate the reason why this is so.

EXPERIMENTAL

Cultured cells. Cultured tobacco cells of BY-2 and Xanthi were produced from seedlings in 1968 [10]. Crown gall cells were obtained from the tumor gall formed on a plant of the *N. tabacum* cultivar, Hicks-2 by *Agrobacterium tumefaciens* in 1970 [11]. The cultured cells of *Daucus carota*, *Lycopersicum esculentum* and *Lactuca scariola* were produced from seedlings and *Datula tatula* cells were obtained from leaves of a parent plant in 1973. Stock suspension cultures were routinely subcultured on Linsmaier-Skoog medium [12] every week.

Culture medium. Linsmaier–Skoog inorganic medium containing (per l.) 30 g sucrose, 1.0 mg of thiamine HCl-ide and 100 mg of myo-inositol was used as the basal medium. This was supplemented either with (per l.) 0.2 mg of 2,4-D for BY-2 cells, with 0.5 mg of 2,4-D and 0.2 mg of kinetin for Xanthi cells, or with twice the amount of KH_2PO_4 and 75 μg nicotinic acid for crown gall cells [13]. The initial pH of these media were adjusted to 6.0 with 0.5 N NaOH.

Culture conditions and harvesting of cultured tobacco cells. Incubation was carried out in 500 ml Erlenmeyer flasks containing 100 ml of medium for 10 days at 28° in the dark on a reciprocal shaker (100 strokes/min; 2.0 cm in length). Seed cultures were incubated for 7 days, and 10 ml of the culture was inoculated into each flask. Five flasks were harvested every 24 hr after inoculation for analysis. Cultured cells were separated from culture media by filtration through filter paper and weighed for fr. wt determination and then lyophilized.

Extraction of ubiquinone-10. To 1 g of the lyophilized cells were added 20 ml of 10% pyrogallol in EtOH and 15 ml of 20% NaOH. The mixture was refluxed for 30 min and then cooled immediately, and diluted with 65 ml H_2O and extracted with 3 × 100 ml of *n*-hexane. The extract was dried (Na_2SO_4) and conc *in vacuo* at 30 to 40° to dryness [6, 7, 14].

Determination of ubiquinone-10. The determination of ubiquinone-10 was carried out using the Craven assay essentially according to Folkers *et al.* [15]. The unsaponifiable substance extracted by *n*-hexane was dissolved in 10 ml EtOH and 4.0 ml was treated with 1.0 ml of ethyl cyanoacetate followed by 1.0 ml of 0.2 N KOH: the blue color was measured at 625 nm after 12 min. The results by this method were in good agreement with those obtained by spectrophotometric borohydride-reduction method [16].

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