Cribrarione B, a New Naphthoquinone Pigment from the Myxomycete *Cribraria cancellata*

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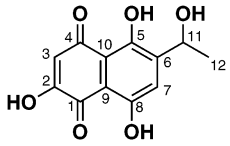
Cribrarione B (**1**), a new naphthoquinone pigment, has been isolated from the myxomycete *Cribraria cancellata*, and its structure was elucidated as 2,5,8-trihydroxy-6-(1-hydroxyethyl)-[1,4]-naphthoquinone by NMR and mass spectral data.

The myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryotes, and chemical studies on the secondary metabolites of the myxomycetes are limited so far.[1] During our search for natural products from myxomycetes,[2,3] we recently investigated a field-collected sample of fruit bodies of *Cribraria cancellata* (Cribrariaceae). Here we describe the isolation and structure elucidation of a new naphthoquinone pigment, cribrarione B (**1**).



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Cribrarione B (**1**) was obtained as a brown-red solid and showed a quasi-molecular ion peak at *m*/*z* 249 $(M - H)^-$ in its negative FAB mass spectrum. The molecular formula of **1** was revealed as $C_{12}H_{10}O_6$ by the HRFABMS data [*m*/*z* 249.0404, $(M - H)^-$, Δ +0.5 mmu]. The UV spectrum of **1** showed absorption maxima at 254, 299, and 484 nm, indicating the presence of conjugated system(s). The ^{1}H NMR spectrum of **1** in CD_3OD showed only four signals due to two aromatic (or olefinic) protons [δ_H 7.17 (1H, d, J = 0.8 Hz) and 5.67 (1H, s)], one oxymethine [δ_H 5.13 (1H, qd, J = 6.6 and 0.8 Hz)], and a secondary methyl [δ_H 1.43 (3H, d, J = 6.6 Hz)] group. The ^{13}C NMR data of **1** showed 12 signals assignable to two carbonyls (δ_C 189.0 and 188.6), eight other sp^2 carbons, one sp^3 oxymethine (δ_C 63.9), and a methyl (δ_C 22.3) carbon. Since six out of eight unsaturation equivalents were accounted for from the ^{13}C NMR data, **1** was inferred to have two rings. The ^{1}H−^{1}H COSY spectrum of **1** showed that the oxymethine proton was adjacent to the secondary methyl group. In the HMBC spectrum of **1**, the aromatic proton at δ_H 5.67 (H-3) showed $^3J_{C-H}$ long-range connectivities with a carbonyl carbon at δ_C 189.0 (C-1) and an sp^2 quaternary carbon at δ_C 112.1 (C-10), while another aromatic proton at δ_H 7.17 (H-7) was coupled with two oxygen-bearing sp^2 carbons at δ_C 151.2 (C-5) and 157.4 (C-8) and also with two sp^2 quaternary carbons at δ_C 148.9 (C-6) and 111.5 (C-9). The H-7 (δ_H 7.17) showed another HMBC correlation with the sp^3 oxymethine carbon (C-11), while the oxymethine proton (H-11) showed HMBC correlations with two aromatic carbons at δ_C 148.9 (C-6) and 118.9 (C-7) and with the secondary methyl carbon (C-12). On the other hand, the secondary methyl protons showed HMBC cross-peaks with the oxymethine carbon and the C-6 aromatic carbon (δ_C 148.9).

Thus, a naphthoquinone nucleus was constructed for compound **1** and a 1-hydroxyethyl group was attached to C-6. The presence of three other hydroxyl groups was suggested from the molecular formula of **1**, and these hydroxyl groups were assigned to be on C-2, C-5, and C-8 on the naphthoquinone nucleus from their ^{13}C NMR chemical shifts (δ_C 173.7, 151.2, and 157.4, respectively). The low-field resonance of C-2 (δ_C 173.7) was consistent with the ^{13}C chemical shift of the hydroxy-bearing α-carbon of *p*-quinones.[4] From these results, cribrarione B was concluded to be 2,5,8-trihydroxy-6-(1-hydroxyethyl)-[1,4]-naphthoquinone (**1**).[5]

Naphthoquinone pigments in myxomycetes have been previously reported from *Lindbladia tubulina*,[1,6] *Metatrichia floriformis*,[7] and *M. vesparium*.[8] We recently isolated a new naphthoquinone pigment, cribrarione A, from the extract of the wild fruit bodies of *Cribraria purpurea*.[9] No previous chemical studies on the constituents of members of the genus *Cribraria* had been described in the literature. This is therefore the second report on the chemical constituents of the genus *Cribraria*. However, the genus *Lindbladia* belongs to the same family (Cribrariaceae) as *Cribraria*. *Metatrichia* sp. belong to a different family (Trichiaceae). Crude extract of *Cribraria cancellata* exhibited antimicrobial activity against *Bacillus subtilis*, but cribrarione B (**1**) proved inactive against *B. subtilis*.

Experimental Section

General Experimental Procedures. UV spectra were obtained on a Hitachi U-3400 spectrometer. IR spectra were measured from samples on a Hitachi 260-10 infrared spectrophotometer. NMR spectra were recorded on JEOL JNM ecp600 spectrometers. HRFABMS were acquired on a JMS HX-110 mass spectrometer.

Organism. The fruit bodies of *Cribraria cancellata* were collected at Seki, Ohtsu, Kochi-shi in Kochi Prefecture, Japan, in August 2001. A voucher specimen (#21927) is maintained by Y.Y. (Ohtsu-ko, Kochi).

Extraction and Isolation. The air-dried fruit bodies of *Cribraria cancellata* (1.4 g) were extracted with 90% MeOH (100 mL × 2) and 90% acetone (100 mL × 1). The combined MeOH and acetone extracts (0.22 g), which contained brown-red pigments, were subjected to ODS column chromatography (column A, 2.0 × 13 cm) eluted with 0−100% MeOH in H_2O. The fraction (27 mg) of column A eluted with MeOH/H_2O (1:1) was further separated by gel filtration with Sephadex LH-

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20 (column B, 1.0 × 24 cm) eluted with MeOH/H_2O (1:1) to give cribrarione B (**1**, 2.0 mg).

Cribrarione B (1): brown-red solid; $[\alpha]^{22}_D$ +50° ± 20 (*c* 0.025, MeOH); UV λ_{max} (MeOH) 275 (ϵ 9700), 316 (8700), and 510 nm (8100); ^{1}H NMR (CD_3OD) δ_H 7.17 (1H, d, J = 0.8 Hz; H-7), 5.67 (1H, s; H-3), 5.13 (1H, qd, J = 6.6 and 0.8 Hz; H-11), and 1.43 (3H, d, J = 6.6 Hz; H_3-12); ^{13}C NMR (CD_3OD) δ_C 189.0 (C-1), 188.6 (C-4), 173.7 (C-2), 157.4 (C-8), 151.2 (C-5), 148.9 (C-6), 118.9 (C-7), 112.1 (C-10), 111.5 (C-9), 107.0 (C-3), 63.9 (C-11), and 22.3 (C-12); FABMS (negative) *m*/*z* 249 $(M - H)^-$; HRFABMS *m*/*z* 249.0404 [calcd for $C_{12}H_9O_6$, (M − H) 249.0399].

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