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UBIQUINONE CONTENT OF EIGHT PLANT SPECIES IN CELL CULTURE

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Key Word Index—Gramineae; Leguminosae; Rutaceae; Solanaceae; Zingiberaceae; cultured cells; identification; determination; ubiquinone homologues.

Abstract—Crystals of ubiquinone-10 were isolated from soyabean, peanut and *Ruta* cell cultures, while crystals of ubiquinone-9 were obtained from rice and wheat cell cultures. These crystals also contained lesser amounts of lower and higher homologues (ubiquinone-7 to 10). The ubiquinone content of eight higher plants in cell culture was determined. Ubiquinone-9 content of rice was 680 μg per g dry wt, and this was 3–6 times higher than that of the other plants.

INTRODUCTION

Ubiquinones have been detected in all groups of living organisms and they are localized in the organelles which carry out respiratory chain phosphorylation [1]. In recent years, ubiquinone-10 has been used for chemotherapy of congestive heart failure. In 1963, Threlfall and Goodwin reported that the yellow oil extracted from tissue cultures of Paul's scarlet rose was ubiquinone-10 [2]. Recently, Ikeda *et al.* isolated 53 mg of yellow crystals from 455 g dried tobacco cultured cells and characterized them as ubiquinone-10 [3]. They also reported that tobacco cells in suspension culture appeared to contain more ubiquinone-10 than the parent plant and so might be a suitable source for the large scale production of this compound [4]. Moreover, they examined the effects of nutritional and physical factors on the concentration of ubiquinone in tobacco culture cells using the Craven assay [5, 6].

We now report the isolation and identification of ubiquinone crystals from five plants in cell culture and the determination of ubiquinone homologues in eight plants in culture.

RESULTS AND DISCUSSION

Isolation and identification of ubiquinone from plant cultured cells

Yellow crystals (2.4 mg) were obtained from the $CHCl_3$–MeOH extract of 380 g fresh rice cultured cells (36 g dry wt), and they were identified as ubiquinone-9 by comparison with an authentic sample. Ubiquinone-9 (2.5 mg) was also obtained from 470 g fresh wheat cultured cells (43 g dry wt). Though ubiquinone-9 is known to occur in intact plant tissues of maize, wheat and barley [7–9], this is the first report of it in cell cultures of plants of the Gramineae.

Soyabean and alfalfa plants and cell cultures of Paul's scarlet rose and tobacco contain ubiquinone-10 [2, 3, 9, 10]. We have now isolated ubiquinone-10 from 2650 g fresh soyabean cells (106 g dry wt), 1205 g fresh peanut cells (72 g dry wt) and 1520 g fresh *Ruta* cells (81 g dry wt); the yields were 3.4, 2.7 and 2.5 mg, respectively.

Threlfall and Whistance [11] have shown that ubiquinone fractions in the lipids of several higher plants contain not only a major ubiquinone, but also lesser amounts of lower and higher homologues. The five crystalline samples obtained from cell cultures in this experiment also contained lesser amounts of lower and higher homologues (Table 1).

Table 1. Relative amounts of ubiquinone homologues of the crystals from cell cultures

Plant	UQ* homologue (%) UQ-7	UQ-8	UQ-9	UQ-10
Rice	0.35	0.26	98.44	0.96
Wheat	0.39	0.08	98.76	0.78
Soyabean	0.00	0.67	1.75	97.58
Peanut	0.00	0.32	0.63	99.05
Ruta	0.00	0.46	5.43	94.10

* UQ = ubiquinone. Ubiquinone crystals were dissolved in dioxane and analysed by HPLC.

Ubiquinone content of plant tissue cultures

Ikeda *et al.* determined ubiquinone content of cultured tobacco cells as total ubiquinone using the Craven assay [5, 6]. Recently, Abe *et al.* [12] reported that ubiquinone homologues in serum and liver could be determined separately by HPLC. In this experiment, the ubiquinone content of cell cultures was determined by a modification of the method described by Abe *et al.* As shown in Table 2, the ubiquinone content of the cells which produced mainly ubiquinone-10 was 100–230 μg per g dry wt; the content of rice cells which produced mainly ubiquinone-9 was 680 μg, and it was 3–6 times higher than that of the former. The ubiquinone contents of these plant cultures are similar to those reported earlier [4].

EXPERIMENTAL

Cell suspension culture. The cultures used were soyabean (*Glycine max*, Shinmejiro), rice (*Oryza sativa*, Norin 16), *Ruta* (*Ruta graveolens*), tobacco (*Nicotiana tabacum*, Bright Yellow), peanut (*Arachis hypogea*, Chibasundachi), wheat (*Triticum monococcum*), alfalfa (*Medicago sativa*) and *Curcuma* (*Curcuma zedoaria*) cells, all of which had been maintained as callus cultures on agar media. Stock cultures were initiated from the callus and maintained in 200 ml flasks containing 50 ml R-2-V liquid

Table 2. Ubiquinone content of plant cell cultures

Cell	Main UQ*	Culture period (days)	Main UQ content µg/g Dry wt	µg/g Fr. wt	µg/100 ml of medium
Soyabean	UQ-10	8	126	6.7	131
		8	128	5.2	116
Peanut	UQ-10	8	112	5.9	99
		8	100	5.4	89
Ruta	UQ-10	20	104	5.3	72
		18	106	5.2	76
Tobacco	UQ-10	8	144	5.7	130
		8	154	4.9	144
Curcuma	UQ-10	10	221	22.2	128
		10	228	22.9	132
Alfalfa	UQ-10	8	152	10.5	124
		8	114	5.0	69
Rice	UQ-9	8	679	60.5	344
		8	687	45.1	339
Wheat	UQ-9	8	103	10.9	67
		11	157	11.6	103

* UQ = ubiquinone.

medium [13, 14]. The stock cultures were inoculated into 1 l. of the medium in 2 l. Sakaguchi flasks. The flasks were set on a gyratory shaker agitated at 120 rpm at 30° in the dark.

Extraction and identification of ubiquinones. The fresh rice cells (380 g fr. wt, 36 g dry wt) cultured in 9 l. R-2-V liquid medium for 9 days were harvested and homogenized ×3 in $CHCl_3$–MeOH (2:1) and centrifuged at 3000 rpm for 3 min. The aq. layer was extracted with $CHCl_3$. The $CHCl_3$ extracts were combined and concd to dryness after washing with 0.9% NaCl soln. Residue (3.3 g) was dissolved in a small amount of C_6H_6, chromatographed over 120 g Si gel and eluted with C_6H_6–EtOAc (30:1). The ubiquinone fraction (159 mg) was rechromatographed over 70 g Si gel and eluted with *n*-hexane–EtOAc (10:1) and 6.2 mg of a yellow oil was obtained. Recrystallization from EtOH gave 2.4 mg of yellow crystals of ubiquinone-9. Ubiquinone crystals were also extracted from the fresh cells of wheat, soyabean, peanut and *Ruta* cultured in R-2-V liquid medium. The identities of ubiquinones were demonstrated by mp, UV, reversed-phase TLC, IR and HPLC behaviour with authentic standards.

Ubiquinone-9. Ubiquinone-9 crystals were isolated from the cells of rice and wheat. Mp 41.5–42.5°, mmp undepressed. UV λ_{max}^{EtOH} 275 nm, shifted to 290 nm after reduction with $NaBH_4$. R_f of reversed-phase TLC (paraffin-coated Si gel 60 F-254 (Merck), solvent; Me_2CO–H_2O (95:5)), R_t of HPLC and RI (micro-KBr) were identical to authentic ubiquinone-9.

Ubiquinone-10. Ubiquinone-10 crystals were isolated from the cells of soyabean, peanut and *Ruta*. Mp 47.5–48.5°, mmp undepressed. UV, IR, R_f of reversed-phase TLC and R_t of HPLC were identical to authentic ubiquinone-10.

HPLC of ubiquinone crystals from cultured cells. HPLC used in this expt was Shimadzu LC-1 equipped with a variable-wavelength UV detector (Shimadzu SPD-1) and a 50 cm × 2.1 mm i.d. reversed-phase column Permaphase ODS. The column was eluted with dioxane–H_2O (70:30) at a pressure of 12.5 kg/cm² and 25°, and the column effluent was monitored at 275 nm. The ubiquinones from cultured cells were injected as 2–4 µl of a 0.5% soln in dioxane. The content of ubiquinone homologues was calculated from the area of the peaks.

Quantitative determination of ubiquinone. Ubiquinone content of plant cultured cells was determined by a method of Abe *et al.* [12] with some modification. Fresh cells (0.3–1.0 g in dry wt) were extracted with $CHCl_3$–MeOH (2:1) ×3. The aq. layer was extracted with a small amount of $CHCl_3$ and the $CHCl_3$ solns were combined and washed with 0.9% NaCl soln. It was also washed with $CHCl_3$ and the $CHCl_3$ layer was added to the latter $CHCl_3$ soln. The combined $CHCl_3$ was concd *in vacuo* to dryness. The residue was dissolved in 1 ml C_6H_6, chromatographed over 10 g Si gel and eluted with C_6H_6–EtOAc (30:1). The first 28 ml eluate was excluded and the next 25 ml was concd to dryness, dissolved in 200 µl dioxane and 50 µl 0.25% 2,3,6-trimethyl-5-decaprenyl-1,4-benzoquinone soln was added as internal standard (sample soln). 5 µl sample soln was used for the determination of ubiquinone content by HPLC.

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