Ketene Acetal and Spiroacetal Constituents of the Marine Fungus *Aigialus parvus* BCC 5311

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Aigialone (**1**) and aigialospirol (**2**), two structurally unique compounds, were isolated from the mangrove fungus *Aigialus parvus* BCC 5311. The structure of the new ketene acetal **1** was elucidated by spectral analysis, and its relative stereochemistry was determined by X-ray crystallography. The stereochemistry of aigialospirol (**2**), elucidated by NMR spectral analysis, suggested that this compound is possibly derived from hypothemycin (**3**), a metabolite previously isolated from this same fungus.

Marine fungi are of increasing interest in recent years as sources for biologically active compounds with diverse chemical structures.[1] As part of our ongoing research program on bioactive fungal secondary metabolites,[2] we recently isolated five new resorcylic macrolides, aigialomycins A−E, together with hypothemycin, from the mangrove Ascomycete, *Aigialus parvus* BCC 5311.[3] Due to the slow-growing nature of this fungus (BCC 5311) during flask fermentation, we have undertaken further chemical investigation of the cultures from prolonged fermentation. Thus, extension of the incubation period in a liquid medium from 35 to 80 days resulted in the production of compound **1**, named aigialone, which is an entirely different class of compound from those previously isolated and reported from this source.[3,4] A new hypothemycin-related compound, aigialospirol (**2**), was also isolated from the same fermentation broth, together with two major constituents, hypothemycin (**3**)[5,6] and aigialomycin B (**4**). We report herein the isolation and structural elucidation of compounds **1** and **2**.

Aigialone (**1**) possesses the molecular formula $C_{16}H_{26}O_5$, as determined from the HRMS and ^{13}C NMR data. The UV spectrum of **1** showed an intense absorption at λ_{max} 254 nm (log ϵ 4.35), and its IR spectrum showed two sharp hydroxyl absorptions at ν_{max} 3492 and 3245 cm^{-1}, a carbonyl at 1677 cm^{-1}, and a very strong absorption at 1574 cm^{-1}. The structure of **1** was elucidated by analysis of its 2D NMR spectra (Table 1). Most importantly, HMBC correlations were observed from the H-2, H-4, and H-6 signals to an upfield quaternary carbon resonance (δ_C 180.8, C-7a). A downfield olefinic carbon at δ 90.7 (s, C-3a) was attached to C-4 as indicated by HMBC correlations from H-4 and OH-4 (δ_H 3.99) to C-3a. These data required a bicyclic ring skeleton with a ketene acetal linkage. Failing to detect the C-2 configuration by NMR methods, we decided to undertake a X-ray crystallographic study on **1**. These results indicated the relative stereochemistry of **1** as depicted, either in the (2*S*,4*S*,5*R*,6*S*)- or (2*R*,4*R*,5*S*,6*R*)- configuration, and the data confirmed the ketene acetal moiety in this compound (Figure 1). Surprisingly, the *n*-heptyl group on C-6 and the two hydroxyl groups attached to C-4 and C-5 exhibited pseudo-axial orientations, while only the methyl group (on C-5) was pseudo-

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2

3

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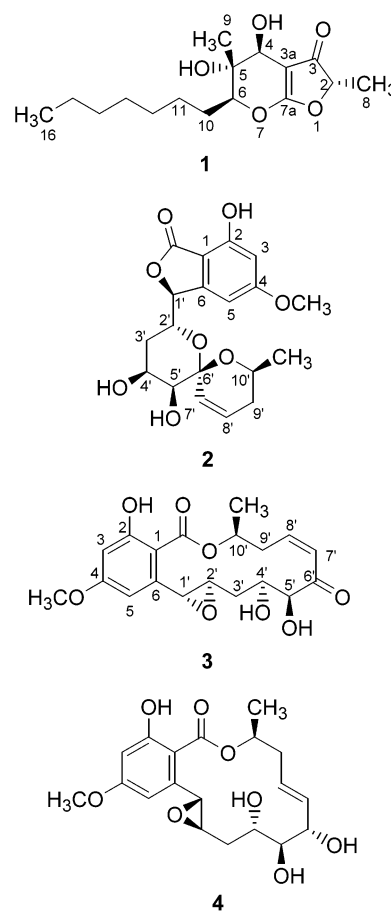
equatorial. This unusual conformation of the crystal structure could be applied to the solution structure, since the observed NOESY correlations were in good agreement with this conformation (Figure 1). For example, both the C-9 methyl protons and OH-5 were correlated with H-4 and H-6. One of the C-10 methylene protons at δ_H 1.92 (H-10a), which is *trans* to H-6 (J = 10.1 Hz), correlated with OH-4. On the other hand, H-10b showed an intense NOESY correlation with the C-9 methyl and H-6 substituents. A correlation between H-4 and H-6 was not observed in the NOESY spectrum in CDCl$_3$, but a weak cross signal was detected in the spectra taken in CD$_3$OD and DMSO-d_6.

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Table 1. NMR Spectral Data for Aigialone (**1**) and Aigialospirol (**2**)

	aigialone (**1**) (CDCl$_3$)				aigialospirol (**2**) (acetone-d_6/D$_2$O, 4:1)		
position	δ_C (mult.)	δ_H (mult., J in Hz)	HMBC (H to C)	position	δ_C (mult.)	δ_H (mult., J in Hz)	HMBC (H to C)
2	84.0 (d)	4.71 (q, 7.1)	3, 7a	1	105.1 (s)		
3	197.9 (s)			2	158.4 (s)		
3a	90.7 (s)			3	102.3 (d)	6.42 (d, 1.9)	1, 2, 4, 5
4	67.5 (d)	4.44 (brs)	5, 7a	4	167.1 (s)		
5	71.7 (s)			5	100.6 (d)	6.61 (dd, 1.9, 0.9)	1, 3, 4, 1'
6	89.6 (d)	4.32 (brd, 10.1)	4, 5, 7a, 9, 10	6	151.8 (s)		
7a	180.8 (s)			1'	81.4 (d)	5.33 (d, 5.2)	6, 2', 3', $-$COO$-$
8	16.0 (q)	1.47 (d, 7.1)	2, 3	2'	66.3 (d)	4.27 (ddd, 12.0, 5.2, 2.6)	
9	18.3 (q)	1.35 (s)	4, 5, 6	3'	32.9 (t)	1.75 (ddd, 13.9, 12.0, 2.9)	2'
10	28.6 (t)	1.92 (m), 1.78 (m)	6, 12			1.65 (ddd, 13.9, 3.4, 2.5)	4', 5'
11	26.5 (t)	1.60 (m), 1.39 (m)		4'	68.3 (d)	4.02 (ddd, 3.4, 3.3, 3.1)	
12	29.13 (t)^a	1.35$-$1.25 (m)^b		5'	70.9 (d)	3.48 (d, 3.6)	7'
13	29.10 (t)^a	1.35$-$1.25 (m)^b		6'	99.1 (s)		
14	31.7 (t)	1.35$-$1.25 (m)^b		7'	127.2 (d)	5.52 (ddd, 10.1, 2.0, 2.0)	6', 9'
15	22.6 (t)	1.35$-$1.25 (m)^b		8'	131.3 (d)	6.04 (ddd, 10.0, 4.3, 3.6)	6'
16	14.0 (q)	0.88 (t, 6.8)	14, 15	9'	31.4 (t)	1.92$-$1.93 (2H, m)	7', 8', 10'
O*H*-4		3.99 (brs)	3a, 4, 5	10'	65.2 (d)	3.93 (m)	C*H*$_3$-10'
O*H*-5		4.57 (brs)	5, 6, 9	$-$COO$-$	170.0 (s)		
				C*H*$_3$-10'	20.7 (q)	1.14 (3H, d, 6.3)	9', 10'
				O*C*H$_3$-4	56.2 (q)	3.76 (3H, s)	4

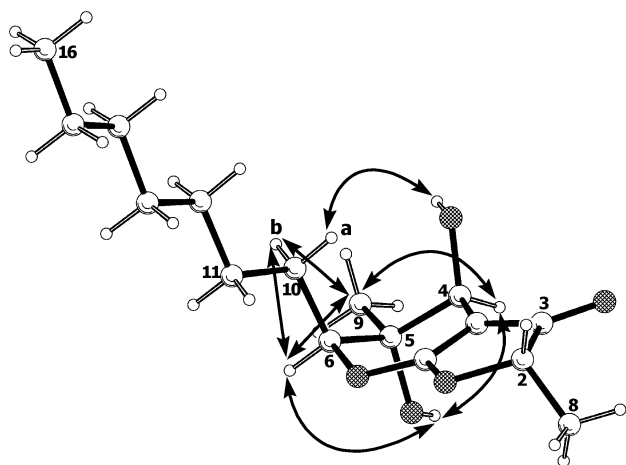^{a} Interchangeable assignments. ^{b} Overlapping signals.

Figure 1. Crystal structure of aigialone (**1**). Selected NOESY correlations are illustrated with solid arrows.

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Unfortunately attempts to prepare α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters[7] of **1** were not successful, and the absolute configuration of **1** remains questionable.

Compounds possessing a ketene acetal functionality are very rare in nature.[8–10] The structure of aigialone (**1**) is closely related to that of benesudon (**5**), isolated from the fungus *Mollisia benesuada* A26-93.[8] Benesudon possesses a C-2—C-8 double bond (exomethylene), and its relative configuration differs from that of **1**. The relative stereochemistry (C-4, C-5, and C-6) of benesudon (**5**) was determined on the basis of the NOESY correlation data, which were in good agreement with pseudo-equatorial orientations of OH-4, the 5-methyl and 6-heptyl substituents, and a pseudo-axial OH-5.[8] However, the stereochemistry shown in Figure 1 also accounts well for the NOESY correlation data recorded for **5**. Therefore our finding casts some doubts on the relative assignment of stereochemistry of benesudon (**5**). The ^{1}H and ^{13}C NMR spectral data of **1** in CD$_3$OD were very similar to those described for compound **5** except for those of the five-membered-ring moiety. In addition, a *W*-coupling of H-4 and H-6 (J = 1.5 Hz), recorded for **5**, was suggestive for the pseudo-equatorial orientations of these two protons in **5**.[11]
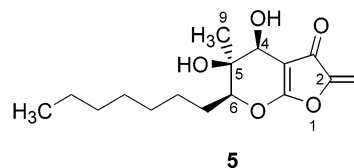
Aigialospirol (**2**) was isolated as a pale yellow amorphous solid, and its molecular formula of C$_{19}$H$_{22}$O$_8$ was suggested by HRMS and its ^{1}H and ^{13}C NMR spectral data. Initially, the NMR spectra of **2** were taken in CDCl$_3$; however, better peak resolution was achieved in acetone-d_6/D$_2$O (4:1). NMR spectral analysis revealed that aigialospirol (**2**) is somewhat related structurally to hypothemycin (**3**) and aigialomycins (e.g., **4**), possessing a resorcylic ester connected at C-6 with an aliphatic polyketide chain. Unlike the 14-membered macrolides, this compound possesses a dihydroisobenzofuranone structure, as indicated by the low-field (δ_H 5.33) shift of H-1' and also by the HMBC correlation from this proton to the ester carbonyl. The linkage from C-1' to C-10', through a quaternary carbon (C-6', δ_C 99.1), was established by analysis of the COSY, HMQC, and HMBC correlations. In addition to HMBC correlation data acquired in acetone-d_6/D$_2$O (Table 1), the spectrum taken in CDCl$_3$ showed a correlation from H-10' to C-6'. Although a HMBC correlation from H-2' to C-6' was not observed, the molecular formula (C$_{19}$H$_{22}$O$_8$) of this compound required a spiroacetal-type structure.

Analysis of the vicinal coupling constant values and NOESY spectral correlations of **2** revealed the relative configuration from C-2' to C-6', demonstrating that the inner tetrahydropyran ring adopts a chair conformation. Thus, an intense NOESY interaction between one of the C-3' methylene protons at δ_H 1.75 (H-3'ax) and H-5' indicated the 1,3-diaxial relationship of these protons. The axial orientation of H-2' was indicated by its large coupling (J = 12.0 Hz) to H-3'ax. The H-4' signal appeared as a doublet of doublets of doublets with small J values (3.4, 3.3, and 3.1 Hz), and this proton showed NOESY correlations to H-3'ax, H-3'eq, and H-5', which placed it in an equatorial position. The olefinic proton, H-7', showed an intense NOESY correlation signal to the axial proton, H-5', which clearly indicated the configuration of the acetal carbon (C-6'), as shown in Figure 2. Configurations of C-1' and C-10' relative to the C-2'—C-6' asymmetric centers
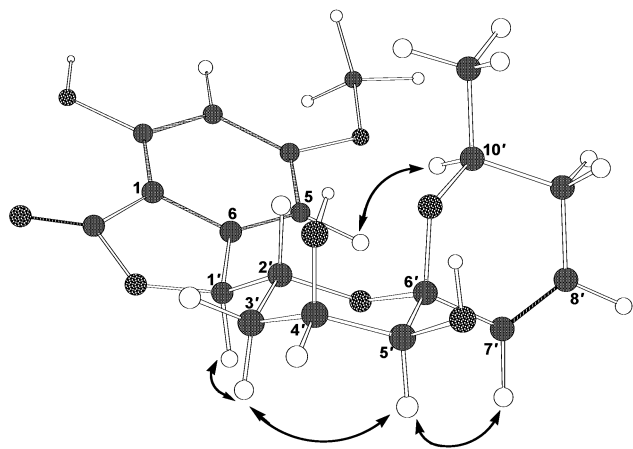
Figure 2. Probable conformation of aigialospirol (**2**). Selected NOESY correlations are illustrated with solid arrows.

were also established by NOESY spectral analysis. The H-1′ proton showed a clear NOESY correlation to H-3′ax, but was only very weakly correlated to H-3′eq. On the other hand, one of the aromatic protons, H-5, showed a NOESY correlation to H-10′, despite the long through-bond distance between these protons. These data demonstrated the relative configuration and plausible conformation of **2**, as shown in Figure 2.

The structure of aigialospirol (**2**) is related to hypothemycin (**3**), the major metabolite in the fungal extract. The relative stereochemistry of the asymmetric carbon centers of **2** was identical to that of **3** except for the opposite configuration at C-1′. Since compound **2** was not detected in the extract from the earlier harvested fermentation culture, it is not unreasonable to assume that this compound may be derived from **3**, and this correlation suggested that the absolute configuration of aigialospirol (**2**) should be that as depicted. It must be noted that aigialospirol (**2**) was not obtained as an isolation artifact. Compound **2** was detected in the HPLC/UV analysis (ODS column, MeCN/H$_2$O) of the crude extract, and this was confirmed by co-injection experiments with the standard.

A plausible biosynthetic pathway from **3** to **2** is the initial epoxide cleavage (hydration) of **3** by attack of a hydroxyl oxygen to the benzylic carbon (C-1′) with stereoinversion (S$_N$2), giving a 1′,2′-diol, followed by γ-lactone formation. Subsequent spiroacetal formation may well proceed smoothly due to the favorable *cis*-olefinic geometry. An alternative pathway, involving the hydrolysis of the ester linkage and subsequent attack at the epoxide (C-1′), may also be plausible.

Compounds **1** and **2** were inactive in our in vitro biological protocols, inclusive of antimalarial (*Plasmodium falciparum* K1), antitubercular (*Mycobacterium tuberculosis* H37Ra), antiviral (HSV-1), antifungal (*Candida albicans*), and cytotoxicity (KB cells, BC-1 cells) assays. In contrast, hypothemycin (**3**) has been shown to exhibit significant antimalarial[3] and antitumor[3,6] activities.

Experimental Section

General Experimental Procedures. Melting points were measured with an Electrothermal IA9100 digital melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Varian CARY 1E UV–visible spectrophotometer. FT-IR spectra were taken on a Perkin-Elmer 2000 spectrometer. NMR spectra were taken on Bruker DRX400 and AV500D spectrometers. ESI-TOF mass spectra were measured with a Micromass LCT mass spectrometer.

Fungal Material. *Aigialus parvus* was collected, identified, and isolated from mangrove wood by Prof. E. B. G. Jones in June 1999. This fungus was deposited at the Thailand BIO-TEC Culture Collection as BCC 5311.[3]

Fermentation and Isolation. *A. parvus* BCC 5311 was grown on potato dextrose agar (PDA) at 22 °C for 35 days, before inoculation into 20 × 1 L Erlenmeyer flasks each containing 250 mL of potato dextrose broth (PDB). Fermentation was conducted under stationary conditions at 22 °C for 80 days. The flask cultures were filtered, and the filtrate (5 L) was extracted with an equal volume of EtOAc to obtain a light brown solid (1.23 g). This crude extract was passed through a Sephadex LH-20 column (elution with MeOH), where an early-eluting fraction (Fr-A, 160 mg) consisted mainly of aigialone (**1**), and a later fraction (Fr-B, 1.05 g) contained aigialospirol (**2**) and 14-membered macrolides. Fr-A was subjected to column chromatography on silica gel (MeOH/CH$_2$Cl$_2$, 3:97, then 5:95) to obtain aigialone (**1**, 124 mg; R_f 0.20, MeOH/CH$_2$Cl$_2$, 5:95) as a colorless solid. Fr-B was fractionated by repeated silica gel column chromatography (MeOH/EtOAc, then MeOH/CH$_2$Cl$_2$), and the following compounds were obtained in the order of elution: **3** (195 mg); **2** (16 mg); and **4** (80 mg). Compound **2** was further purified by preparative HPLC using a reversed-phase column (4.0 × 10.0 cm; MeCN/H$_2$O, 20:80; flow rate, 20 mL/min): t_R 15 min, 13.9 mg.

Aigialone (1): colorless crystals (EtOAc/hexane); mp 132.5–133.0 °C; [α]^{26}$_D$ −193° (*c* 0.65, CHCl$_3$); UV (MeOH) λ$_{max}$ (log ε) 254 (4.35) nm; IR (KBr) ν$_{max}$ 3492, 3245, 2921, 1677, 1574, 1493, 1039, 934 cm^{-1}; NMR data in CDCl$_3$, Table 1; ^{1}H NMR (CD$_3$OD, 400 MHz) δ 4.84 (1H, q, *J* = 7.0 Hz, H-2), 4.42 (1H, brd, *J* = 11.6 Hz, H-6), 4.20 (1H, d, *J* = 1.3 Hz, H-4), 2.03 (1H, m, H-10a), 1.74 (1H, m, H-10b), 1.61 (1H, m, H-11a), 1.49 (3H, d, *J* = 7.0 Hz, H-8), 1.42 (1H, m, H-11b), 1.39 (3H, s, H-9), 1.39–1.33 (8H, m, H-12, H-13, H-14, and H-15), 0.95 (3H, t, *J* = 7.0 Hz, H-16); ^{13}C NMR (CD$_3$OD, 100 MHz) δ 200.0 (s, C-3), 182.2 (s, C-7a), 91.9 (d, C-6), 91.6 (s, C-3a), 84.9 (d, C-2), 72.4 (s, C-5), 67.1 (d, C-4), 33.0 (t, C-14), 30.4 (t, C-10), 30.29 and 30.26 (t, C-12 and C-13), 28.0 (t, C-11), 23.7 (t, C-15), 20.8 (q, C-9), 16.3 (q, C-8), 14.4 (q, C-16); HRMS (ESI-TOF, positive) *m/z* 299.1839 (calcd for C$_{16}$H$_{27}$O$_5$, 299.1858) [M + H]$^+$.

X-ray Crystal Structure Determination of Aigialone (1). Crystal data of aigialone (**1**): C$_{16}$H$_{26}$O$_5$, MW 298.38, monoclinic, *P*2$_1$ (No. 4), *a* = 5.4952(2) Å, *b* = 8.6872(4) Å, *c* = 17.1402(8) Å, β = 93.979(2)°, *V* = 816.27(6) Å^3, *D$_x$* = 1.214 g/cm^3, *Z* = 2. A total of 19 048 reflections, of which 2028 were unique (1882 observed, |*F*$_o$| > 4σ|*F*$_o$|), were measured at room temperature from a 0.20 × 0.15 × 0.10 mm^3 colorless crystal using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a Bruker-Nonius kappaCCD diffractometer. The crystal structure was solved by direct methods using SIR-97, and then all atoms except hydrogen atoms were refined anisotropically by full-matrix least-squares methods on *F*² using SHELXL-97 to give a final *R*-factor of 0.0392 (*R*$_w$ = 0.1001 for all data) with a data-to-parameter ratio of 10.6:1.[12]

Aigialospirol (2): pale yellow amorphous solid; mp 85–89 °C; [α]^{25}$_D$ +47° (*c* 0.50, CHCl$_3$); UV (MeOH) λ$_{max}$ (log ε) 217 (4.29), 257 (3.92), 292 (3.51) nm; IR (KBr) ν$_{max}$ 3453, 1741, 1615, 1455, 1218, 1159, 1167, 1001 cm^{-1}; NMR data in acetone-*d$_6$*/D$_2$O, Table 1; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 6.59 (1H, dd, *J* = 1.7, 1.0 Hz, H-5), 6.47 (1H, d, *J* = 1.8 Hz, H-3), 6.16 (1H, ddd, *J* = 10.0, 4.2, 3.7 Hz, H-8′), 5.66 (1H, ddd, *J* = 10.1, 2.1, 1.9 Hz, H-7′), 5.36 (1H, d, *J* = 6.0 Hz, H-1′), 4.15 (1H, ddd, *J* = 12.0, 6.0, 2.3 Hz, H-2′), 4.13 (1H, m, H-4′), 4.01 (1H, m, H-10′), 3.86 (3H, s, OC*H$_3$*-4), 3.52 (1H, d, *J* = 3.6 Hz, H-5′), 2.05–2.03 (3H, m, H-3′a and H-9′), 1.88 (1H, ddd, *J* = 13.9, 12.1, 3.0 Hz, H-3′b), 1.26 (3H, d, *J* = 6.2 Hz, C*H$_3$*-10′); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 171.3 (s, −*C*OO−), 167.3 (s, C-4), 157.7 (s, C-2), 149.1 (s, C-6), 130.5 (d, C-8′), 126.5 (d, C-7′), 104.3 (s, C-1), 101.6 (d, C-3), 101.2 (d, C-3), 99.1 (s, C-6′), 82.3 (d, C-1′), 70.7 (d, C-5′), 68.3 (d, C-4′), 65.8 (d, C-2′), 65.0 (d, C-10′), 56.0 (q, O*C*H$_3$-4), 33.6 (t, C-3′), 31.4 (t, C-9′), 21.1 (q, *C*H$_3$-10′); HRMS (ESI-TOF, positive) *m/z* 401.1228 (calcd for C$_{19}$H$_{22}$O$_8$-Na, 401.1212) [M + Na]$^+$.

Supporting Information Available: NMR and IR spectra of compounds **1** and **2** and ORTEP diagram and crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 1−6. (b) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1−49.

(2) (a) Isaka, M.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2001**, *66*, 4803−4808. (b) Isaka, M.; Jaturapat, A.; Rukseree, K.; Danwisetkanjana, K.; Tanticharoen, M.; Thebtaranonth, Y. *J. Nat. Prod.* **2001**, *64*, 1015−1018. (c) Nilanonta, C.; Isaka, M.; Kittakoop, P.; Trakulnaleamsai, S.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron* **2002**, *58*, 3355−3360. (d) Isaka, M.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron Lett.* **2000**, *41*, 1657−1660.

(3) Isaka, M.; Suyarnsestakorn, C.; Tanticharoen, M.; Kongsaeree, P.; Thebtaranonth, Y. *J. Org. Chem.* **2002**, *67*, 1561−1566.

(4) A separate study on the time-course of metabolite production revealed that aigialone (**1**) started to be produced after a long period of incubation, while the concentration of resorcylic macrolides in the fermentation broth reached a maximum within four weeks. This may be the reason compound **1** was not observed in the initial chemical investigation of the 35-day culture.[3]

(5) (a) Nair, M. S. R.; Carey, S. T. *Tetrahedron Lett.* **1980**, *21*, 2011−2012. (b) Nair, M. S. R.; Carey, S. T.; James, J. C. *Tetrahedron* **1981**, *37*, 2445−2449.

(6) Revised structure of hypothemycin (**3**): Agatsuma, T.; Takahashi, A.; Kabuto, C.; Nozoe, S. *Chem. Pharm. Bull.* **1993**, *41*, 373−375.

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(9) Anke, H.; Casser, I.; Schrage, M.; Steglich, W. *J. Antibiot.* **1988**, *41*, 1681−1684.

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(11) Also the following coupling for aigialone (**1**) was observed; $J_{4,6} = 1.3$ Hz in CD_3OD. Cf.: Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon Press: Oxford, 1969; p 334.

(12) Crystallographic data of compound **1** have been deposited at the Cambridge Crystallographic Data Centre under the reference number CCDC 218502. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk).

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