Lindbladione and Related Naphthoquinone Pigments from a Myxomycete *Lindbladia tubulina*

Yae ISHIKAWA,[a] Masami ISHIBASHI,*[,a] Yukinori YAMAMOTO,[b] Masahiko HAYASHI,[c] and Kanki KOMIYAMA[c]

[a] *Graduate School of Pharmaceutical Sciences, Chiba University; Chiba 263–8522, Japan:* [b] *Kochi Kita High School; 160 Higashiishidate, Kochi 780–8039, Japan: and* [c] *The Kitasato Institute; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8642, Japan.* Received March 12, 2002; accepted May 8, 2002

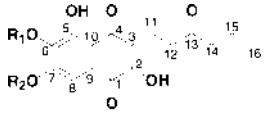
Lindbladione (1), 7-methoxylindbladione (2), and 6,7-dimethoxylindbladione (3) have been isolated from a myxomycete *Lindbladia tubulina* and their structures were elucidated by spectral data.

Key words myxomycete; naphthoquinone; pigment

The Myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryotes. As their fruit bodies are very small and it is very difficult to collect much quantity of slime molds, chemical studies on the secondary metabolites of the myxomycetes to date have been limited. Early explorative studies carried out by Steglich[1)] and Asakawa's[2)] groups have demonstrated that myxomycetes have developed a rather unique secondary metabolism. During our studies on the search for natural products from myxomycetes,[3)] we recently investigated a field-collected sample of fruit bodies of *Lindbladia tubulina*. Here we describe the isolation and structure elucidation of three naphtoquinone pigments, lindbladione (**1**), 7-methoxylindbladione (**2**), and 6,7-dimethoxylindbladione (**3**). The name and structure of lindbladione (**1**) were found in a symposium proceedings by Steglich's group,[1)] but a SciFinder® search found no description of experimental details or spectral data of compound **1** in the literature. This is the first report on full characterizations of compound **1** and isolation of two new compounds **2** and **3**.

The fruit bodies of *Lindbladia tubulina*, collected in Kochi Prefecture, Japan, were extracted with MeOH. The MeOH extract was partitioned between EtOAc and water, and the water-soluble fraction containing pigments was then subjected to chromatography on ODS and/or Sephadex LH-20, followed by further purification with HPLC on ODS to give three pigments (**1**—**3**).

Lindbladione (**1**) was obtained as a dark brown solid, and shown to have the molecular formula $C_{16}H_{14}O_7$ by the high resolution (HR)-FAB-MS data (*m*/*z* 319.0816, $[M+H]^+$, Δ −0.1 mmu). The UV spectrum of **1** showed absorption maxima at 265 and 367 nm, which were shifted to 271 and 379 nm, respectively, with the 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 206.4, 189.6, 184.1), ten sp^2 olefinic or aromatic carbons, and three sp^3 ones (δ_C 42.4, 20.2, 14.3). The ^{1}H-NMR spectrum of **1** in CD_3OD showed only six signals due to one aromatic proton (δ_H 6.97, 1H, s), one *E*-olefin (δ_H 8.07, 7.45, each 1H, d, J=16.0 Hz), and one *n*-propyl group [δ_H 2.53 (2H, t, J=7.6 Hz), 1.60 (2H, m), 0.90 (3H, t, J=7.6 Hz)]. The presence of the *n*-propyl group was confirmed by its ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum. Since eight out of ten unsaturation degrees were accounted for by ^{13}C-NMR data, **1** was inferred to have two rings. The presence of the *n*-propyl group was further corroborated by the heteronuclear multiple bond connectivity (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 attached to the carbonyl group resonating at δ_C 206.4 (C-13) by the HMBC correlations (from H_2-14 to C-13 and from H_2-15 to C-13). In the HMBC spectrum of **1**, one olefinic proton at δ_H 8.07 (H-11) showed long-range connectivities with two carbonyl carbons at δ_C 206.4 (C-13) and 189.6 (C-4) and also with sp^2 carbons at δ_C 176.4 (C-2) and 122.5 (C-12), while another olefinic proton at δ_H 7.45 (H-12) showed correlations with the carbonyl carbon at δ_C 206.4 (C-13) and the sp^2 carbon at δ_C 113.3 (C-3). From these observations, a 3-oxo-hex-1-enyl group was inferred to be at-



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

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1**—**3** (in CD_3OD)

	1		**2**		**3**	
	δ_H/Hz	δ_C	δ_H/Hz	δ_C	δ_H/Hz	δ_C
1		184.1		183.9		184.3
2		176.4		176.4		175.6
3		113.3		113.4		113.6
4		189.6		189.4		189.3
5		151.6		151.2		156.5
6		141.5		143.2		143.7
7		149.5		151.3		157.0
8	6.97 s	108.6	7.14 s	104.4	7.12 s	103.8
9		125.0		124.5		129.0
10		112.2		113.7		113.8
11	8.07 d 16.0	140.6	8.04 d 16.0	140.4	8.03 d 16.0	140.1
12	7.45 d 16.0	122.5	7.34 d 16.0	122.7	7.44 d 16.0	123.1
13		206.4		206.4		206.3
14	2.53[a)] t 7.6	42.4	2.52[a)] t 7.3	42.5	2.52[a)] t 7.4	42.5
15	1.60[a)] m	20.2	1.58[a)] m	20.1	1.58[a)] m	20.1
16	0.90[b)] t 7.6	14.3	0.88[b)] t 7.3	14.3	0.88[b)] t 7.4	14.3
CH_3O-6					3.81[b)] s	61.1
CH_3O-7			3.84[b)] s	56.6	3.84[b)] s	56.6

a) 2H. *b*) 3H.

* To whom correspondence should be addressed. e-mail: mish@p.chiba-u.ac.jp

tached to the sp^2 carbon resonating at δ_C 113.3 (C-3). The aromatic proton at δ_H 6.97 (H-8) showed HMBC correlations to carbonyl carbon at δ_C 184.1 (C-1) and sp^2 carbons at δ_C 141.5 (C-6), 149.5 (C-7), 125.0 (C-9), and 112.2 (C-10). By interpreting these spectral data, a naphtoquinone nucleus with four hydroxyl groups at C-2, C-5, C-6, and C-7 was constructed for compound **1**. 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-11 to the C-4 carbonyl group. Thus, the structure of lindbladione was concluded to be 2,5,6,7-tetrahydroxy-3-(3-oxo-hex-1-enyl)-[1,4]naphthoquinone (**1**).

The molecular formula of compound **2** was suggested to be $C_{17}H_{17}O_7$ from the HR-FAB-MS data (*m/z* 333.0961, $[M+H]^+$, Δ −1.4 mmu). The ^{1}H- and ^{13}C-NMR data of **2** were almost parallel to those of lindbladione (**1**), except for a signal due to a methoxy group (δ_H 3.84 and δ_C 56.6). The position of the methoxy group was revealed on C-7 from the HMBC correlation from H_3CO (δ_H 3.84) to C-7 (δ_C 151.3) as well as the NOE observations between H_3CO (δ_H 3.84) and H-8 (δ_H 7.14). The H-8 of **2** (δ_H 7.14) resonated in the lower-field, compared with that of **1** (δ_H 6.97). From these findings, compound **2** was deduced to be 7-*O*-methyllindbladione. Compound **3** had a molecular formula of $C_{18}H_{19}O_7$ from the HR-FAB-MS data (*m/z* 347.1149, $[M+H]^+$, Δ +1.8 mmu). The ^{1}H- and ^{13}C-NMR data of **3** showed signals due to two methoxy groups (δ_H 3.84, δ_C 56.6; δ_H 3.81, δ_C 61.1). These two groups were located on C-6 and C-7, which was suggested by the following HMBC correlations: from one methoxy proton (δ_H 3.84) to C-7 (δ_C 157.0); from the other methoxy proton (δ_H 3.81) to C-6 (δ_C 143.7). The C-6 and C-7 carbons showed HMBC correlations with H-8 (δ_H 7.12). The former methoxy proton at δ_H 3.84 showed NOE with H-8 (δ_H 7.12), thus assigning this methoxy proton (δ_H 3.84) on C-7. Compound **3** was therefore shown to be 6,7-di-*O*-methyllindbladione.

Several pigments naphthoquinone derivatives in myxomycetes were previously reported,[4,5] and Steglich[1] said that lindbladione (**1**) was responsible for the color change from dark to red on treatment of the plasmodia of *Lindbladia tubulina* with mineral acid. However, no spectral data of **1** (except for UV data[6]) could be found in the literature. Here we isolated lindbladione (**1**) and its methoxy derivatives (**2**, **3**) from wild fruit bodies of *L. tubulina*. The cytotoxic activity of compound **1** against murine leukemia P388 cells was examined, but it proved to be inactive at 25 μg/ml. It might be possible that these naphtoquinones are derived from polyketides, but no biosynthetic proof has been provided experimentally.

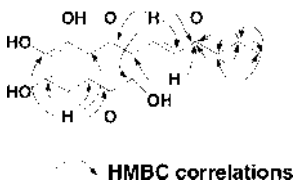


Fig. 1. HMBC Correlations Observed for Compound **1**

Experimental

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 was acquired on a JMS HX-110 mass spectrometer.

Extraction and Isolation The air-dried fruit bodies of *Lindbladia 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 contained no pigments. The water-soluble fraction was, without evaporation, subjected to ODS column chromatography (column A; 2.4×13 cm) and eluted with 0—100% MeOH/H_2O. A part (274 mg) of the fraction (499 mg) of column A eluted with 100% H_2O was further separated by gel filtration with Sephadex LH-20 (column B; 2.0×23 cm) eluted with MeOH/H_2O (1 : 2) to give lindbladione (**1**, 66 mg) in the fraction of the 200—270 ml elution. The fraction (8.1 mg) of the 70—90 ml elution of column B was further purified by HPLC on ODS (Develosil ODS HG-5, 10×250 mm; eluent, 40% MeOH; detection, UV at 367 nm; flow rate, 1.8 ml/min) to give compound **2** (1.9 mg). The fraction (2.0 mg) of column A eluting with MeOH/H_2O (1 : 4) was further separated with HPLC on ODS (Develosil ODS HG-5, 10×250 mm; eluent, 50% MeOH; detection, UV at 367 nm; flow rate, 1.8 ml/min) to afford compound **3** (1.2 mg).

Lindbladione (**1**): Brown-red solid; IR (film) ν_{max} 3300, 1620, 1540, and 1360 cm^{-1}; UV λ_{max} (MeOH) 265 (ε 13000) and 367 (29000) nm; UV λ_{max} (MeOH+NaOH) 271 (ε 12000) and 379 (28000) nm; ^{1}H- and ^{13}C-NMR (Table 1); FAB-MS *m/z* 319 $(M+H)^+$ and 357 $(M+K)^+$; HR-FAB-MS *m/z* 319.0816 [Calcd for $C_{16}H_{15}O_7$, (M+H) 319.0817].

7-*O*-Methyllindbladione (**2**): Brown-red solid; IR (film) ν_{max} 3400, 1620, 1540, and 1350 cm^{-1}; UV λ_{max} (MeOH) 264 (ε 19000) and 367 (34000) nm; ^{1}H- and ^{13}C-NMR (Table 1); FAB-MS *m/z* 333 $(M+H)^+$, 355 $(M+Na)^+$, and 371 $(M+K)^+$; HR-FAB-MS *m/z* 333.0961 [Calcd for $C_{17}H_{17}O_7$, (M+H) 333.0975].

6,7-Di-*O*-methyllindbladione (**3**): Brown-red solid; IR (film) ν_{max} 3540, 1600, 1540, and 1360 cm^{-1}; UV λ_{max} (MeOH) 266 (ε 11000) and 365 (21000) nm; ^{1}H- and ^{13}C-NMR (Table 1); FAB-MS *m/z* 347 $(M+H)^+$ and 385 $(M+K)^+$; HR-FAB-MS *m/z* 347.1149 [Calcd for $C_{17}H_{17}O_7$, (M+H) 347.1131].

Acknowledgments This work was partly supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by a Grant-in-Aid from the Uehara Memorial Foundation and the Japan Securities Scholarship Foundation.

References

1) Steglich W., *Pure Appl. Chem.*, **61**, 281—288 (1989).
2) Hashimoto T., Yasuda A., Akazawa K., Takaoka S., Tori M., Asakawa Y., *Tetrahedron Lett.*, **35**, 2559—2560 (1994).
3) Ishibashi M., Iwasaki T., Imai S., Sakamoto S., Yamaguchi K., Ito A., *J. Nat. Prod.*, **64**, 108—110 (2001).
4) Kopanski L., Li G.-R., Besl H., Steglich W., *Liebigs Ann. Chem.*, **1982**, 1722—1729 (1982).
5) Kopanski L., Darbach D., Selbitschka G., Steglich W., *Liebigs Ann. Chem.*, **1982**, 1722—1729 (1982).
6) Steglich W., "Progr. Chem. Org. Nat. Prod.," Vol. 51, ed. by Herz W., Grisebach H., Kirby G. W., Tamm Ch., Springer-Verlag, Wien, 1987, pp. 172—173.