Dihydrolindbladiones, Three New Naphthoquinone Pigments from a Myxomycete *Lindbladia tubulina*

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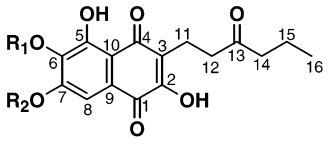
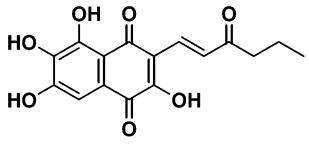
Three new naphthoquinone pigments, 6,7-dimethoxydihydrolindbladione (**1**), dihydrolindbladione (**2**), and 6-methoxydihydrolindbladione (**3**), have been isolated from a myxomycete *Lindbladia tubulina*, and their structures were elucidated by spectral data. Compound **3** appreciably exhibited a reversal effect of multidrug resistance. Lindbladione (**4**), the major pigment of this myxomycete, was also isolated from *Cribraria intricata*.

The myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryote. During our studies on the search for new bioactive substances from the myxomycetes,[1] we recently isolated three naphthoquinone pigments, lindbladione (**4**) and its related compounds, from a field-collected sample of fruiting bodies of *Lindbladia tubulina* collected at Kochi prefecture.[2] We further investigated the less polar fraction of the extract of this myxomycete, and here we describe isolation and structure elucidation of three new naphthoquinone pigments, 6,7-dimethoxydihydrolindbladione (**1**), dihydrolindbladione (**2**), and 6-methoxydihydrolindbladione (**3**). We also examined the extract of field-collected fruiting bodies of another myxomycete, *Cribraria intricata*, and isolated lindbladione (**4**), the major pigment of *L. tubulina*.[2,3]



1 R_1=R_2= Me
2 R_1=R_2= H
3 R_1=Me, R_2= H



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The fruiting bodies of *L. tubulina*, collected in Kochi Prefecture, Japan, were extracted with MeOH. The MeOH extract was partitioned between EtOAc and water, and the EtOAc-soluble fraction was then subjected to chromatographies on silica gel and Sephadex LH-20 to give three pigments (**1**−**3**).

6,7-Dimethoxydihydrolindbladione (**1**), which was the major pigment of the EtOAc-soluble fraction, was obtained as a red solid and shown to have the molecular formula $C_{18}H_{20}O_7$ by the HRFABMS data (*m*/*z* 349.1307, $[M + H]^+$, Δ +2.0 mmu). The UV spectrum of **1** showed absorption maxima at 264 and 324 nm, which were shifted to 275 and 331 nm, respectively, on addition of alkali (NaOH), indicating the presence of phenol group(s). The ^{13}C NMR spectrum of **1** (Table 1) showed signals for three carbonyls (δ_C 210.3, 190.1, and 179.8), eight sp^2 olefinic or aromatic carbons, two methoxy groups (δ_C 61.1 and 56.5), and five other sp^3 carbons (δ_C 44.4, 40.1, 17.3, 17.2, and 13.7). The ^1H NMR spectrum of **1** showed signals due to one aromatic proton (δ_H 7.26, 1H, s), two methoxy groups (δ_H 4.00 and 3.98, each 3H, s), one ethylene (CH_2CH_2) unit (δ_H 2.75 and 2.70, each 2H, t, J = 7.5 Hz), and one *n*-propyl group [δ_H 2.45 (2H, t, J = 7.2 Hz), 1.61 (2H, m), 0.91 (3H, t, J = 7.5 Hz)]. The $^1H-^1H$ COSY spectrum of **1** revealed cross-peaks (from H_2-11 to H_2-12, from H_2-14 to H_2-15, and from H_2-15 to H_3-16) confirming the presence of the ethylene and *n*-propyl groups. Since seven out of nine unsaturation degrees were accounted for from ^{13}C NMR data, **1** was inferred to have two rings. The presence of an *n*-propyl group was further corroborated by HMBC correlations (from H_3-16 to C-15 and C-14, from H_2-15 to C-16 and C-14, and from H_2-14 to C-16 and C-15), and this *n*-propyl group was shown to be

Table 1. ^1H and ^{13}C NMR Data of Compounds **1**−**3**

	1 (in $CDCl_3$)		**2** (in CD_3OD)		**3** (in $CDCl_3$)	
	δ_H/Hz	δ_C	δ_H/Hz	δ_C	δ_H/Hz	δ_C
1		179.8		180.9		179.6
2		153.8		157.2[c]		155.0
3		122.1		122.1		122.1
4		190.1		191.7		190.2
5		155.7		157.9[c]		153.3
6		143.2		141.1		140.1
7		156.7		151.9		153.8
8	7.26 s	104.2	7.11 s	109.6	7.28 s	108.0
9		124.6		123.1		125.1
10		110.5		110.3		110.0
11	2.75[a] t 7.5	17.3	2.74[a] t 7.0	18.3	2.82[a] t 7.5	17.3
12	2.70[a] t 7.5	40.1	2.65[a] t 7.0	41.8	2.70[a] t 7.5	40.6
13		210.3		213.3		210.2
14	2.45[a] t 7.2	44.4	2.48[a] t 7.2	45.2	2.44[a] t 7.5	44.5
15	1.61[a] m	17.2	1.59[a] m	18.2	1.63[a] m	17.2
16	0.91[b] t 7.5	13.7	0.92[b] t 7.5	14.0	0.93[b] t 7.5	13.7
CH_3O-6	4.00[b] s	61.1			4.13[b] s	61.2
CH_3O-7	3.98[b] s	56.5				
HO-5	12.57 s				12.81 s	

[a] 2H. [b] 3H. [c] Interchangeable signals.

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attached on the carbonyl group resonating at δ_C 210.3 (C-13) by additional HMBC correlations (from H_2-14 to C-13 and from H_2-15 to C-13). In the HMBC spectrum of **1**, the C-11 methylene protons of the ethylene unit at δ_H 2.75 (H_2-11) showed long-range connectivities with two carbonyl carbons at δ_C 210.3 (C-13) and 190.1 (C-4), with sp^2 carbons at δ_C 153.8 (C-2) and 122.1 (C-3), and with an sp^3 methylene carbon at δ_C 40.1 (C-12), while the other methylene protons at δ_H 2.70 (H_2-12) showed correlations with the carbonyl carbon at δ_C 210.3 (C-13) and an sp^3 methylene carbon at δ_C 17.3 (C-11). From these observations, a 3-oxohexyl group was inferred to be attached on the sp^2 carbon resonating at δ_C 122.1 (C-3). The aromatic proton at δ_H 7.26 (H-8) showed HMBC correlations to the carbonyl carbon at δ_C 179.8 (C-1) and sp^2 carbons at δ_C 143.2 (C-6), 156.7 (C-7), 124.6 (C-9), and 110.5 (C-10). By interpreting these spectral data, a [1,4]naphthoquinone nucleus with hydroxy or methoxy groups at C-2, C-5, C-6, and C-7 positions was constructed for compound **1**. A hydroxy proton resonating at δ_H 12.57 showed HMBC correlations with C-5 (δ_C 155.7), C-6 (δ_C 143.2), and C-10 (δ_C 110.5), suggesting this hydroxy group was on C-5. The methoxy protons at δ_H 4.00 and 3.98 showed HMBC correlations to C-6 (δ_C 143.2) and C-7 (δ_C 156.7), respectively, both of which in turn showed HMBC cross-peaks with H-8 (δ_H 7.26), implying that two methoxy groups were attached at the C-6 and C-7 positions. The remaining hydroxy group, therefore, had to be located on C-2 by a process of elimination, and this assignment was consistent with the ^{13}C chemical shift of C-2 (δ_C 153.8). There was no possibility of inverse location of substituents on C-2 and C-3 on the basis of observation of the HMBC correlations (^3J) from H-8 to the C-1 carbonyl group and from H_2-11 to the C-4 carbonyl group. Thus, the structure of compound **1** was concluded as 2,5-dihydroxy-6,7-dimethoxy-3-(3-oxohexyl)-[1,4]naphthoquinone. This structure corresponds to the 11,12-dihydro-6,7-di-*O*-methyl derivative of lindbladione (**4**).[2,3]

The molecular formula of compound **2** was suggested as $C_{16}H_{16}O_7$ from the HRFABMS data (*m/z* 321.0999, $[M + H]^+$, Δ +2.5 mmu), corresponding to the loss of two CH_2 (C_2H_4) groups from compound **1**. The ^1H and ^{13}C NMR data of **2** were almost parallel to those of compound **1**, except for the absence of signals due to two methoxy groups. The spectral data of **2** were also similar to those of lindbladione (**4**) previously isolated from a water-soluble fraction of the extract of *L. tubulina*.[2,3] ^1H and ^{13}C NMR spectra of compound **2** showed signals due to an ethylene unit instead of the signals due to an *E*-olefin observed for lindbladione (**4**). The HMBC spectrum of **2** revealed that the ethylene unit was located at the C-11−C-12 position (H_2-11 to C-3 and C-12; H_2-12 to C-3, C-11, and C-13). From these findings, compound **2** was deduced to be 11,12-dihydrolindbladione.

Compound **3** had a molecular formula of $C_{17}H_{18}O_7$ from the HRFABMS data (*m/z* 335.1130, $[M + H]^+$, Δ −0.1 mmu). In the ^1H and ^{13}C NMR spectra of **3** signals due to one methoxy group (δ_H 4.13, 3H, s; δ_C 61.2) were observed. This methoxy group was located at the C-6 position, which was suggested by the HMBC correlations from the methoxy protons (δ_H 4.13) to C-6 (δ_C 140.1). The C-6 carbon showed HMBC correlation with H-8 (δ_H 7.28), and the higher-field resonance of C-6 (δ_C 140.1) than the other two phenol-bearing carbons (C-5: δ_C 153.3; C-7: δ_C 153.8) implied that this methoxy-bearing benzene-ring carbon (C-6) was located between two hydroxy-bearing benzene-ring carbons (C-5 and C-7).[4] In addition, a hydroxy proton resonating at δ_H 12.81 showed HMBC correlations with C-5 (δ_C 153.3), C-6 (δ_C 140.1), and C-10 (δ_C 110.0), suggesting this hydroxy group was on C-5 and the methoxy group was located on C-6. Compound **3** was therefore revealed as 6-*O*-methyl-11,12-dihydrolindbladione.

Table 2. Cytotoxic Activity (IC_{50} values, μg/mL) of Compounds **1**−**3**[a]

compound	P388/S	P388/VCR(−)	P388/VCR(+)
1	>50	>50	>50
2	12.1	14.0	12.6
3	>50	>50	13.8

[a] P388/VCR is a vincristine-resistant P388 cell line, while P388/S is a sensitive P388 cell line. Tests toward P388/VCR cell lines were carried out in the absence (−) and presence (+) of 0.004 μg/mL of VCR, which did not affect the growth of the cells.

Herein we report the first three dihydrolindbladione derivatives (**1**−**3**) from fruiting bodies of a wild-type strain of *L. tubulina*. The cytotoxic activities of these compounds against murine leukemia P388 cells were examined, and the IC_{50} values (μg/mL) against vincristine (VCR)-resistant P388 cells (P388/VCR) as well as those against a sensitive P388 strain (P388/S) are presented in Table 2. Compound **2** was cytotoxic against all P388 cell lines, while compound **3** appreciably showed a reversal effect of multidrug resistance,[5] since it was inactive against P388/S and P388/VCR cells in the absence of VCR but was active against P388/VCR cells in the presence of VCR.

We recently also examined the pigments contained in the fruiting bodies of *Cribraria intricata*, collected in Kochi Prefecture, Japan, and the extract of this myxomycete was subjected to chromatography using ODS eluted with acetonitrile and water to afford lindbladione (**4**) as a major pigment constituent (0.3% yield). Myxomycetes, *L. tubulina* and *C. intricata*, belong to different genera but to the same family, Cribrariaceae. It may be possible that lindbladione (**4**) is one of the common red pigments in myxomycetes of the family Cribrariaceae.[6]

Experimental Section

General Procedures. UV spectra were obtained on a Hitachi U-3400 spectrometer. NMR spectra were recorded on JEOL JNM ecp600 spectrometers. HRFABMS were acquired on a JMS HX-110 mass spectrometer.

Organism. The fruiting bodies of *Lindbladia tubulina* were collected at Takamagahara, Ohtsu, Kochi-shi in Kochi Prefecture, Japan, in August 2001. The fruiting bodies of *Cribraria intricata* were also collected at Takamagahara, Ohtsu, Kochi-shi in Kochi Prefecture, Japan, in August 2001. Voucher specimens (#37120 for *L. tubulina*, and #21516 and 21517 for *C. intricata*) are maintained by Y.Y. at Kochi Kita Highschool.

Extraction and Isolation. The air-dried fruiting bodies of *L. tubulina* (7.21 g) were extracted with 90% MeOH (200 mL × 2) and 90% acetone (200 mL × 1). The combined MeOH and acetone extract (1.66 g) was partitioned between EtOAc (150 mL × 3) and water (150 mL). The EtOAc-soluble fraction was evaporated under reduced pressure to give a residue (343 mg), which was subjected to silica gel column chromatography (column A; 2.6 × 22 cm) eluted with 50−100% EtOAc in hexane. A fraction (15.2 mg) of column A eluted with 50% EtOAc/hexane was further separated on a silica gel column (column B; 1.2 × 23 cm) eluted with 1−100% MeOH/$CHCl_3$ to give 6,7-dimethoxydihydrolindbladione (**1**, 9.8 mg) in the fraction of the 30−55 mL elution of column B. The fraction (3.5 mg) of the 55−115 mL elution of column B was further purified on a silica gel column (column C; 1.0 × 28 cm) eluted with 5% MeOH/$CHCl_3$ to afford 6-methoxydihydrolindbladione (**3**, 1.7 mg). Another fraction (33.8 mg) of column A eluted with 66% EtOAc/hexane was further separated on a Sephadex LH-20 column (column D; 1.0 × 35 cm) eluted with 100% MeOH

to give dihydrolindbladione (**2**, 1.9 mg) in the fraction of the 22−27 mL elution.

6,7-Dimethoxydihydrolindbladione (1): red solid; UV λ_{max} (MeOH) 264 (ϵ 23 000), 324 (12 000), and 403 (12 000) nm; ^{1}H and ^{13}C NMR (Table 1); FABMS *m*/*z* 349 $(M + H)^+$; HRFABMS *m*/*z* 349.1307 [calcd for $C_{18}H_{21}O_7$, (M + H) 349.1287].

Dihydrolindbladione (2): brown-red solid; UV λ_{max} (MeOH) 273 (ϵ 21 000), 325 (11 000), and 419 (5100) nm; ^{1}H and ^{13}C NMR (Table 1); FABMS *m*/*z* 321 $(M + H)^+$; HRFABMS *m*/*z* 321.0999 [calcd for $C_{16}H_{17}O_7$, (M + H) 321.0974].

6-Methoxydihydrolindbladione (3): red solid; UV λ_{max} (MeOH) 269 (ϵ 27 000), 324 (13 000), and 389 (6000) nm; ^{1}H and ^{13}C NMR (Table 1); FABMS *m*/*z* 335 $(M + H)^+$; HRFABMS *m*/*z* 335.1130 [calcd for $C_{17}H_{19}O_7$, (M + H) 335.1131].

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