Bahiensol, a New Glycerolipid from a Cultured Myxomycete *Didymium bahiense* var. *bahiense*

Yuka MISONO,^a Masami ISHIBASHI,^{*,a} and Akira ITO^b

^a Graduate School of Pharmaceutical Sciences, Chiba University; Chiba 263–8522, Japan; and ^b Kyorin Pharmaceutical Co., Ltd.; 2–5 Kanda-Surugadai, Chiyoda-ku, Tokyo 101–8311, Japan.

Received February 19, 2003; accepted March 6, 2003; published online March 6, 2003

Bahiensol (1), a new glycerolipid with antimicrobial activity has been isolated from a cultured plasmodium of myxomycete *Didymium bahiense* var. *bahiense* and its planar structure was elucidated by spectral data.

Key words myxomycete; glycerolipid; cultured plasmodium

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 studies on the search for natural products from myxomycetes,¹⁾ we recently investigated laboratory culture of myxomycetes and isolated sterols and pyrroloiminoquinone pigments.²⁾ Here we describe the isolation and structure elucidation of a new antimicrobial glycerolipid, bahiensol (**1**) from the cultured plasmodium of the myxomycete *Didymium bahiense* var. *bahiense*.

The fruit bodies of the myxomycetes *D. bahiense* var. *bahiense* were collected at Ina, Nagano Prefecture, Japan. The plasmodium of this myxomycete obtained in a plate culture^{2,3)} was mass cultured in the laboratory by agar plates with oatmeal.^{2,3)} The harvested plasmodial cells were extracted with 90% MeOH and 90% acetone, and the combined extract was partitioned between hexane and 90% MeOH. The 90% MeOH layer was subjected to silica gel column chromatography, and the fraction exhibiting antimicrobial activity against *Bacillus subtilis* was further separated by the flash chromatography on ODS to give bahiensol (**1**) as a colorless oil.

Bahiensol (**1**) was shown to have the molecular formula C₁₉H₄₀O₅ by the high resolution (HR)-FAB-MS data (*m/z* 349.2950, [M+H]⁺, Δ –0.4 mmu), implying that compound **1** possessed zero degree of unsaturation. The ¹³C-NMR spectrum of **1** in CDCl₃ (Table 1) showed signals due to three oxymethines (δ_C 64.1, 70.1, 72.6), three oxymethylenes (δ_C 70.6, 70.9, 72.1), one methyl (δ_C 14.2) carbons. All other twelve signals were assignable to *sp*³ methylene carbons (δ_C 22.7, 25.5, 25.6, 25.8, 29.4, 29.7, 29.8, 31.9, 36.5, 37.4, 37.5, 37.6), and no signals due to *sp*² carbons were observed. Treatment of **1** with acetic anhydride and pyridine afforded a tetraacetate (**2**), implying that compound **1** contains four hydroxyl groups. Analyses of the ¹H–¹H correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) spectra of **1** and **2** suggested the presence of a glycerol unit (C-1' to C-3'), and that C₁₆ aliphatic chain (C-1 to C-16) is connected to C-3' of glycerol unit through an

ether linkage. Two secondary hydroxyl groups [δ_H 3.72 (1H, m), 3.51 (1H, m)] were included in the C₁₆ aliphatic chain, and one of the two secondary hydroxyl groups was shown to be located on C-3 position from the COSY (H₂-1/H₂-2, H₂-2/H-3) and HMBC correlations (H₂-1/C-2, H₂-1/C-3, H₂-2/C-1, H₂-2/C-3). The position of the other secondary hydroxyl group (position 'ω') was implied to be between C-6 to C-13, and MS/MS and ¹H-detected heteronuclear multiple quantum coherence (HMQC)-total correlation spectroscopy (TOCSY) experiments were carried out to determine the exact position 'ω', but were unsuccessful at this time. From these results, structure of bahiensol (**1**) was concluded to be 3-*O*-(3,ω-dihydroxyhexadecanyl)-glycerol.

Bahiensol (**1**) is a previously unknown monoalkylglycerol, first isolated from the cultured plasmodium of the myxomycete *Didymium bahiense* var. *bahiense*. It was weakly positive on an antimicrobial activity test against *Bacillus subtilis* with a diameter of inhibition zone 12.5 mm at 500 μg per paper disc (8 mm in diameter).

Experimental

General Procedures 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.

Organism and Culture The fruit bodies of the myxomycete *Didymium bahiense* (Order Physarales; Family Didymiaceae) were collected at Ina,

Table 1. ¹H- and ¹³C-NMR Data of Compound **1** (in CDCl₃)

	1	
	δ _H	δ _C ^{c)}
1'	3.57 m	64.1
	3.65 m	
2'	3.80 m	72.0
3'	3.48 ^{a)} m	72.6
1	3.55 m	70.1
	3.67 m	
2	1.67 m	36.5
	1.62 m	
3	3.72 m	70.6
4	1.48 ^{a)} m	37.4
ω	3.51 m	70.9
14	1.26 ^{a)} m	31.9
15	1.28 ^{a)} m	22.7
16	0.81 ^{b)} t, <i>J</i> = 7.5 Hz	14.2

a) 2H. b) 3H. c) ¹³C-NMR signals of methylene carbons C-5–C-13 except C-ω: δ_C 37.6, 37.5, 29.8, 29.7, 29.4, 25.8, 25.6, and 25.5.

Nagano Prefecture, Japan, in August, 1999, and the procedures for the laboratory culture of this organisms are described previously.²⁾

Extraction and Isolation The harvested plasmodial cells from 793 plate cultures (9 cm ϕ) were lyophilized to give 84.3 g of material (dry weight), which was extracted with 90% MeOH (*ca.* 900 ml $\times$ 2) and 90% acetone (750 ml $\times$ 1). The combined MeOH and acetone extract was partitioned between 90% aqueous MeOH (100 ml) and hexane (25 ml $\times$ 3). The 90% MeOH layer was evaporated under reduced pressure to give a residue (6.5 g), which was subjected to silica gel column chromatography (column A; 4.0 $\times$ 60 cm) and eluted with 20–100% MeOH in CHCl₃. A fraction (99.7 mg) of column A eluted with 20% MeOH in CHCl₃ was further separated by ODS column (column B; 1.6 $\times$ 24 cm) eluted with 67–100% MeOH in H₂O to give bahiensol (**1**, 2.5 mg) in the fraction eluted with 80% MeOH in H₂O. The fraction (26.2 mg) of the column B eluted with 67–80% MeOH in H₂O was separated again by ODS column (1.0 $\times$ 22 cm) to give compound **1** (3.1 mg) additionally.

Bahiensol (**1**): Colorless oil; $[\alpha]_D^{25} +23^\circ$ (*c*=0.5, MeOH); IR (film) $\nu_{\max}$ 3345, 2920, and 1460 cm⁻¹; ¹H- and ¹³C-NMR (Table 1); FAB-MS *m/z* 349 (M+H)⁺; HR-FAB-MS *m/z* 349.2950 [Calcd for C₁₉H₄₀O₅, (M+H) 349.2954]. Tetraacetate (**2**, 0.9 mg) was prepared by treatment of bahiensol (**1**, 0.9 mg) with acetic anhydride (0.1 ml) and pyridine (0.2 ml) at room temperature for 14 h.

2: ¹H-NMR (CDCl₃) δ_H 5.16 (1H, m; H-2'), 4.95 (1H, m; H-3), 4.85 (1H, m; H- ω), 4.32 (1H, dd, *J*=11.5, 3.6 Hz; H-1'), 4.15 (1H, dd, *J*=11.5, 6.3 Hz; H-1'), 3.53 (2H, m, *J*=7.1 Hz; H₂-3'), 3.46 (2H, m; H₂-1), 2.08 (3H, s), 2.06 (3H, s), 2.03 (6H, s), 1.79 (2H, m; H₂-2), and 0.87 (3H, t; H₃-16); ¹³C-NMR (CDCl₃) δ_C 14.1, 20.7, 21.0, 21.2, 21.3, 22.6, 25.1, 25.2, 25.3, 29.2, 29.4, 29.5, 31.8, 34.1, 34.2, 34.4, 62.9, 68.1, 69.1, 70.2, 71.6, 74.3, 170.3, 170.6, 170.6, and 170.9.

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. We are grateful to Dr. K. Yamaguchi and Dr. H. Seki of our university for instrumental analyses.

References

- 1) Ishikawa Y., Ishibashi M., Yamamoto Y., Hayashi M., Komiyama K., *Chem. Pharm. Bull.*, **50**, 1126–1127 (2002).
- 2) Ishibashi M., Iwasaki T., Imai S., Sakamoto S., Yamaguchi K., Ito A., *J. Nat. Prod.*, **64**, 108–110 (2001).
- 3) Ishikawa Y., Kono Y., Iwasaki T., Misono Y., Nakatani S., Ishibashi M., Ito A., Matsumoto J., *Nat. Med.*, **55**, 312 (2001).