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Cribrarione A, a new antimicrobial naphthoquinone pigment from a myxomycete *Cribraria purpurea*

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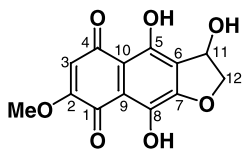
Abstract—Cribrarione A (**1**), a new dihydrofuranonaphthoquinone pigment with antimicrobial activity against *Bacillus subtilis* has been isolated from a myxomycete *Cribraria purpurea* and its structure was elucidated by spectral data. The $^{1}H-^{13}C$ long-range couplings through an intramolecular hydrogen bond were clearly observed in the HMBC spectrum of **1**.

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

The fruit bodies of *Cribraria purpurea* (0.85 g) collected in Kochi Prefecture, Japan, were extracted with 90% MeOH and 90% acetone. The combined extract (0.12 g) having antimicrobial activity against *B. subtilis* was subjected to chromatographies on silica gel and Sephadex LH-20 to give cribrarione A (**1**) in 0.8% yield.



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Cribrarione A (**1**) was obtained as brown–red solid, and showed a molecular ion peak at *m/z* 278 in its EI mass spectrum. The molecular formula of **1** was revealed as $C_{13}H_{10}O_7$ by ^{13}C NMR aided with the DEPT and HMQC data along with the HRFABMS data (*m/z* 279.0494, $[M+H]^+$, Δ −1.1 mmu). The UV spectrum of **1** showed absorption maxima at 275, 316, and 510 nm, which were shifted to 310, 537, and 568 nm, respectively, on addition of alkali (NaOH), indicating the presence of phenol group(s). The ^{1}H NMR spectrum of **1** in $CDCl_3$ (Table 1) showed signals due to one aromatic proton [δ_H 6.23 (1H, s)], three hydroxyl protons [δ_H 13.2 (1H, s), 12.3 (1H, s), and 2.78 (1H, br s)], one methoxy group [δ_H 3.94 (3H, s)], one oxymethine [δ_H 5.70 (1H, dd, J=7.3, 3.0 Hz)], and one oxymethylene [δ_H 4.81 (1H, dd, J=11.0, 7.3 Hz) and 4.71 (1H, dd, J=11.0, 3.0 Hz)] groups. The ^{13}C NMR spectrum of **1** (Table 1) showed signals for two carbonyls (δ_C 180.9 and 176.2), eight other sp^2 carbons, and each one sp^3 oxymethine (δ_C 69.9), oxymethylene (δ_C 81.1), and methoxy (δ_C 56.8) carbons. Since, six out of nine

Table 1. ^{1}H and ^{13}C NMR data of compound **1** in $CDCl_3$

	δ_H (Hz)	δ_C	HMBC
1		176.2	
2		159.8	
3	6.23 s	110.1	C-1, C-2, C-4, C-10
4		180.9	
5		162.5	
6		124.7	
7		158.0	
8		155.2	
9		113.7	
10		106.0	
11	5.70 dd 7.3, 3.0	69.9	C-5, C-6
12	(a) 4.81 dd 11.0, 7.3	81.1	C-6, C-11
	(b) 4.71 dd 11.0, 3.0		C-6, C-11
MeO-2	3.94[a] s	56.8	C-2
HO-5	13.2 s		C-3,[b] C-4,[b] C-5, C-6, C-10
HO-8	12.3 s		C-7, C-8, C-9
HO-11	2.78 br s		

[a] 3H.
[b] $^{1}H-^{13}C$ couplings assigned to those through a hydrogen bond.

Keywords: myxomycete; dihydrofuranonaphthoquinone; antimicrobial activity; $^{1}H-^{13}C$ coupling; hydrogen bond.

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unsaturation degrees were accounted for from ^{13}C NMR data, **1** was inferred to have three rings. The $^{1}H-^{1}H$ COSY spectrum of **1** showed that oxymethylene protons (δ_H 4.81 and 4.71; H_2-12) were adjacent to the oxymethine proton (δ_H 5.70; H-11), which in turn was coupled with the hydroxyl proton at δ_H 2.78 (OH-11). In the HMBC spectrum of **1**, the aromatic proton at δ_H 6.23 (H-3) showed long-range connectivities with two carbonyl carbons at δ_C 176.2 (C-1) and 180.9 (C-4) and also with sp^2 carbons at δ_C 106.0 (C-10) and 159.8 (C-2), and the C-2 was in turn coupled with the methoxy protons at δ_H 3.94, implying the methoxy group was attached on C-2. The hydroxyl proton at δ_H 13.2 showed HMBC correlations with the sp^2 carbons at δ_C 106.0 (C-10), 162.5 (C-5), and 124.7 (C-6), while the oxymethine proton at δ_H 5.70 (H-11) showed connectivities to C-6 and C-5, thus suggesting that the hydroxyl group at δ_H 13.2 was placed on C-5 (δ_C 162.5) and the two sp^3 carbon unit (C-11 and C-12) was connected to C-6 (δ_C 124.7). The other hydroxyl proton at δ_H 12.3 was indicated to be present on C-8 position, since this hydroxyl proton showed the HMBC correlations to ^{13}C signals at δ_C 113.7 (C-9), 155.2 (C-8), and 158.0 (C-7).

By interpreting these spectral data describe above, a naphthoquinone nucleus with two hydroxyl groups at C-5 and C-8 and a methoxy group on C-2 was constructed for compound **1**. It should be noteworthy that in the HMBC spectrum of **1** (J_{C-H}=8 Hz) cross-peaks were clearly observed from OH-5 (δ_H 13.2) to C-4 (δ_C 180.9) and C-3 (δ_C 110.1) (Fig. 1). These HMBC correlations may be attributable to 2- and 3-bond J_{C-H} couplings, respectively, through a rigid intramolecular hydrogen bond[3] between the hydroxyl proton (OH-5) and the oxygen of the C-4 carbonyl group, which further corroborated the proposed naphthoquinone structure.

The ^{13}C chemical shift of C-7 (δ_C 158.0) implied that this carbon bore an oxygen atom, and the unsaturation degree of **1** had suggested the presence of one more ring other than the naphthoquinone. The C-7, therefore, had to be connected with the sp^3 methylene carbon (C-12) through an ether–oxygen atom to give rise to a dihydrofuran ring moiety. The oxymethine proton (H-11) exhibited a clear NOE to one of the oxymethylene proton (δ_H 4.81; H-12a), while the NOE between H-11 and the other oxymethylene proton (δ_H 4.71; H-12b) was obscure, thus suggesting that H-12a (δ_H 4.81) was *cis* and H-12b (δ_H 4.71) was *trans* to H-11. The coupling constant for H-11/H-12a (*cis*) was 7.3 Hz and that for H-11/H-12b (*trans*) was 3.1 Hz; these *J*-values may be consistent for the vicinal protons of a five-membered ring.

Cribrarione A (**1**) was shown to be optically active from the CD spectrum. The absolute stereochemistry of the C-11 chiral center of cribrarione A (**1**), however, remained undefined, since MTPA esterification easily led to dehydration of 11-hydroxyl group to give a furan. From these results, the structure of cribrarione A was concluded as **1**.

Several pigments naphthoquinone derivatives in myxomycetes were previously reported from *Lindbladia tubulina*,[1,2] *Metatrichia floriformis*,[4] and *Metatrichia vesparium*.[5] Chemical studies on the constituents of the myxomycetes of the genus *Cribraria* had never been described in the literature to the best of our knowledge. This is therefore, the first report on the chemical constituents of the genus *Cribraria* sp., although the genus *Lindbladia* belongs to the same family (Cribrariaceae) as *Cribraria*. *Metatrichia* sp., however, belongs to a different family (Trichiaceae). Crude extract of *Cribraria purpurea* exhibited antimicrobial activity against *Bacillus subtilis*, and this activity was revealed to be ascribable to cribrarione A (**1**), since **1** was substantially active against *B. subtilis*with a diameter of inhibition zone 11 mm at 5 μg per paper disc (8 mm in diameter).

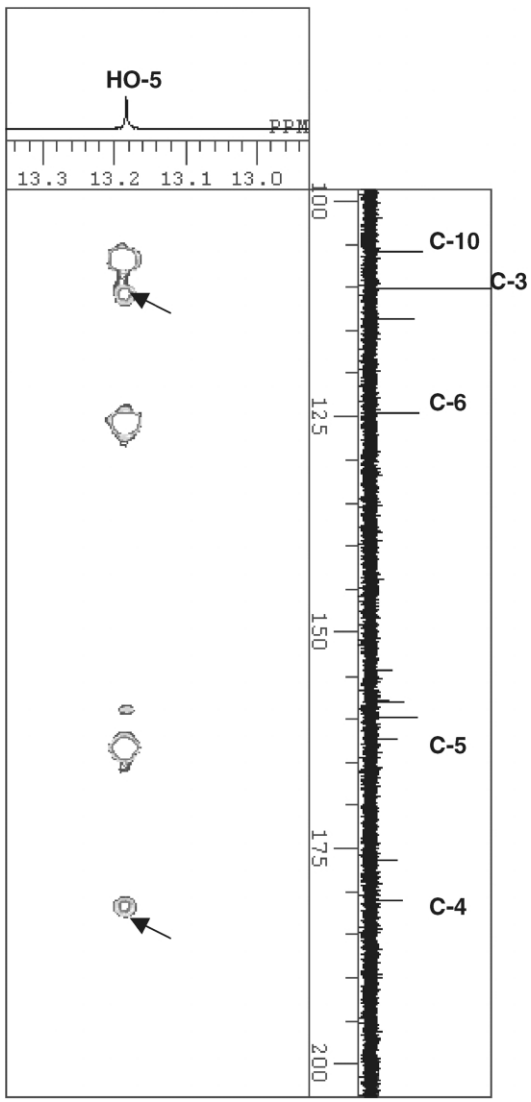

Figure 1. HMBC correlations observed from HO-5 to C-4 and C-3. Two arrows show the HMBC correlations from HO-5 to C-4 and C-3.

1. Experimental

1.1. General 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. HR-

FAB-MS were acquired on a JMS HX-110 mass spectrometer.

1.2. Material organism

The fruit bodies of *Cribraria purpurea* were collected at Mt. Shiraga, Monobe-mura in Kochi Prefecture, Japan, in November 2001. A voucher specimen (#22101) is maintained by Y. Y. at Kochi Kita Highschool.

1.3. Extraction and isolation

The air-dried fruit bodies of *Cribraria purpurea* (0.85 g) were extracted with 90% MeOH (30 mL×2 and 150 mL) and 90% acetone (150 mL×1). The combined MeOH and acetone extract (0.12 g), which contained purple pigments, was subjected to silica gel column chromatography (column A; 2.0×17 cm) eluted with 0–100% $CHCl_3$ in acetone. The fraction (10 mg) of column A eluted with 100% $CHCl_3$ was further separated by gel filtration with Sephadex LH-20 twice (column B, 1.0×47 cm; column C, 1.0×50 cm) eluted with 100% MeOH to give cribrarione A (**1**, 7.0 mg).

1.3.1. Cribrarione A (1). Brown–red solid; dextrorotatory; CD (MeOH) λ_{ext} 214 ($\Delta\varepsilon$ −3.1), 231 (+5.4), 276 (+0.76), and 315 nm (−1.5); IR (film) ν_{max} 3450, 1620, 1540, and 1460 cm^{-1}; UV λ_{max} (MeOH) 275 (ε 9700), 316 (8700), and 510 nm (8100); UV λ_{max} (MeOH+NaOH) 310 (ε 7800), 537 (ε 17000) and 568 (16000) nm; ^1H and ^{13}C NMR (Table 1); EIMS *m/z* 278 (M^+) and 260; HRFABMS *m/z* 279.0494 [calcd for $C_{13}H_{11}O_7$, (M+H) 279.0505].

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