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

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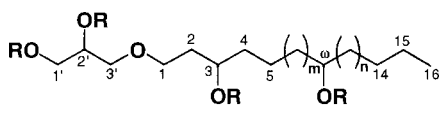
Bahiensol (1), a new glycerolipid with antimicrobial activity has been isolated from a cultured plasmodium of myxomycete *Didymium bahiense* var. *bahiense* and its planar structure was elucidated by spectral data.

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 $C_{19}H_{40}O_5$ 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 ^{13}C-NMR spectrum of **1** in $CDCl_3$ (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 (δC 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 ^{1}H–^{1}H correlation spectroscopy (COSY) and heteronuclear multiple bone connectivity (HMBC) spectra of **1** and **2** suggested the presence of a glycerol unit (C-1′ to C-3′), and that C_{16} 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 C_{16} aliphatic chain, and one of the two secondary hydroxyl groups was shown to be located on C-3 position from the COSY (H_2-1/H_2-2, H_2-2/H-3) and HMBC correlations (H_2-1/C-2, H_2-1/C-3, H_2-2/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 ^{1}H-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.



1 R = H
2 R = $COCH_3$
m+n=7

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. ^{1}H- and ^{13}C-NMR Data of Compound **1** (in $CDCl_3$)

	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, J=7.5 Hz	14.2

a) 2H. *b*) 3H. *c*) ^{13}C-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.

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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 cmϕ) were lyophilized to give 84.3 g of material (dry weight), which was extracted with 90% MeOH (*ca.* 900 ml×2) and 90% acetone (750 ml×1). The combined MeOH and acetone extract was partitioned between 90% aqueous MeOH (100 ml) and hexane (25 ml×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 (colum A; 4.0×60 cm) and eluted with 20—100% MeOH in $CHCl_3$. A fraction (99.7 mg) of column A eluted with 20% MeOH in $CHCl_3$ was further separated by ODS column (colum B; 1.6×24 cm) eluted with 67—100% MeOH in H_2O to give bahiensol (**1**, 2.5 mg) in the fraction eluted with 80% MeOH in H_2O. The fraction (26.2 mg) of the column B eluted with 67—80% MeOH in H_2O was separated again by ODS column (1.0×22 cm) to give compound **1** (3.1 mg) additionally.

Bahiensol (**1**): Colorless oil; $[\alpha]_D^{25}$ +23° (c=0.5, MeOH); IR (film) ν_{max} 3345, 2920, and 1460 cm^{-1}; ^{1}H- and ^{13}C-NMR (Table 1); FAB-MS m/z 349 $(M+H)^+$; HR-FAB-MS m/z 349.2950 [Calcd for $C_{19}H_{40}O_5$, (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: ^{1}H-NMR ($CDCl_3$) 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; H_2-3′), 3.46 (2H, m; H_2-1), 2.08 (3H, s), 2.06 (3H, s), 2.03 (6H, s), 1.79 (2H, m; H_2-2), and 0.87 (3H, t; H_3-16); ^{13}C-NMR ($CDCl_3$) 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, 34.1, 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.

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