Bisindole Alkaloids from Myxomycetes *Arcyria denudata* and *Arcyria obvelata*

Kazuaki Kamata,[†] Tomoko Suetsugu,[†] Yukinori Yamamoto,[‡] Masahiko Hayashi,[§] Kanki Komiyama,[§] and Masami Ishibashi*,[†]

Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 263-8522, Japan, Ohtsu-ko, Kochi 781-5102, Japan, and The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan

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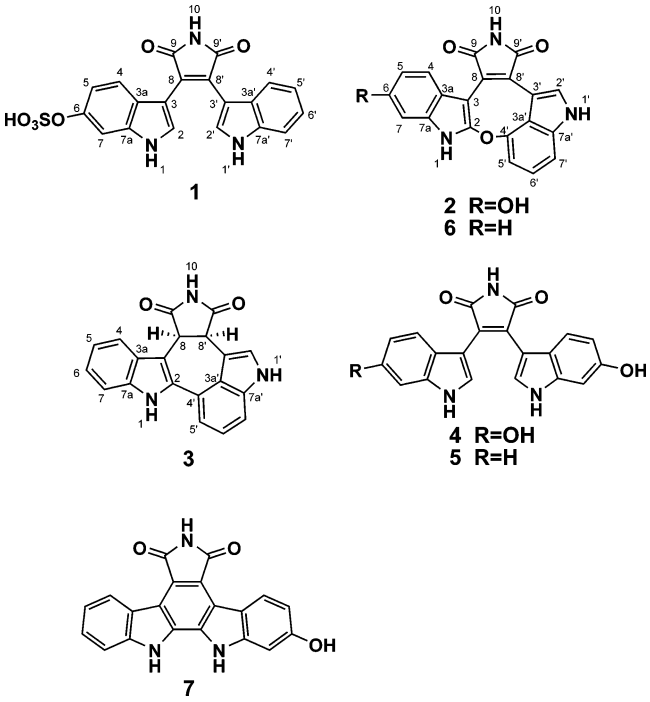
A new bisindole sulfate (**1**) and arcyroxocin B (**2**) were isolated from wild fruiting bodies of *Arcyria denudate*, along with three known bisindoles (**4**−**6**). Dihydroarcyriacyanin A (**3**) was obtained from wild fruiting bodies of *Arcyria obvelata*, along with a known bisindole (**7**). The structures of these compounds were elucidated on the basis of spectroscopic data, and this is the first report of full characterizations of arcyroxocin B (**2**) and dihydroarcyriacyanin A (**3**). Compounds **2**, **3**, and **6** showed cytotoxicity against Jurkat cells.

Considerable attention has been focused on bisindole alkaloids with a bisindolylmaleimide core such as staurosporine,[1] UCN-01,[2] and rebeccamycin,[3] which are produced by various *Streptomyces*, *Actinomycetes*, and *Saccharothrixes*. These metabolites are reported to cause topoisomerase I mediated DNA cleavage, potent inhibition of protein kinase C and cell-cycle-regulating cyclin-dependent kinase (CDK), and cell-cycle checkpoint inhibition.[4] Bisindole alkaloids have also been isolated from myxomycetes;[5,6] the myxomycetes (true slime molds) are an unusual group of primitive organisms that may be assigned to one of the lowest classes of eukaryotes. During our search for bioactive natural products from myxomycetes,[7] we recently investigated field-collected samples of fruiting bodies of *Arcyria denudata* and *Arcyria obvelata* collected at Kochi Prefecture and obtained several bisindole alkaloids. Here we describe the isolation and structure elucidation of a new sulfated bisindole (**1**) together with the known bisindoles **2**−**7**. The names and structures of arcyroxocin B (**2**) and dihydroarcyriacyanin A (**3**) were found in symposium proceedings,[8] but no literature on **2** or **3** was found by a SciFinder search, and their spectral data are not available in the literature. This report is the first full characterization of compounds **2** and **3**. These bisindole alkaloids were tested for cytotoxicity against Jurkat cells.

The fruiting bodies of *A. denudata*, collected in Kochi Prefecture, Japan, were extracted with 90% MeOH and 90% acetone. The combined extracts were subjected to silica gel chromatography, followed by fractionations on a Sephadex LH-20 column and by ODS HPLC to give bisindoles **1**, **2**, and **4**−**6**. Three of these alkaloids were identified as arcyriarubin C (**4**),[9] arcyriarubin B (**5**),[9] and arcyroxocin A (**6**)[10] on the basis of comparison of physical constants and spectroscopic data, while compound **1** was revealed to be a new compound and compound **2** was found to be an uncharacterized compound.

Compound **1** was shown to have a molecular formula of $C_{20}H_{13}O_6N_3S$ by the negative HRFABMS data (*m*/*z* 422.0459, [M − H]$^-$, Δ +1.2 mmu). Its UV spectrum showed absorption maxima at 374 and 278 nm, suggesting the presence of conjugated or aromatic system(s). The ^{1}H NMR spectrum of **1** in acetone-d_6 (Table 1) showed proton signals for nine aromatic protons and three broad singlet signals due to OH or NH (δ_H 10.80, 10.60, and 9.57). Analysis of the ^{13}C NMR (Table 1) and HMQC spectra of **1** suggested the presence of 20 carbons including nine sp^2 methines, nine sp^2 quaternary carbons, and two carbonyl carbons. Comparison of the ^{1}H and ^{13}C NMR spectral data of **1** with those of arcyriarubin B (**5**) implied that they had the same bisindole backbone skeleton



with a maleimide moiety. The molecular weight of **1** was 80 amu higher than that of **5** (*m*/*z* 422 [M − H]$^-$ for **1** in FABMS and *m*/*z* 343 [M$^+$] for **5** in EIMS); 80 amu corresponded to one sulfur and three oxygen atoms, thus implying that **1** contained a sulfate ester group attached to the bisindole nucleus of **5**. The ^{1}H−^{1}H COSY spectrum showed connectivity between the aromatic protons H-4 and H-5 and also showed the presence of four consecutive aromatic protons (H-4′, H-5′, H-6′, and H-7′). H-5 showed *meta*-coupling (J = 2.1 Hz) with an aromatic proton resonating at δ_H 7.48, which was assignable to H-7. This observation suggested that the sulfate ester group was attached at C-6, which was consistent with the low-field resonance of C-6 (δ_C 151.1). These assignments were also suggested by the HMBC correlations observed for H-4/C-6, H-4/C-7a, H-5/C-3a, H-5/C-7, H-7/C-5, H-7/C-6, and H-7/C-3a. From these results, the structure of **1** was revealed as arcyriarubin B 6-*O*-sulfate. Sulfate ester compounds such as **1** have not been reported previously from myxomycetes.

Compound **2** had the molecular formula $C_{20}H_{11}N_3O_4$ as shown by the HRFABMS data (*m*/*z* 357.0745, [M$^+$], Δ −0.5 mmu). The ^{1}H NMR spectrum of **2** in acetone-d_6 (Table 1) showed seven aromatic proton signals. The analysis of the ^{13}C NMR (Table 1) and HMQC spectra of **2** suggested the presence of 20 carbons, including seven sp^2 methines, 11 sp^2 quaternary carbons, three of

* To whom correspondence should be addressed. Tel & Fax: +81-43-290-2913. E-mail: mish@p.chiba-u.ac.jp.
† Chiba University.
‡ Ohtsu-ko, Kochi.
§ Kitasato Institute.

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**3** in Acetone-d_6

	1			**2**			**3**		
position	δ_H (J, Hz)		δ_C	δ_H (J, Hz)		δ_C	δ_H (J, Hz)		δ_C
1	10.80	br s					10.76	br s	
2	7.78	d 1.8	129.8			151.3			134.7
3			107.2[a]			94.2			106.5
3a			122.3			119.7			132.4
4	6.73	d 8.7	121.9	7.55	d 8.8	123.5	7.68	d 8.0	119.4
5	6.57	dd 8.7, 2.1	115.2	6.67	dd 8.8, 2.2	110.9	7.05	ddd 8.0, 6.9, 0.9	120.1
6			151.1			154.7	7.14	ddd 8.0, 6.9, 1.1	123.1
7	7.48	d 2.1	104.3	6.76	d 2.2	97.1	7.42	d 8.0	111.6
7a			137.3[b]			132.5			137.3
8			129.6			126.1[c]	5.32	d 7.5	44.7
9			173.3			172.7[d]			178.3
10	9.57	br s					9.88	br s	
1′	10.60	br s					10.67	br s	
2′	7.70	d 1.8	129.7	8.13	s	130.2	7.63	dd 2.7, 1.5	125.7
3′			107.5[a]			106.0			110.6
3a′			126.9			120.3			123.0
4′	7.15	d 8.0	122.5			152.5			124.6
5′	6.74	dd 8.0, 7.2	120.5	7.45	dd 7.5, 1.6	110.7	7.65	dd 7.7, 0.8	115.8
6′	7.01	dd 8.4, 7.2	122.7	7.18	t 7.5	123.6	7.21	t 7.7	122.7
7′	7.41	d 8.4	112.3	7.21	dd 7.5, 1.6	113.1	7.40	dd 7.7, 0.8	112.0
7a′			137.6[b]			139.9			137.9
8′			129.6			129.7[c]	4.58	dd 7.5, 1.5	45.6
9′			173.3			172.3[d]			178.7

[a−d]Signals may be reversed.

which were oxygenated (δ_C 154.7, 152.5, and 151.3), and two carbonyl carbons (δ_C 172.7 and 172.3) ascribable to a maleimide moiety. The ^{1}H−^{1}H COSY spectrum showed that compound **2** had two vicinal aromatic protons [δ_H 7.55 (H-4) and 6.67 (H-5)] and three other successive aromatic protons [δ_H 7.45 (H-5′), 7.18 (H-6′), and 7.21 (H-7′)]. H-5 (δ_H 6.67) and H-7 (δ_H 6.76) showed *meta*-coupling (J = 2.2 Hz) with each other. By analysis of the HMBC spectrum, compound **2** was suggested to contain an indole nucleus with a hydroxyl group on C-6 (δ_C 154.7) from the cross-peaks for H-4/C-3, H-4/C-6, H-4/C-7a, H-5/C-3a, H-5/C-7, H-7/C-3a, H-7/C-5, and H-7/C-6. Another indole moiety, oxygenated at C-4′ (δ_C 152.5), was apparent from the correlations for H-2′/C-3′, H-2′/C-3a′, H-2′/C-7a′, H-5′/C-3a′, H-5′/C-7′, H-6′/C-4′, H-6′/C-7a′, H-7′/C-3a, and H-7′/C-5′. Furthermore, compound **2** apparently had one more ring from consideration of unsaturation degrees. Thus, the remaining sp^2 carbon of low-resonance (δ_C 151.3) was assigned to C-2, forming an eight-membered ring through an ether linkage between C-2 and C-4′, a feature also found in arcyroxocin A (**6**).[10] Accordingly, the structure of **2** was concluded to be 6-hydroxyarcyroxocin A; this structure appears with the name arcyroxocin B in a symposium proceedings paper,[8] with no characterization or spectral data, and no literature on the structure **2** was found by a SciFinder search. This, therefore, represents the first full characterization of arcyroxocin B (**2**).

The wild fruiting bodies of *A. obvelata*, collected in Kochi Prefecture, Japan, were extracted with 90% MeOH and 90% acetone. The combined extracts were separated by chromatography on silica gel and ODS HPLC to give compound **3** and arcyriaflavin B (**7**).[9,11]

Compound **3** was shown to have the molecular formula $C_{20}H_{13}N_3O_2$ by the HRFABMS data (m/z 327.1003, $[M]^+$, Δ −0.5 mmu). The ^{1}H NMR spectrum of **3** in acetone-d_6 (Table 1) showed 13 signals for eight aromatic protons, two sp^3 methine protons, and three OH or NH signals (δ_H 10.76, 10.67, and 9.88). Analysis of the ^{13}C NMR (Table 1) and HMQC spectra of **3** revealed the presence of 20 carbons, including eight sp^2 methines, eight sp^2 quaternary carbons, two sp^3 methines (δ_C 45.6 and 44.7), and two carbonyl carbons (δ_C 178.7 and 178.3). Analysis of the ^{1}H−^{1}H COSY and HMBC spectra of compound **3** indicated that it possessed two sets of indole moieties from observation of the following cross-peaks: ^{1}H−^{1}H COSY: H-4/H-5, H-5/H-6, H-6/H-7, H-5′/H-6′, and H-6′/H-7′; HMBC: H-4/C-3, H-4/C-6, H-4/C-7a, H-5/C-3a, H-5/C-7, H-6/C-4, H-6/C-7a, H-7/C-3a, H-7/C-5, H-2′/C-3′, H-2′/C-3a′, H-2′/C-7a′, H-5′/C-3a′, H-5′/C-7′, H-6′/C-4′, H-6′/C-7a′, and H-7′/C-3a. Also indicated was a dihydromaleimide moiety from the HMBC correlations for H-8/C-9, H-8/C-8′, H-8′/C-8, and H-8′/C-9′. In addition, compound **3** was inferred to have one more ring from the degrees of unsaturation, and the presence of a seven-membered ring through a carbon−carbon linkage between C-2 and C-4′ was suggested from the HMBC correlations from H-5′ to C-2 and from H-8 to C-2. Thus, the structure of compound **3** corresponded to an 8,8′-dihydro derivative of arcyriacyanin A.[8,12] NOE correlations were observed between H-8 and H-8′, implying that these hydrogens were *syn*-configured. The structure of compound **3** appeared with the name dihydroarcyriacyanin A in the symposium proceedings paper[8] with no characterization or spectroscopic data, and no literature on the structure **3** was found by a SciFinder search. Thus, this is the first complete characterization of dihydroarcyriacyanin A (**3**).

Arcyroxocin B (**2**), dihydroarcyriacyanin A (**3**), and arcyriarubin B (**5**) exhibited cytotoxic activity against Jurkat cells[13] with IC_{50} values of 7, 7, and 10 μg/mL, respectively, while the new sulfate compound (**1**) was inactive (IC_{50}: >25 μg/mL).

Experimental Section

General Experimental Procedures. UV spectra were obtained on a Shimadzu UV mini-1240 spectrometer. IR spectra were measured on a Hitachi 260-10 infrared spectrophotometer. NMR spectra were recorded on a JEOL JNM ecp600 spectrometer. HRFABMS were acquired on a JMS HX-110 mass spectrometer.

Organism. The fruiting bodies of *Arciria denudata* and *Arciria obvelata* were collected in Kochi Prefecture, Japan, in May 2004 and in July 2004, respectively. Voucher specimens (*A. denudata*, #26149−26153; *A. obvelata*, #26359 and 26427) are maintained by Y.Y. (Ohtsuko, Kochi).

Extraction and Isolation. The air-dried fruiting bodies of *A. denudata* (1.8 g) were extracted with 90% MeOH (150 mL × 2) and 90% acetone (150 mL × 1) at room temperature. The combined extracts (0.3 g) were subjected to silica gel column chromatography (column A; 25 × 200 mm) eluted with $CHCl_3$, $CHCl_3$/MeOH (98:2, 95:5, 9:1, 8:2, 1:1), and MeOH. Arcyriarubin C (**4**, 20.9 mg) was obtained in a fraction from column A eluted with $CHCl_3$/MeOH (8:2). A fraction (27 mg) from column A eluted with $CHCl_3$/MeOH (9:1) was further separated by Sephadex LH-20 column chromatography (15 × 580 mm) eluted with MeOH to give arcyroxocin B (**2**, 10.8 mg) and arcyriarubin

B (**5**, 10.2 mg). A fraction (34 mg) eluted with $CHCl_3$/MeOH (1:1) was further purified using a Sephadex LH-20 column (20 × 600 mm) eluted with $CHCl_3$/MeOH (1:1) to give **1** (1.8 mg). A fraction (11 mg) eluted with $CHCl_3$/MeOH (95:5) was further purified on Sephadex LH-20 (column B; 15 × 580 mm) eluted with MeOH. A fraction (6 mg) from column B eluted with MeOH was purified by HPLC on ODS (Develosil ODS-HG-5, 10 × 250 mm; eluent, 60% MeOH; flow rate, 1.8 mL/min; UV detection at 254 nm) to give arcyroxocin A (**6**, 4.1 mg).

The air-dried fruiting bodies of *A. obvelata* (330 mg) were extracted with 90% MeOH (50 mL) and 90% acetone (50 mL). The combined MeOH and acetone extracts (54.7 mg) were subjected to silica gel column chromatography (column C; 15 × 175 mm) eluted with $CHCl_3$, $CHCl_3$/MeOH (98:2, 95:5, 9:1, 8:2, 1:1), and MeOH. Arcyriaflavin B (**7**, 6.1 mg) was obtained in a fraction eluted with $CHCl_3$/MeOH (9:1). The combined fractions (4 mg) from column C eluted with $CHCl_3$/MeOH (98:2 and 95:5) were further purified by HPLC on ODS (Mightysil RP-18 GP, 10 × 250 mm; eluent, 70% MeOH, flow rate, 1.8 mL/min; UV detection at 254 nm) to give dihydroarcyriacyanin A (**3**, 1.4 mg).

Arcyriarubin B 6-*O*-sulfate (1): amorphous powder; UV λ_{max} (MeOH) 374 (ϵ 15 000) and 278 (ϵ 37 000); IR (film) ν_{max} 3290, 1700, 1620, 1530, 1350, and 1240 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 422 $(M - H)^-$; HRFABMS *m*/*z* 422.0459 [calcd for $C_{20}H_{12}N_3O_6S$, $(M - H)^-$ 422.0447].

Arcyroxocin B (2): amorphous powder; UV λ_{max} (MeOH) 474 (ϵ 10 000) and 212 (ϵ 91 000); IR (film) ν_{max} 3270, 1750, 1700, 1620, 1580, and 1560 cm^{-1}; ^1H and ^{13}C NMR (Table 1); EIMS *m*/*z* 357 (M^+); HRFABMS *m*/*z* 357.0745 [calcd for $C_{20}H_{11}N_3O_4$, (M^+) 357.0750].

Dihydroarcyriacyanin A (3): amorphous powder; UV λ_{max} (MeOH) 363 (ϵ 32 000) and 344 (ϵ 38 000); IR (film) ν_{max} 3390, 1710, and 1340 cm^{-1}; ^1H and ^{13}C NMR (Table 1); FABMS *m*/*z* 327 (M^+); HRFABMS *m*/*z* 327.1003 [calcd for $C_{20}H_{13}N_3O_2$, (M^+) 327.1008].

Cytotoxic Activity. For Jurkat cells, 3.5×10^5 cells/mL of the cells were pipetted in 95 μL of culture medium per well in 96-well microtiter plates, were treated with 5 μL of graded concentrations of samples in the absence or presence of 0.5 μg/mL of TRAIL, and were then incubated for 42 h at 37 °C in a 5% CO_2/95% air atmosphere. Cell viability was determined by the colorimetric assay using Alamar blue.[13]

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