

ISOLATION OF UBIQUINONE Q₉ FROM TRICHOPHYTON MENTAGROPHYTES (ROBIN) BLANCHARD

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Abstract. A yellow pigment was isolated from *Trichophyton mentagrophytes* (Robin) Blanchard by using thin-layer chromatography. The absorption spectrum and the IR spectrum of the pigment correspond to the spectra of ubiquinone. By means of reverse phase chromatography, the ubiquinone is shown to be ubiquinone Q₆.

Ubiquinone or coenzyme Q was originally isolated from mammalian tissue by Crane and co-workers in 1957 (2) and by Morton and co-workers in 1958 (14). It was described as a yellow, crystalline product, extracted from non-saponifiable lipid with a melting point of 48°-49° and with oxidation-reduction properties. Spectrophotometric examination shows a characteristic band at 275 nm, which disappears on reduction with borohydride. After reduction a new band appears at 290 nm. The constitution formula is a benzoquinone with variable numbers of isoprenoid chains in the 6-position.

Apart from many kinds of mammalian tissues, ubiquinones with a variable number of isoprenoid units in their side chain have been isolated from plants and microorganisms including different species of fungi. Q₆ has been isolated from *Saccharomyces cerevisiae*, and Q₈ and Q₇ from *Torulopsis* species (12). Livate & Bentley (9) studied various moulds and isolated Q₉ from *Aspergillus niger*, *Mucor abundans*, *Penicillium notatum*, *Penicillium brevi-compactum*, *Penicillium charlesii* and *Penicillium chrysogenum*. Q₁₀ was isolated from *Aspergillus fumigatus* and *Cladosporium fulvum*, while dihydro Q₁₀ and tetrahydro Q₁₀ were isolated from various *Penicillium* species (10, 11).

The function of coenzyme Q, which is located mostly in mitochondria, is still a subject for discussion. It seems clear that ubiquinone is in-

volved in the electron transfer process in the succinate oxidase and NADH oxidase system. Its role as a vitamin is under debate (15, 3). Histochemical assays have shown ubiquinone in the mitochondria of the dermatophytes of the genera *Trichophyton*, *Microsporum*, *Epidermophyton* and *Keratinomyces* (13). Experiments concerning the pigments of the dermatophytes suggest some of them to be quinonoid in nature and to have oxidation-reduction properties (7, 8).

The present study on the pigments of *Trichophyton mentagrophytes* has revealed a bright yellow lipid pigment, which is identified as coenzyme Q₉. No other traces of ubiquinones with other numbers of isoprenoid chains have been isolated from this species.

MATERIAL AND METHODS

The material consisted of strains of *Trichophyton mentagrophytes* isolated from patients suffering from foot mycosis and a strain of *Trichophyton mentagrophytes* (variety nodular) (4). The latter was an atypical strain isolated from a patient suffering from nail mycosis. Spore solutions were prepared by pouring distilled water over 1-week-old cultures kept on Sabourauds solid medium. The spore solution was inoculated on Sabourauds liquid medium, containing 4% dextrose and 1% peptone and with a pH of 6.5. The cultures were grown at room temperature in 500 ml Erlenmeyer flasks containing 150 ml substrate. After an incubation period of 2-4 weeks the cultures were killed by heating to 120° for 1 hour. A control for contamination by other fungi and bacteria was made before heating, by inoculation on Sabourauds solid medium. The cultures were washed several times to remove the substrate and blotted dry with filter paper. The mycelium was homogenised in an Ultra-Turrax homogeniser at 20 000 rpm in 60 ml methanol. Saponification was done with 200 ml per 20 g mycelium of a so-

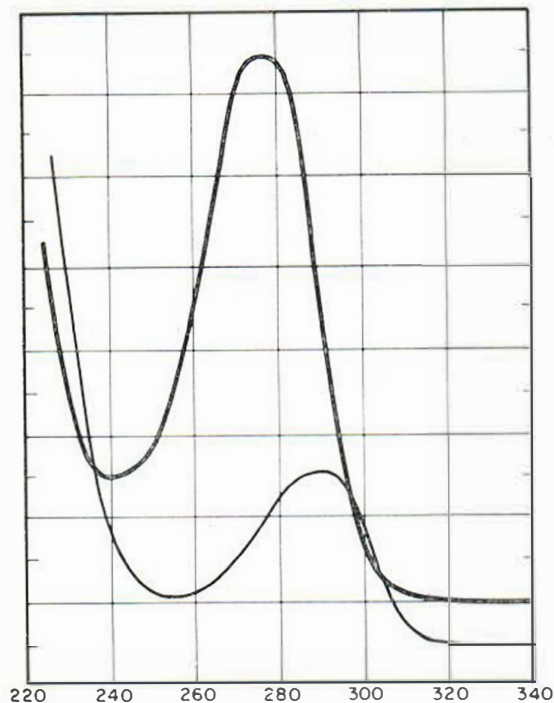


Fig. 1. Absorption spectrum of the ubiquinone in ethanol. The thick line represents the oxidized form, the thin line the reduced form.

lution of methanol with 5% pyrogallol and 15% KOH. The mixture was heated on a boiling water bath for 30 min and cooled rapidly (1). The non-saponifiable lipid was extracted three times by 200 ml petroleum benzene, boiling range 40°–60°. The petroleum benzene was washed ten times with 1 liter distilled water and dried over Na_2SO_4 for 1 hour. After evaporation under a N_2 stream the lipid was dissolved in a small amount of iso-octane and stored in a refrigerator overnight in order to precipitate the ergosterol. After washing several times with iso-octane the ergosterol was discharged and the iso-octane evaporated under N_2 stream. During the evaporation more ergosterol was precipitated, and a bright yellow lipid was left. The lipid was redissolved in 1–2 ml iso-octane and applied to the starting line of a 20×20 cm thin-layer chromatogram of silica gel G together with known amounts of standard ubiquinones, dissolved in iso-octane. The solvent system was iso-octane–benzene–ethylacetate 25:20:5, and the developing length was 16 cm in about 45 minutes (1). The distinct yellow zones were removed and eluted twice with 3 ml of ethanol. After evaporation of the ethanol in N_2 stream the yellow pigment was dissolved in ethanol and assayed spectrophotometrically in a Beckman DB spectrophotometer. When sterols contaminated the spectra, the lipid was re-chromatographed in the solvent system iso-octane–benzene–acetone 25:25:1.5 (1). A few grains of potassium borohydride were added to the ethanolic solution to reduce the ubiquinone (5). For determination

of the number of isoprenoid side chains of the ubiquinone, reverse phase TLC was used (16). The plates were prepared with 5% liquid paraffin (Merck) in diethylether using the ascending technique. After application of the samples and the standards the plates were run in the solvent system acetone–water 95:5 (16). The standards were Q_{10} , Q_8 , Q_7 and Q_6 . Q_8 were not available. In order to detect non-visible amounts of ubiquinone with different numbers of isoprenoid chains the plates were sprayed with a solution of rhodamine B 0.5% in ethanol (6). The ubiquinone was then visible in ultraviolet light as dark violet spots. The ubiquinone concentration was calculated from the value of the extinction $E_{1\text{cm}}^{1\%}$ at 275 nm in ethanol = 185, or if the eluate was impure, from the value of $\Delta E_{1\text{cm}}^{1\%}$ ox.-red. at 275 nm = 158 (5).

An IR spectrophotometer (Perkin-Elmer) was used for determining the infrared spectrophotogram by the tablet method.

A non-incubated Sabourauds medium was extracted in the same way as the fungus mat and the extract chromatographed in the solvent described.

The chemicals were all purified. The standards were obtained from Hoffmann-La Roche, Basel.

RESULTS

After an incubation period of 14–16 days the ordinary strains of *Trichophyton mentagrophytes* had a powdery whitish surface and a light yellow back side with brown spots. No pigment was seen in the medium. The strain of *Trichophyton mentagrophytes* (variety nodular) had a velvety greyish surface and a bright orange-yellow back side. The medium had changed its ordinary light colour to a darker, orange colour. The growth of this strain was slow, and the fungus was harvested as 15–20-day-old cultures. The pH of the medium ranged from 8.3–8.8 at the time of harvest after sterilisation. Contamination by bacteria or moulds was not observed. The largest amount of wet mycelium in one batch was 31 g, and amounts smaller than 14 g yielded too small amounts of lipid for estimation of the ubiquinone.

The thin-layer chromatography of the extracted lipid in the solvent system iso-octane–benzene–ethylacetate yielded several visible spots. Two spots were bright yellow with a R_f value of 0.51 and 0.33. The spectrophotometric curve of the eluate of the spot with the R_f value of 0.51 showed a maximum at 275 nm in ethanol and, after reduction with potassium borohydride, a maximum at 290 nm, suggesting ubiquinone. In order to purify the ubiquinone, a separation was done in the solvent system iso-octane–benzene–acetone. After elution the ubiquinone was run in

Table I. Amounts of ubiquinone Q₉ in four different strains of *Trichophyton mentagrophytes*

Strain	Age of culture (days)	Wet weight (g)	pH of medium	Ubiquinone Q ₉ (μg per g wet weight)
K. H. ^a	26	31.29		9.5
9675	20	14.4	8.52	8.8
9675	15	24.2	8.60	12.8
9621	19	23.6	8.81	7.8
9621	18	22.6	8.77	10.7
9670 B	16	17.4	8.34	10.0
9670 B	15	17.3	8.30	9.0

^a *Trichophyton mentagrophytes* (variety nodular).

reverse phase chromatography together with the ubiquinone standards. The isolated ubiquinone had the same R_f value as the ubiquinone Q₉ = 0.7. Fig. 1 shows the UV spectrum of the oxidized and the reduced ubiquinone. The ubiquinone was readily crystallized in cold ethanol. The IR spectrum of the isolated Q₉ corresponded to the IR spectrum of the standard ubiquinone.

No other ubiquinones were detectable after spraying with rhodamine B. The estimated values of the ubiquinone are given in Table I. The recovery of the ubiquinone after TLC was low, ranging from 63 to 80% in spite of an instant elution and spectrography after the chromatography.

The yellow pigment with an R_f value of 0.33 showed a distinct band in visible light at 348 nm, and was extracted in the largest amount from the strain of *Trichophyton mentagrophytes* (variety nodular). The pigment was unstable and lost its colour and its characteristic band after few days' storage in darkness at 4°. In the same solvent the ergosterol had an R_f value of 0.88, and was very often a contaminant of the ubiquinone.

DISCUSSION

The ubiquinones are labile to strong alkali. This is the reason why the saponification procedure must have destroyed some of the ubiquinone. On the other hand, the large amount of lipid extracted from a non-saponified material made it difficult to obtain a clear separation in TLC. The addition of pyrogallol to the alkali solution should,

to some extent, prevent the destruction of the ubiquinone (2). Destruction of the ubiquinone during chromatography and the possibility that not all the ubiquinone was extracted from the material, results in non-total values of the ubiquinone found in the material.

The rhodamine B spraying of the TLC plates is sensitive to ubiquinones with a limit of 0.5 μg (16). Smaller amounts of ubiquinone with of isoprenoid chain numbers other than Q₉ may be present in *Trichophyton mentagrophytes* although not detectable in the rather small batches of mycelium used in this study.

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