Cribrarione C, a Naphthoquinone Pigment from the Myxomycete *Cribraria meylanii*

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Chemical investigation of field-collected fruit bodies of the myxomycete *Cribraria meylanii* resulted in the isolation of a naphthoquinone pigment, cribrarione C, and its structure was elucidated by spectral data as 2,5,6,7-tetrahydroxy-1,4-naphthoquinone (1). This compound (1) had been synthesized previously, while it was isolated here for the first time as a natural product, and its NMR and MS data are described in this study.

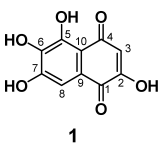
Key words myxomycete; *Cribraria meylanii*; naphthoquinone; pigment

During our search for bioactive natural products from myxomycetes, we have isolated a number of new bioactive secondary metabolites with unique bioactivities, such as a chlorinated polyene-pyrone, tyrosine-kinase inhibitory bis-indole alkaloids, Wnt-signal inhibitory peptide, TNF-related apoptosis inducing ligand (TRAIL) resistance overcoming cycloanthranilylproline, and cytotoxic triterpenoid aldehyde lactone.[1)] 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 of the secondary metabolites of the myxomycetes had been limited before our studies.[2)] We recently investigated field-collected fruit bodies of *Cribraria meylanii*, having a dull crimson 1—2 mm broad sporangium with a 5-mm long dark brown stalk. Here we describe the isolation and structure elucidation of a naphthoquinone pigment, cribrarione C (**1**), which had been synthesized previously but was isolated here as a natural product for the first time and its NMR and MS data are described in this study.

Results and Discussion

The fruit bodies of myxomycete *Cribraria meylanii*, collected in Kochi Prefecture, Japan, were extracted with 90% MeOH and 90% acetone. The combined extracts were subjected to chromatography on silica gel and ODS, followed by further purification with a Sephadex LH-20 column to give a dark red pigment compound, named cribrarione C (**1**), in 0.17% yield.

Cribrarione C (**1**), obtained as a brown-red solid, showed a molecular ion at *m*/*z* 222 in its EI mass spectrum. The molecular formula of **1** was revealed as $C_{10}H_6O_6$ by HR-EI-MS data [*m*/*z* 222.0161 (M^+), Δ −0.3 mmu]. The UV spectrum of **1** showed absorption maxima at λ_{max} 273, 323, and 414 nm, indicating the presence of conjugated systems, and IR absorption bands at 3320 and 1630 cm^{-1} suggested the presence of hydroxy group(s) and carbonyl group(s). The ^{1}H-NMR spectrum of **1** in DMSO-d_6 (Table 1) showed five signals due to two aromatic protons [δ_H 5.72 (1H, s) and 6.98 (1H, s)] and three hydroxy protons [δ_H 13.52 (br s), 10.11 (1H, br s), and 9.67 (1H, br s)]. The ^{13}C-NMR spectrum aided with heteronuclear multiple quantum coherence (HMQC) spectrum of **1** revealed signals assignable to two carbonyls (δ_C 189.6, 181.2), two sp^2 methine carbons (δ_C 108.3, 108.0), and five other sp^2 quaternary carbons (δ_C 150.6, 149.1, 140.0, 122.3, 109.3), with no sp^3 carbons being observed. In the heteronuclear multiple bond coherence (HMBC) spectrum of **1** (Fig. 1), the aromatic proton at δ_H 5.72 (H-3) showed J_{C-H} long-range connectivities with a carbonyl carbon at δ_C 181.2 (C-1) and an sp^2 quaternary carbon at δ_C 109.3 (C-10); these two carbon signals were also correlated in its HMBC spectrum with another aromatic proton at δ_H 6.98 (which was finally assigned to H-8), and this proton also showed HMBC correlations clearly with δ_C 140.0 (C-6)



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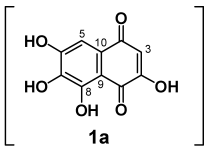

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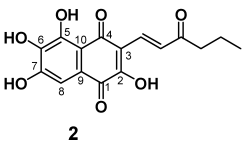

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of **1** in DMSO-d_6

Position	δ_H	δ_C	HMBC (^{1}H to ^{13}C)	δ_C of **2**[c)]
1		181.2		184.1
2		—[a)]		176.4
3	5.72 (s)	108.0	181.2, 109.3	113.3
4		189.6		189.6
5		150.6		151.6
6		140.0		141.5
7		149.1		149.5
8	6.98 (s)	108.3	181.2, 149.1,[b)] 140.0, 122.3,[b)] 109.3	108.6
9		122.3		125.0
10		109.3		112.2
OH	9.67 (br s)[d)]			
	10.11 (br s)[d)]			
	13.52 (s)[d)]			

a) Not observed in both experiments with a long-range delay of 62.5 ms and 125 ms. *b*) Observed weakly with a long-range delay of 62.5 ms, but observed clearly with a long-range delay of 125 ms. *c*) For naphthoquinone moiety from ref. 3 (in CD_3OD). *d*) Disappeared on addition of D_2O.

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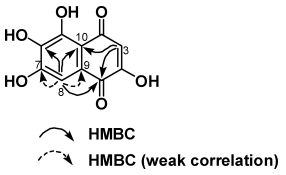

Fig. 1. HMBC Correlations Observed for **1** with a Long-Range Delay of 62.5 ms, Which Corresponds to a $1/^nJ_{CH}$ of 8 Hz

and weakly with δ_C 149.1 (C-7) and 122.3 (C-9). These ^{1}H- and ^{13}C-NMR data, including the HMBC correlations observed for **1**, were reminiscent of a naphthoquinone structure, and the presence of another hydroxyl group attached to a quaternary carbon (C-2) of the naphthoquinone nucleus was deduced from consideration of the molecular formula of **1**, although the fourth hydroxyl proton signal or the C-2 carbon signal was not visible. In addition, comparison of its ^{1}H- and ^{13}C-NMR chemical shift data with those of other naphthoquinone pigments, such as lindbladione (**2**, Table 1),[3] and its analogues[4] previously isolated from a myxomycete *Lindbladia tubulina* also suggested the whole structure of **1** having a naphthoquinone nucleus bearing four hydroxyl groups at C-2, C-5, C-6, and C-7. An alternative structure (**1a**) with a hydroxyl group at C-8 and a hydrogen at C-5 position could be proposed; however, this possibility was excluded by the following observations. The signal at δ_C 109.3 was firmly assigned to C-10 since this carbon signal showed an HMBC correlation with H-3. Thus, the remaining unoxygenated quaternary carbon resonating at δ_C 122.3 had to be assigned to C-9. When the HMBC spectrum was recorded with a long-range delay of 62.5 ms, which corresponds to a $1/^nJ_{CH}$ of 8 Hz, the proton signal resonating at δ_H 6.98 showed only a weak HMBC correlation with C-9 (*vide supra*), while by using a long-range delay of 125 ms, which corresponds to a $1/^nJ_{CH}$ of 4 Hz, the HMBC correlation between the proton signal at δ_H 6.98 and C-9 was observed clearly. Since in aromatic rings $^2J_{CH}$ values are characteristically smaller than $^3J_{CH}$ values (*e.g.*, in benzene, $^2J_{CH}$=1.0 Hz and $^3J_{CH}$=7.6 Hz),[5] the HMBC correlation between the proton at δ_H 6.98 and C-9 was not through $^3J_{CH}$ but through $^2J_{CH}$. Thus, the proton signal resonating at δ_H 6.98 was assigned not to H-5 but to H-8. The HMBC experiment with a long-range delay of 125 ms also showed a clear correlation from H-8 to C-7 ($^2J_{CH}$). From these results, the structure of cribrarione C was concluded to be 2,5,6,7-tetrahydroxy-1,4-naphthoquinone (**1**).

We previously studied pigment constituents of myxomycetes of the genus *Cribraria*, and have isolated two naphthoquinone pigments; cribrarione A from *Cribraria purpurea*[6] and cribrarione B from *Cribraria cancellata*.[7] This is the third report on the chemical constituents of the genus *Cribraria*. These naphthoquinones are fine pigments, which may be one of the meanings of natural occurrence of these kind of molecules. 2,5,6,7-Tetrahydroxy-1,4-naphthoquinone (**1**, cribrarione C) was previously synthesized from gallic acid by Natori and Kumada,[8] but its spectral data were described incompletely. Compound **1** was here isolated for the first time as a natural product and its NMR and MS data are described for the first time in this study.

The cytotoxicity of cribrarione C (**1**) was examined against HeLa cells, but it proved to be inactive (IC_{50} value, >100 μM), while this compound (**1**) showed mild TRAIL-resistant overcoming activity against TRAIL-resistant human gastric adenocarcinorma (AGS) cells.[9] Treatment of the cell with TRAIL (100 ng/ml) alone or compound **1** (75 μM) alone resulted in only a slight decrease in cell viability (87% and 96%, respectively), whereas treatment of the cells with compound **1** at 75 μM in the presence of TRAIL (100 ng/ml) reduced cell viability to 71%, which was 16% and 25% more than TRAIL only or **1** only, respectively, suggesting a possible synergism between the two agents. Antimicrobial activity of compound **1** was also examined against *Staphylococcus aureus*, but it was inactive at 50 μg/ml.

Experimental

General IR spectra were recorded on ATR in a Jasco FT-IR 230 spectrophotometer, and UV spectra were obtained on a Shimadzu UV mini-1240 spectrometer. The NMR spectra were recorded on a JEOL JNM ecp600 spectrometer. EI-MS and HR-EI-MS were recorded on a JEOL GC-Mate spectrophotometer.

Organisms The fruiting bodies of *Cribraria meylanii* were collected in Kochi Prefecture, Japan, in December 2007. Voucher specimens (#31198, 31199, and 31200) are maintained by Y.Y. (Ohtsu-ko, Kochi).

Extraction and Isolation Air-dried fruiting bodies of *Cribraria meylanii* (2.2 g) were extracted with 90% MeOH (100 ml×1 and 50 ml×1) and 90% acetone (50 ml×1) at rt. The combined extracts (908 mg) were subjected to silica gel column chromatography (20×440 mm) with gradient elution of 0—100% MeOH in $CHCl_3$. The fraction (110 mg) of the silica gel column eluted with 100% MeOH was separated by ODS column chromatography (10×260 mm) eluted with 50% MeOH in the presence of 0.1% TFA, and further separated by Sephadex LH-20 column chromatography (15×380 mm) eluted with MeOH to give cribrarione C (3.8 mg).

Cribrarione C (**1**): Red-brown solid; UV λ_{max} (EtOH) 273 (ε 9000), 323 (3800), and 414 nm (2100); IR (ATR) ν_{max} 3320 and 1630 cm^{-1}; ^{1}H- and ^{13}C-NMR (Table 1); EI-MS *m/z* (%) 222 (M^+, 81), 194 (100), and 153 (69); HR-EI-MS *m/z*: 222.0161 [Calcd for $C_{10}H_6O_6$, (M^+) 222.0164].

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