Production of 5,7-Dihydroxy-6-hydroxymethyl-2-methoxy-1,4-naphthoquinone by the Cultured Lichen Mycobiont of Opegrapha sp. No. 9771836

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A new pigment was isolated from an osmotically-stressed culture of the lichen mycobiont of Opegrapha sp. and the structure was determined to be 5, 7-dihydroxy-6-hydroxymethyl-2-methoxy-1, 4-naphthoquinone by spectroscopic analyses.

Lichens are symbiotic organisms which are comprised of fungi and algae. It is known that lichens produce a number of unique secondary metabolites, the production of which is thought to be mainly associated with the metabolism of the fungal portion (mycobiont) of the symbiotic system. On the other hand, previous studies have shown that the mycobionts from lichens, in some cases, produce new compounds that are not found in natural lichens when cultured under osmotically-stressed conditions [1–4]. In this paper, we report the isolation and structural characterization of a new pigment, which is produced in the culture of spore-derived mycobiont from a strain of Opegrapha sp.

Structures of compounds 1–5.

A lichen sample of Opegrapha sp. was collected from the bark of a tree on Iriomote Island, Okinawa, Japan (24°N, ca. 50 m altitude) in July 1997. The voucher specimen was identified by Prof. M. Nakanishi, Hiroshima University, and was then deposited at Osaka City Institute of Public Health and Environmental Sciences under registration No. 9771836. The sample contained no characteristic aromatic component, as evidenced by TLC analysis of the thallus extract.

The spore-derived mycobiont was cultured on a malt-yeast extract medium (malt extract 1 g, yeast extract 4 g, agar 15 g in 1 l H\textsubscript{2}O, pH 7) supplemented with sucrose at a concentration of 100 g l\textsuperscript{-1}. The culture was maintained at 18 °C in the dark. After 9 months, the culture media turned dark red in color, as the result of pigment production. Subsequently, the culture media along with the mycelial colonies (2.9 g) was extracted with acetone. TLC and/or HPLC analyses of the extract indicated the presence of a single red-colored component (1), which was purified by silica gel column chromatography (Wakogel C-200) using a solvent mixture of \textit{\theta}-hexane and ethyl acetate, and by reverse phase HPLC using a Cosmosil C18 column (10 x 250 mm) and a mobile phase composed of MeOH-H\textsubscript{2}O-CH\textsubscript{3}COOH (50:50:1). Pure compound 1 was obtained as a solid (10.6 mg).

HR-EI-MS indicated that the molecular formula of 1 was C\textsubscript{12}H\textsubscript{10}O\textsubscript{9}, based on the molecular ion, M\textsuperscript{+}, at \textit{m}/\textit{z} 250.0476 (calcd. 250.0477). The degree of unsaturation for 1 was calculated to be 8. The \textsuperscript{13}C NMR spectrum showed 12 resonance signals, including two signals consistent with carbonyl carbons (\textdelta C 189.4 and 178.9 ppm) and 8 aromatic
carbons (δC 162.5, 161.3, 160.4, 131.0, 120.5, 109.4, 107.8, and 106.7 ppm). Of the latter aromatic carbon signals, 3 signals (δC 162.5, 161.3, and 160.4 ppm) resonated of relatively low field, indicating that each is connected to an electronegative atom (oxygen). 1H NMR data showed the presence of a chelated phenolic proton (δH 12.8 ppm), two aromatic protons (δH 7.05 and 6.21 ppm), an oxygen-bound methylene group (δH 4.52 ppm) and a methoxy group (δH 3.86 ppm). All these signals appeared as singlets and no primary spin-interactions were indicated. The δC values of the two carbonyl carbons, along with IR absorption bands at 1670 and 1625 cm⁻¹, suggest that 1 is a quinone derivative. Treatment of 1 with acetic anhydride in the presence of a catalytic amount of sulfuric acid gave a triacetyl derivative, which could be confirmed by both MS and 1H NMR, and thus, the presence of three hydroxyl groups in 1 was indicated.

The connectivity of these components in 1 was established by observing the long-range C-H coupling by HMBC experiments, as shown in Fig. 1. A close spatial relationship between the methoxy protons (δH 3.86) and an aromatic proton (δH 6.21) was also indicated by the observed NOE.

Collectively, the above data are consistent with 1 being 5, 7-dihydroxy-6-hydroxymethyl-2-methoxy-1, 4-naphthoquinone. This compound has hitherto never been described in the literature.

Compound 1 appears to be of polyketide origin. A possible biosynthetic pathway is shown in Fig. 2. It is likely that the carbon skeleton of 1 is constructed by the acetate-malonate pathway. Important intermediates, therefore, should include flavolin (2), which has been isolated from such fungal cultures as Aspergillus citricus [5], Phoma wasabiae [6] and Verticillium dahliae [7]. The introduction of C₁ units to C-6 and to the oxygen of hydroxyl moiety attached to C-2 of 2, followed by oxidation of the introduced methyl group at C-6, lead to the formation of 1. Derivatives of flavolin, 3 and 4, have been isolated from Hendersonula toruloidea, a filamentous fungus [8], and a strain of Streptomyces [9], respectively. Compound 1 is also related to squamarone (5), which has been isolated from a lichen Squamarina cartilaginea [10]. The production of derivatives of naphthazarine, 5, 8-dihydroxy-1, 4-naphthoquinone, has been reported in the cultures of the mycobiont of the lichen Cladonia cristatella [11].

Experimental

1H and 13C NMR spectra were recorded on Bruker AC 300 and/or ARX 500 spectrometers in DMSO-d₆ with TMS as an internal standard.

5,7-Dihydroxy-6-hydroxymethyl-2-methoxy-1, 4-naphthoquinone (1). A red solid. EI-MS (70 eV) m/z (rel. int.): 250.0479 [M]+ (28%) (calcd for C₁₂H₁₀O₉: 250.0477), 232 (100). UV (MeOH) nm (log ε): 434 (3.79), 304 (4.23), 266 (4.42), 219 (4.76). IR (KBr) νmax cm⁻¹: 3450, 3050, 1670, 1625,
1595. $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 12.8 (s, -OH), 7.02 (s, 1H), 6.21 (s, 1H, 2.6% NOE was observed on the irradiation of the signal at $\delta$ 3.85), 4.51 (s, 2H, -CH$_2$-), 3.85 (s, 3H, -OCH$_3$, an NOE was observed on irradiation of the signal at $\delta$ 3.85), 4.51 (s, 2H, -CH$_2$-), 3.85 (s, 3H, -OCH$_3$, an NOE was observed on irradiation of the signal at $\delta$ 6.21). $^{13}$C NMR (DMSO-$d_6$, 75 MHz): $\delta$ 189.4, 178.9, 162.5, 161.3, 160.4, 130.9, 120.5, 109.4, 107.8, 106.7, 56.7, 51.4.

5,7-Diacetyl-6-acetoxymethyl-2-methoxy-1,4-naphthoquinone. A small amount of 1 was treated with Ac$_2$O, which contained a catalytic amount of H$_2$SO$_4$. After chromatographic work-up, the product was analyzed by API-MS and $^1$H NMR. API-MS $m/z$ (rel. int.): 377 [M+H]$^+$ (1), 317(23), 275(17), 233(100). $^1$H NMR (DMSO-$d_6$, 300 MHz): $\delta$ 7.85 (s, 1H), 6.24 (s, 1H), 5.11 (s, 2H, -CH$_2$-), 3.85 (s, 3H, -OCH$_3$), 2.37 (s, 3H, -OCOCH$_3$), 2.35 (s, 3H, -OCOCH$_3$), 1.99 (s, 3H, -OCOCH$_3$).

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