Isolation and Identification of Ubiquinone 10 from Cultured Cells of Tobacco

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Ubiquinones which act as the redox carriers in the electron transport system in mitochondria have been recognized in animals, plants, and microorganisms. As for cultured plant cells, the ubiquinones have not been isolated yet, although Threfall et al. assumed the occurrence of ubiquinone 10 in the cultured cells of Paul’s scarlet rose by thin-layer chromatography, paper chromatography and UV spectroscopy. In the course of our studies on the chemical constituents of cultured tobacco cells, the occurrence of an orange pigment in the lipid fraction was fairly noticeable. The isolation and characterization of the pigment revealed it to be ubiquinone 10, and this paper deals with the results.

The cell culture of Nicotiana tabacum L. var. BY-2 was carried out for 5 days according to the cultural condition described in a previous paper. The cultured cells were separated from the culture broth by filtration through filter paper and were immediately lyophilized. The dried cells (455 g) were homogenized with a 20-fold amount of 2:1 chloroform-methanol mixture. The homogenate was filtrated through filter paper and the residue was further extracted twice by the same procedure. The combined extract was washed with one-fifth volume of 0.9% aqueous sodium chloride and was dried up in vacuo at 40 to 50°C, yielding 15.4 g of crude lipid. The chloroform solution of the crude lipid (1.5 g) was added onto the top of a silicic acid column (2.5 × 20 cm) which was previously equilibrated with chloroform. The elution was carried out with 200 ml of chloroform and the eluate was collected in 15 ml fractions. The yellow-colored fraction which were eluted early were combined and the combined solution was concentrated in vacuo to dryness, giving an orange oil. The oil (751 mg from 455 g of dry cells) was further purified by thin-layer chromatography on silica-gel plate with benzene as developing solvent. The final purification was carried out by recrystallization from a mixture of chloroform-methanol. Two recrystallizations gave 53 mg of yellow needles: mp 48~49°C; Found: C, 82.08; H, 10.68. Calcd. for C₉₀H₁₆O₄: C, 82.08; H, 10.51%. The NMR spectrum (Table I) suggested the presence of a long isoprenoid side-chain and methoxy groups but the absence of aromatic ring protons.

In the mass spectrum, the dominant feature was the appearance of an intense peak at m/e 235 and 197 which were due to the characteristic decomposition of ubiquinone molecules. The UV spectrum of the pigment exhibited a single absorption peak (λ_max, ethanol, 275 nm; E_1%1cm 151) characteristic to ubiquinones and reduction with sodium boron hydride caused the absorption maximum to shift to 290 nm (ethanol, E_1%1cm 84) which is also characteristic to ubiquinones.

These results strongly suggest that the pigment is ubiquinone 10. The IR, UV, NMR and mass spectra of the isolated pigment were all perfectly identical to those of authentic ubiquinone 10. The paper chromatographic behaviors of the isolated compound using several solvent systems for the separation of ubiquinone homologs were also perfectly identical to those of the authentic one. The
mixed melting point of the isolated with authentic ubiquinone 10 (47–48°C) was 47–49°C showing no depression. These results clearly demonstrated that the isolated quinone is ubiquinone 10.

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REFERENCES