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Isolation of Bisindole Alkaloids that Inhibit the Cell Cycle from Myxomycetes *Arcyria ferruginea* and *Tubifera casparyi*

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Abstract—From a myxomycete *Arcyria ferruginea*, dihydroarcyriarubin C (**1**), a new bisindole alkaloid, has been isolated together with two known bisindoles, arcyriarubin C (**2**) and arcyriaflavin C (**3**), and arcyriaflavin C (**3**) was also isolated from *Tubifera casparyi* together with arcyriaflavin B (**4**). Arcyriaflavin C (**3**) exhibited cell cycle inhibition effect at G1 and G2/M stage at 10 and 100 ng/mL, respectively.

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 search for natural products from myxomycetes,[1,2] we recently investigated a field-collected sample of fruit bodies of *Arcyria ferruginea* and *Tubifera casparyi* collected at Kochi prefecture. Here we describe isolation of a new bisindole alkaloid, dihydroarcyriarubin C (**1**), along with known bisindoles, arcyriarubin C (**2**) and arcyriaflavins C (**3**) and B (**4**). It was revealed that arcyriaflavin C (**3**) was cytotoxic against HeLa cells at 1 μg/mL and it exhibited cell cycle inhibition effects at lower concentrations.

The fruit bodies of *A. ferruginea*,[3] collected in Kochi Prefecture, Japan, were extracted with 90% MeOH and 90% acetone. The combined extracts[4,5] were subjected to chromatographies on silica gel and Sephadex LH-20 to give dihydroarcyriarubin C (**1**, 0.07% yield), together with arcyriarubin C (**2**, 0.9% yield) and arcyriaflavin C (**3**, 0.14% yield). The fruit-bodies of *T. casparyi*, also collected in Kochi Prefecture, Japan were extracted with 90% MeOH and 90% acetone. The combined extracts[4,5] were partitioned between ethyl acetate and water, and the ethyl acetate-soluble fraction was subjected to chromatographies on silica gel and Sephadex LH-20 to give arcyriaflavin C (**3**, 0.2% yield) and arcyriaflavin B (**4**, 0.02% yield). Compounds **2**, **3**, and **4** were previously isolated from *Arcyria denudata*[6] and **3** was also obtained from *Metatrichia vesparium*,[7] and they were identified from comparison of their spectral data.

Dihydroarcyriarubin C (**1**)[8] was obtained as colorless solids, and its ^{1}H NMR spectrum (in acetone-d_6, Table 1) showed eight signals for four aromatic (or olefinic) protons (δ_H 7.25, 7.15, 6.85, and 6.61), one sp^3 methine proton (δ_H 4.44), and three OH or NH signals (δ_H 10.29, 9.88, and 7.92). The ^{13}C NMR data of **1** was obtained from the F1 axis projections of its HMQC and HMBC spectra, which gave ten signals including eight aromatic (or olefinic) carbons (δ_C 154.1, 138.6, 121.7, 120.4, 119.5, 111.3, 109.6, and 97.1), one sp^3 methine carbon (δ_C 48.2), and one carbonyl carbon (δ_C 177.9). These ^{1}H and ^{13}C NMR signals of sp^2 region of **1** suggested the presence of a 6-hydroxyindole moiety from comparison with those signals of arcyriarubin C (**2**), which was further supported by its ^{1}H–^{1}H COSY and HMBC spectra. In the HMBC spectrum of **1**, the hydroxyl proton at δ_H 7.92 (br s, HO-6) showed correlations to C-5 (δ_C 109.6) and C-7 (δ_C 97.1). The H-5 (δ_H 6.61) and H-7 (δ_H 6.85) were coupled to each other by $J = 2.2$ Hz, implying that these two hydrogens were *meta*. The H-5 was also coupled with H-4 (δ_H 7.25) by $J = 8.5$ Hz, suggesting that H-4 and H-5 was *ortho*. The H-4 showed an HMBC correlation to C-3 (δ_C 111.3)

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Table 1. ^{1}H and ^{13}C NMR data of compound **1** (in CD_3COCD_3)

	δ_H (Hz)	δ_C	HMBC
1,1′	9.88 br s		
2,2′	7.15 d 2.2	121.7	138.6, 120.4, 111.3
3,3′		111.3	
3a,3a′		120.4	
4,4′	7.25 d 8.5	119.5	154.1, 138.6, 111.3
5,5′	6.61 dd 8.5, 2.2	109.6	120.4, 97.1
6,6′		154.1	
7,7′	6.85 d 2.2	97.1	154.1, 120.4, 109.6
7a,7a′		138.6	
8,8′	4.44 s	48.2	177.9, 121.7, 120.4, 111.3, 48.2[a]
9,9′		177.9	
6,6′-OH	7.92 br s		120.4, 97.1
10-NH	10.29 br s		

[a]HMBC correlation from H-8 to C-8′ and from H-8′ to C-8.

and C-6. From these observations, the hydroxyl group was confirmed to be located on C-6. The sp^3 methine signal (δ_H 4.44) was observed as a singlet, which showed HMBC correlations to C-3 and C-3a (δ_C 120.4) of the indole nucleus, and also to the carbonyl carbon at δ_C 177.9. These observations along with comparison with spectral data of arcyriarubin C (**2**) suggested that the sp^3 methine was assignable to C-8. The HMBC spectrum clearly showed a cross peak from H-8 to C-8 itself (δ_C 48.2), and this HMBC correlation may be assigned to H-8 to C-8′ (or H-8′ to C-8), thus implying that compound **1** is also dimeric. From these results, the structure of compound **1** was concluded as 8,8′-dihydroarcyriarubin C. Bergman et al. described the synthesis of bisindolemaleimide derivatives[9] including preparation of *cis*- and *trans*-8,8′-dihydroarcyriarubin C (**5**). From the comparison of their NMR data, the relative stereochemistry of compound **1** was likely to be *trans* [C-8: δ_C 44.7 (*cis*-**5**), δ_C 47.0 (*trans*-**5**), and δ_C (acetone-d_6) 48.2 (**1**)].

Several bisindole alkaloids were previously isolated from myxomycetes.[3,4,10,11] and recently considerable attention has been focused on the metabolites belonging to bisindolylmaleimides such as staurosporine (**6**),[12] UCN-01,[13] and rebeccamycin,[14] which were produced by the family of *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.[15] We here examined the cell cycle inhibition effects of arcyriarubin C (**2**) and arcyriaflavin C (**3**) by flow cytometry studies[5] on HeLa cells. Compound **3** showed considerable increase of subG1 phase at more than 1 μg/mL, implying that this compound was cytotoxic at these concentrations. Arcyriaflavin C (**3**), however, exhibited significant increase of G2/M and G1 phases at 100 ng/mL (=280 nM) and 10 ng/mL (=28 nM), respectively (Fig. 1), suggesting that at these concentrations of **3** the cell cycle was inhibited at these stages, respectively. These findings were consistent with the previous results on staurosporin (**6**), described by Zong et al.[16] Staurosporin (**6**)[12] corresponds to one of glycoside derivatives of arcyriaflavin C (**3**) analogue, and it was reported that staurosporin (**6**) showed G1 arrest at lower concentration (20 nM/mL) and G2/M arrest at higher concentration (200 nM/mL), respectively.[16] The cell cycle inhibition effect of arcyriarubin C (**2**) was weak, compared with **3**; no significant change in flow cytometry study was observed at 10 μg/mL of **2**, while appreciable increase of subG1 phase and decrease of G1 phase was observed at 50 μg/mL of **2**. This finding suggested that the presence of a C–C bond between C-2 and C-2′ positions appears to be important for the cell-cycle inhibition effect.

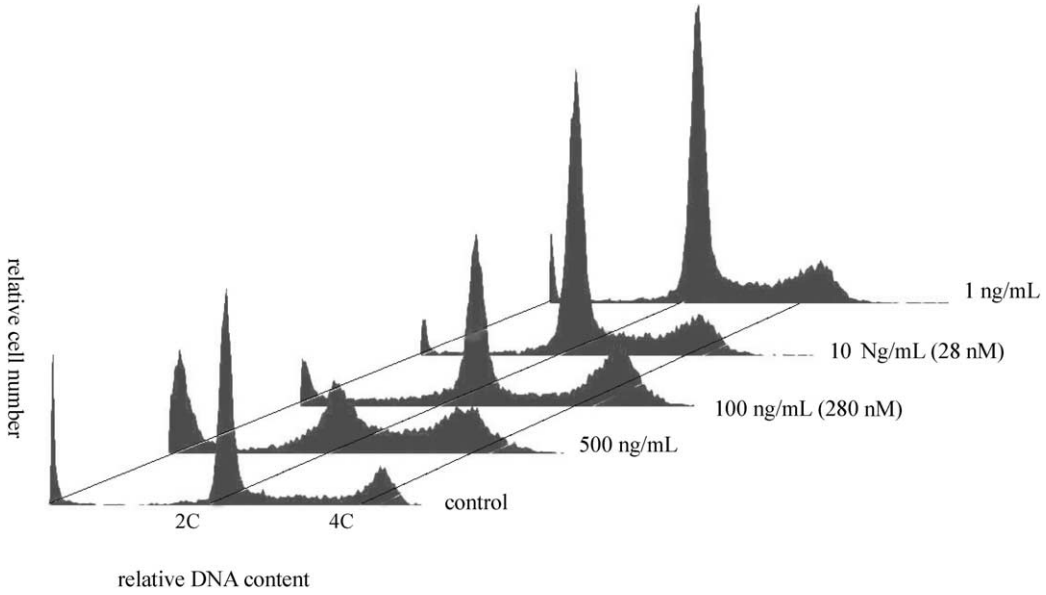
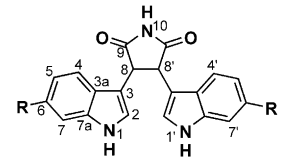
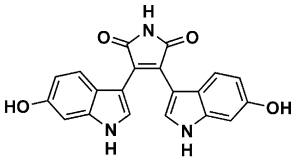


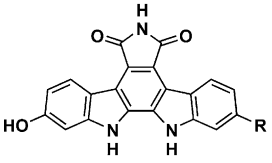
Figure 1. Effects of arcyriaflavin C (**3**) on DNA distribution pattern of HeLa cells. Arcyriaflavin C (**3**) arrested cell in G1 phase (=2C) at a lower concentration (10 ng/mL) and in G2/M phase (=4C) at a higher concentration (100 ng/mL).



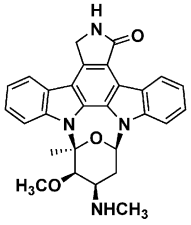
1 R=OH
5 R=H



2



3 R=OH
4 R=H



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