Isolation and Structure Elucidation of a Tetrahydrogenated Isoprenoid Side-Chain Ubiquinone with Ten Isoprene Units Isolated from *Chaetomium funicola* JS 525

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A new ubiquinone homologue was isolated from *Chaetomium funicola* JS 525. Its chemical structure was found to be 2,3-dimethoxy-5-methyl-6-IX,X-tetrahydrofarnesylfarnesylgeranyl-geranyl-1,4-benzoquinone based on the results of mass and $^1$H-NMR spectra containing a spin decoupling experiment. This fungus also contains dihydrogenated ubiquinone with ten isoprene units as a minor component, and this ubiquinone was presumed to be composed of two different homologues saturated at the 9th and 10th (terminal) units from a benzoquinone ring.

In yeasts and yeast-like fungi, various ubiquinone (Q) homologues, which differ structurally in the number of isoprene units in the side chain, namely, Q-5, Q-6, Q-7, Q-8, Q-9, Q-10, and dihydrogenated Q-10 (Q-10 (H$_2$)) in a few species, have been reported as major components of the ubiquinone system.1 ~ 7) Dihydrogenated ubiquinone Q-10 is widely distributed in various taxa of the Ascomycotina.8)

During the chemotaxonomic study of filamentous fungi, a new homologue was found in the species of *Chaetomium* and of other several genera.8) This ubiquinone had a longer elution time than that of Q-10(H$_2$) on high-performance liquid chromatography with a reverse-phase column and was presumed to be tetrahydrogenated ubiquinone-10, Q-10 (H$_4$).

In this paper, we purified Q-10(H$_4$) to homogeneity from *Chaetomium funicola* JS 525 which contains it as a major ubiquinone, and found the positions of saturated units in the isoprenoid side chain of this new ubiquinone by mass spectrometry and proton magnetic resonance ($^1$H-NMR) spectrometry. We also showed the position of saturation in the dihydrogenated ubiquinones which were found as minor components of the ubiquinone system in this organism.

**MATERIALS AND METHODS**

**Fungal strains used.** Strain JS 525 was isolated by one of the authors (J.S.) as a culture contaminant and identified by us in this study. *Emericella unguis* JCM 2727 (=CBS 132.55) was chosen as a representative strain having Q-10 (H$_2$) without any Q-10 (H$_4$).

**Growth conditions.** *Chaetomium funicola* JS 525 was grown in 5-liter conical flasks containing 500 ml of a potato infusion medium with 20 g/l of sucrose at pH 5.5 for 4 days with continuous rotary agitation. The seed cultures were prepared for 48 hr in test tubes which contained 10 ml of the same medium. All cultivations were done at 30°C. The fungal cells were harvested by filtration on a Buchner funnel, washed with distilled water, and stored at -20°C.

**Extraction and purification of ubiquinone.** The harvested cells were lyophilized and ground with a pestle and mortar. For 1 g of the cell powder, 200-ml portions of methanol were added to obtain the quinone fraction and kept at 60°C. After 20 min, the mycelia were removed by filtration. The methanol solution thus obtained was evaporated to dryness in vacuo, and the dry matter was
extracted with acetone. After evaporation of acetone, the crude ubiquinone fraction was extracted with n-hexane.

The evaporated n-hexane fraction was dissolved in a small amount of diethyl ether, and methanol was added until milky turbidity appeared. The solution was kept at -20°C overnight, and centrifuged at -10°C, 1500 x g for 10 min.

The separated two fractions, in which ubiquinones were contained, were each chromatographed on thin layer plates of Merck Kieselgel GF254 (20 x 20 cm) which were developed with a mixture of petroleum benzine (bp 50 ~ 90°C)-diethyl ether (6:1, v/v). The ubiquinone homologue mixture collected from the plates was then separated on a silica gel plate impregnated with 9% silver nitrate (w/v) with 2-butanone-n-hexane (1:4, v/v) as a solvent system.9)

After removal of Ag+ from quinones on the TLC plates without silver nitrate, the eluted quinones were further purified by high-performance liquid chromatography (HPLC) using a Shimadzu LC-3A with a Zorbax ODS prepacked column (250 x 4.6 mm i.d.) at 25°C. Methanol-isopropyl ether (5:1, v/v) and (3:1, v/v) were used as eluents for the purification and identification of homologues, respectively.

Mass spectroscopy. Mass spectra of the ubiquinone homologues were measured on a Shimadzu LKB 9000 mass spectrometer using a direct-inlet system with an electron energy of 70 eV, and a temperature range of 150 ~ 160°C.

1H-NMR spectrometry. 1H-NMR spectrum of Q-10 (H2) in CDCl3 was recorded on a JEOL JNM-GX 400 NMR spectrometer (400.5 MHz) using tetramethyl silane as an internal reference standard.

RESULTS

Strain JS 525 was identified as Chaetomium funicola Cooke, for which the brief description is given below.

Chaetomium funicola Cooke

Colonies on oatmeal agar greenish black, with scanty white aerial mycelium. Ascomata dark olive-brown, superficial, scattered, globose to ellipsoidal 100 ~ 130 x 90 ~ 120 μm, ostiolate, covered by densely hairs. Terminal hairs of two kinds: (1) unbranched, long and straight, gradually tapering to blunt tips, and (2) shorter, dichotomously branched at acute angles, tips rounded; both types coarsely roughened. Lateral hairs similar to unbranched terminal hairs. Asci clavate, evanescent, 14.5 ~ 19 x ca. 8.5 μm, 8-spored. Ascospores ovate to limoniform with one end usually rounded and the other one pointed, dark olive-brown, 5.0 ~ 6.5 x 3.5 ~ 5.0 μm.

Strain examined: Isolated as a culture contaminant, Tokyo, 1. iii. 1982, J. Sugiyama # 525. It was deposited in the Culture Collection of the Institute of Applied Microbiology, The University of Tokyo, Tokyo, with the accession number IAM 13491.

The characteristics of strain JS 525 agreed well with the description of Chaetomium funicola provided by Cooke,10) Udagawa,11) Ames,12) Seth,13) and Domsch et al.14)

The ubiquinone composition of C. funicola JS 525 consists of a new homologue as a major component, and Q-10(H2) and Q-10 as minor components with elution times of 20.8, 18.4, and 16.5 min, respectively, using the eluent system for identification (Fig. 1). For the identification of the homologues with HPLC, Q-10 kindly given by the Mitsubishi Gas Chemical Co., Inc., and Q-10(H2) from Gibberella fujikuroi G-2 kindly given by Dr. A. Murobuse, The University of Tokyo, were used as standard reagents.

Fig. 2 shows the mass spectra of ubiquinones. The mass spectrum of Q-10 had the molecular ion peak (M+) at m/z 862, hydroquinone ion peak (M+ + 2) at m/z 864, intense fragment ion peaks at m/z 197 and 235 which

![Fig. 1. HPLC Elution Pattern of Ubiquinones Extracted from Chaetomium funicola JS 525. Methanol-isopropyl ether (3:1, v/v) was used as an effluent.](image)
A Ubiquinone Homologue, Q-10(H₄), from *Chaetomium funicola*.

Fig. 2. Mass Spectra of Ubiquinones.

(a), Q-10 from a bacterium (preparation of Mitsubishi Gas Chemical Co., Inc.); (b), Q-10(H₄) from *Chaetomium funicola* JS 525; (c), Q-10(H₂) from *C. funicola* JS 525; (d), Q-10(H₂) from *Emmericella unguis* JCM 2727.

Fig. 3. ¹H-NMR Spectra of Q-10(H₄) Isolated from *Chaetomium funicola* JS 525.

(a), the enlarged spectrum from 0.80 to 0.92 ppm; (b), the above spectrum in the decoupling experiment.
were assignable to the benzylium ion (C₁₀H₁₃O₄⁺) and 7,8-dimethoxy-2,5-dimethyl-6-cyclohexadienone-1-pyrylium ion (C₁₃H₁₅O₄⁺), respectively, and at m/z 847 (M⁺–15) corresponding to the expulsion of a methyl radical. In addition, small peaks due to successive losses of isoprene units were also at m/z 796, 728, 660, 592, 524, 456, 388, 320, and 252. This sequential fragmentation pattern is typical for ubiquinone-10 with no saturated isoprene unit (Fig. 2a).

A shift up of fragment ion peaks by 3 mass units than those expected from successive losses of isoprene units should conceivably have resulted from a rearrangement (1 mass unit) and dismutation (2 mass units) reactions in the mass spectrometer. The latter gives hydroquinone ions. The peaks derived from the fragmentations without such reactions (no 3 mass units shift up) were also observed with less intensities.

The UV-absorption spectra of the new homologue dissolved in ethanol from C. funicola JS 525 showed typical features of ubiquinone with maxima at 274.5 nm and 290 nm in oxidative and reduced conditions, respectively.

### Table I. ¹H-NMR Signals of Q-10(H₄) in CDCl₃

<table>
<thead>
<tr>
<th>Signal</th>
<th>Assignment* (number of protons)</th>
<th>Chemical shift (ppm)</th>
<th>Multiplicity and coupling constant (J in Hz)</th>
<th>Signal</th>
<th>Assignment (number of protons)</th>
<th>Chemical shift (ppm)</th>
<th>Multiplicity and coupling constant (J in Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>( \text{CH}_{2} ) (7H)</td>
<td>5.11</td>
<td>m</td>
<td>g</td>
<td>( \text{CH}_{2} ) (3H)</td>
<td>1.90 ~ 2.11</td>
<td>m</td>
</tr>
<tr>
<td>b</td>
<td>( \text{CH}_{2} ) (1H)</td>
<td>4.94</td>
<td>t (J=8.5)</td>
<td>h</td>
<td>( \text{CH}_{2} ) (21H)</td>
<td>1.56 ~ 1.62</td>
<td>7 x s</td>
</tr>
<tr>
<td></td>
<td>(next to quinone)</td>
<td></td>
<td></td>
<td></td>
<td>(trans)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>( \text{H}_2\text{CO} )</td>
<td>3.99</td>
<td>2 x s</td>
<td>i</td>
<td>( \text{CH}_{2} ) (11H)</td>
<td>1.00 ~ 1.56</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td>( \text{CH}_{3} ) (6H)</td>
<td>4.00</td>
<td></td>
<td></td>
<td>( \text{CH}_{3} ) (terminal unit)</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>( \text{CH}_{2} ) (2H)</td>
<td>3.19</td>
<td>d (J=8.5)</td>
<td>j</td>
<td>( \text{CH}_{3} ) (6H)</td>
<td>0.87 ~ d (J=6.6)</td>
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<tr>
<td></td>
<td>(next to quinone)</td>
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<td></td>
<td></td>
<td>( \text{CH}_{3} ) (terminal unit)</td>
<td>1.56</td>
<td></td>
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<tr>
<td>e</td>
<td>( \text{CH}_{2} ) (3H)</td>
<td>2.01</td>
<td>s</td>
<td>k</td>
<td>( \text{CH}_{3} )</td>
<td>0.84 ~ d (J=6.3)</td>
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<tr>
<td>f</td>
<td>( \text{CH}_{3} ) trans</td>
<td>1.74</td>
<td>s</td>
<td>l</td>
<td>( \text{CH}_{3} )</td>
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</tbody>
</table>

* The protons assigned are shown by underlines.

* s, singlet; d, doublet; t, triplet; m, multiplet.

* except for the singlet e at δ 2.01 (ppm).
The mass spectrum of the new homologue from *Chaetomium funicola* JS 525 (Fig. 2b), with the molecular ion peak at \( m/z \) 866 (M⁺), hydroquinone ion peak at \( m/z \) 868 (M⁺ + 2), intense fragment ion peaks at \( m/z \) 197, 235, and at \( m/z \) 851 (M⁺ − 15) corresponding to the expulsion of a methyl radical, suggested that this homologue is ubiquinone-10 having a tetrahydrogenated isoprenoid side-chain, namely, Q-10(H₄).

The positions of the saturated units are suggested from the fragmentation pattern of the mass spectrum. That is, the mass spectrum showed no peaks at \( m/z \) 800 (M⁺ − 69 + 3) or \( m/z \) 732 (M⁺ − 69 − 68 + 3) but, instead, had peaks at \( m/z \) 660 ~ 252 corresponding to M⁺ − 71 − 68 x n + 3 (n is 1 to 7). The fact strongly indicated that the 9th and terminal units from the quinone ring were saturated.

The 'H-NMR spectrum of Q-10(H₄) and the assignment of the proton signals are shown in Fig. 3 and Table I, respectively. The signal at δ 3.19 was assigned to a methylene group next to the quinone moiety, as shown by Yamada et al. The numbers of protons of each signal were calculated based on the integration of the two-proton signal at δ 3.19. Two doublets at δ 0.87 (for 6H) and δ 0.84 (for 3H) were assigned to three methyl groups attached to the saturated carbon atoms, and the multiplet appearing at δ 1.00 ~ 1.56 were assigned to methylene and methine protons on saturated carbon atoms.

In a spin decoupling experiment, a 6-proton doublet at δ 0.87 (Fig. 3a) was decoupled to a singlet by irradiation of the one-proton multiplet at δ 1.47 ~ 1.56 (Fig. 3b). This indicated that two methyl groups were attached to a saturated carbon atom bearing a methine proton and that the groups were at the end of the side chain.

Summarizing the data obtained from mass and 'H-NMR spectra, the new ubiquinone isolated from *Chaetomium funicola* JS 525 was identified as Q-10 (H₄), that is, 2,3-dimethoxy-5-methyl-6-IX,X-tetrahydrofarnesylfarnesylgeranyl-geranyl-1,4-benzoquinone (Fig. 4).

The structure of Q-10 (H₂) obtained from *Chaetomium funicola* JS 525 as a minor component was presumed on the basis of results from the mass spectra, in which peaks at \( m/z \) 798 and 728 corresponding to (M⁺ − 69 + 3) and (M⁺ − 71 − 68 + 3), respectively, were detected in addition to other fragments due to successive losses of isoprene units at \( m/z \) 252 ~ 660 (Fig. 2c). On the other hand, the mass spectrum of Q-10(H₂) obtained from *Emericella unguis* JCM 2727, which was confirmed to have no Q-10(H₄) in its ubiquinone system by HPLC, had small peaks due to successive losses of isoprene units at \( m/z \) 252 ~ 728 with a peak loss due to saturation of a terminal unit, and revealed its structure, i.e., ubiquinone-10 with a saturated isoprene unit at the terminal position (Fig. 2d). These results could assume that Q-10(H₂) obtained from *Chaetomium funicola* JS 525 is a mixture of two homologues; one has a side chain with the 9th unit saturated, and another with the terminal unit saturated (Fig. 5).

**DISCUSSION**

This paper showed the saturation points of the side chain of tetrahydrogenated Q-10
newly found in the fungus Chaetonium funicola JS 525. On the other hand, it is known that menaquinones and their relative compounds have various isoprene units and degrees of saturation in the multiprenyl chains. Azerad et al.\textsuperscript{16,17} found the saturated position of menaquinone (MK)-9(H\textsubscript{2}) of Mycobacterium phlei to be in the second isoprene unit from the quinone ring. Collins et al.\textsuperscript{18} elucidated that a derivative of menaquinone-6, i.e., II,III-tetrahydro-\omega-(2,6,6-trimethylcyclohex-2-enylmethyl)menaquinone-6 obtained from Nocardia brasiliensis DSM 43009 had a saturation unit at the same position of isoprene unit as the side-chain of MK-9(H\textsubscript{2}) in Mycobacterium phlei. Yamada et al.\textsuperscript{19} demonstrated that the saturation positions of MK-8(H\textsubscript{4}) and MK-9(H\textsubscript{4}) obtained from Brevibacterium lipolyticum AJ 1450 and Oerskovia turbata AJ 9191, respectively, were located continuously in the second and third units of the chain, even though the numbers of units were different. Furthermore, MK-9(H\textsubscript{3}) isolated from Actinomadura madurae AJ 9136 has a saturated isoprene unit at the second position from the terminal of the side chain in addition to the second and third units from the quinone ring.\textsuperscript{9,20} The fourth position of saturation in MK-9(H\textsubscript{4}) obtained from Streptomyces albus IFO 13014 was at the end of the side chain.\textsuperscript{21,22} Ishii et al.\textsuperscript{23,24} determined the structure of a menaquinone-like substance, tetrahydrogenated 2-methylthio-1,4-naphthoquinone-7 (MTK-7), isolated from a thermophilic hydrogen-oxidizing bacterium, Hydrogenobacter thermophilus TK-6, and indicated that the units 6 and 7 of the isoprenoid side chain were saturated, differing from the saturation positions of MK-8(H\textsubscript{4}) and MK-9(H\textsubscript{4}) mentioned above.\textsuperscript{19} Furthermore, although the MTK-like quinone, caldariellaquinone, isolated from the extremely thermophilic and acidophilic bacterium Caldariella acidophila, possessed benzo[\textit{b}]thiophen-4,7quinone as a nucleus quinone molecule, its side chain was saturated in all of its six isoprene units.\textsuperscript{25,26}

On the other hand, the saturation position of ubiquinone-10(H\textsubscript{2}) obtained from Gibberella fujikuroi, a gibberellic acid producing strain,\textsuperscript{27} Penicillium stipitatum NRRL 2104,\textsuperscript{28} and Rhodotorula hasegawa IFO 1058\textsuperscript{7,14} was the terminal (10th) isoprene unit of the side chain. Q-10(H\textsubscript{2}) isolated from Chaetomium funicola JS 525 as a minor component, was assumed to be a mixture of units saturated at the 9th or 10th positions. Therefore, one of the homologues of Q-10(H\textsubscript{2}) having the saturated unit at the 9th position seems to be a unique example of the saturation position. Tetrahydrogenated Q-10 from Chaetomium funicola JS 525 has two saturated positions in the 9th and 10th side chain units. These facts show that the arrangement of the saturated isoprene unit(s) differs completely between the side chains of ubiquinone and menaquinone, but the arrangement of tetrahydrogenated Q-10 was consistent with that of tetrahydrogenated MTK-7 on the terminal two isoprene units.

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