ORGANIC LETTERS

2008
Vol. 10, No. 12
2481−2484

Ariakemicins A and B, Novel Polyketide-peptide Antibiotics from a Marine Gliding Bacterium of the Genus *Rapidithrix*

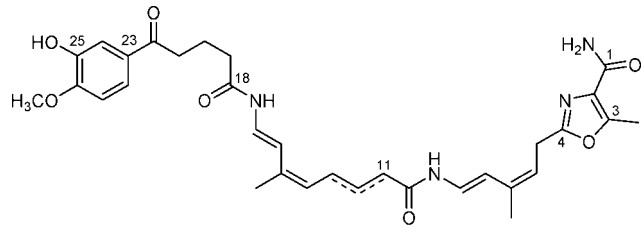
Naoya Oku,*,†,§ Kyoko Adachi,† Satoru Matsuda,† Hiroaki Kasai,† Akira Takatsuki,‡ and Yoshikazu Shizuri†

Applied Bioorganic Chemistry Group, Marine Biotechnology Institute Co. Ltd., 3-75-1 Heita, Kamaishi, Iwate 026-0001, Japan, and Department of Frontier Bioscience, Faculty of Bioscience and Applied Chemistry, Hosei University, 3-7-2 Kajino, Koganei, Tokyo 184-8584, Japan

noku@riken.jp

Received April 1, 2008

ABSTRACT



Ariakemicin A: Δ^{12}
Ariakemicin B: Δ^{11}

Ariakemicins A (1) and B (2), unusual linear hybrid polyketide-nonribosomal peptide antibiotics, were discovered from the fermentation extract of the marine gliding bacterium *Rapidithrix* sp. These metabolites were positional isomers with regard to a double bond and chromatographically inseparable, rendering the structure study on a mixture basis. The ariakemicins were composed of threonine, two ω-amino-(ω-3)-methyl carboxylic acids with diene or triene units, and δ-isovanilloylbutyric acid. The antibiotics selectively inhibited the growth of Gram-positive bacteria.

Gliding bacteria of the phylum *Bacteroidetes* are Gram-negative, nonfruiting chemoheterotrophs that are found in a variety of environments including soil, aquatic sediment, fresh- and seawater, on the surface of aquatic organisms, in the gut of animals, and even in the tissues of arthropods[1] or in the cytosol of amoeba[2] as endosymbionts. They represent a substantial proportion of the bacterial community in marine coastal ecosystems and play a major role in the consumption of high-molecular-weight dissolved organic matter.[3] A growing number of new *Bacteroidetes* species are described each year, most of them coming from marine environments.[4] At present, however, knowledge on the secondary metabolites from marine species remains quite limited; these include *N*-(3-acyloxyacyl)glycines from *Cytophaga* sp.,[5] neoverrucosane diterpenoids from *Saprospira grandis*,[6] an algal morphogen thallusin from a strain belonging to the genus *Zobellia*,[7]

† Marine Biotechnology Institute Co., Ltd.
‡ Hosei University.
§ Current address: Osada Antibiotics Laboratory, RIKEN, Wako, Saitama 351-0198, Japan.

(1) Zchori-Fein, E.; Perlman, S. J. *Mol. Ecol.* **2004**, *13*, 2009–2016.
(2) Horn, M.; Wagner, M. *J. Eukaryotic Microbiol.* **2004**, *51*, 509–514.
(3) Cottrell, M. T.; Kirchman, D. L. *Appl. Environ. Microbiol.* **2000**, *66*, 1692–1697.
(4) Bergey's taxonomic outline. Available via the Internet at http://141.150.157.80/bergeysoutline/main.htm.
(5) Morishita, T.; Sato, A.; Hisamoto, M.; Oda, T.; Matsuda, K.; Ishii, A.; Kodama, K. *J. Antibiot.* **1997**, *50*, 457–468.

10.1021/ol8007292 CCC: $40.75
Published on Web 05/22/2008

Table 1. NMR Data for Ariakemicins A (**1**) and B (**2**) in CD_3OD

	1			**2**		
no.	δ_C[a]	δ_H[b], mult, *J* in Hz, integration	HMBC[c]	δ_C[a]	δ_H[b], mult, *J* in Hz, integration	HMBC[c]
1	166.6			166.6		
1-N*H*$_2$		(6.96, br,6.46, br)[d]			(6.96, br, 6.46, br)[d]	
2	129.9			129.9		
3	155.0			155.0		
3-CH_3	11.6	2.55, s, 3H	1, 2, 3	11.6	2.55, s, 3H	1, 2, 3
4	162.3			162.3		
5	27.2	3.59, d, 7.4, 1H	4, 6, 7, 7-CH_3, 8	27.2	3.59, d, 7.4, 1H	4, 6, 7, 7-CH_3, 8
6	120.4	5.43, td, 7.3, 1.0,1H,	4, 5, 8	120.5	5.43, td, 7.3, 1.0, 1H,	4, 5, 8
7	135.4			135.5		
7-CH_3	20.38	1.89, s, 3H	4, 6, 7, 8	20.38	1.89, s, 3H	4, 6, 7, 8
8	111.4	6.36, dd,14.4, 0.7, 1H	6, 7, 7-CH_3, 9	111.4	6.36, dd, 14.4, 0.7, 1H	6, 7, 7-CH_3, 9
9	125.6	7.03, d,14.3, 1H	6, 7, 8, 10	15.7	7.10, d, 14.3, 1H	7, 10
9-N*H*		(9.16, d, 9.8,1H)[d]			(9.33, d, 9.8, 1H)[d]	
10	171.6			165.8		
11	41.0	3.11, d, 7.2, 2H	10, 12, 13, 14, 15	124.1	35.94, ddd, 15.4, 1.7, 1.7, 1H	10, 13
12	125.5	5.68, ddd,15.1, 7.4,7.1,1H	11, 14	146.0	6.91, ddd, 15.3, 6.1, 6.0, 1H	10, 13, 14
13	130.5	6.57, dd,11.2,14.9, 1H	11, 14, 15	30.9	3.05, t, 6.7, 2H	10, 11, 12, 14, 15
14	128.0	5.86, d,11.1, 1H	12, 13, 15-CH_3, 16	123.6	5.27, td, 7.3, 1.5, 1H	12, 13, 15-CH_3, 16
15	133.5			134.6		
15-CH_3	20.45	1.87, s, 3H	14, 15, 16	20.42	1.88, s, 3H	12, 14, 15,
16	111.9	6.45, d,14.3, 1H	14, 15, 15-CH_3, 17	111.6	6.23, dd, 14.4, 0.6, 1H	14, 15, 15-CH_3, 17
17	124.7	6.97, d,14.4, 1H	14, 15, 16, 18	124.9	7.00, d, 14.4, 1H	15, 16, 18
17-N*H*		(9.19, d,10.5,1H)[d]			(9.19, d, 10.5, 1H)[d]	
18	173.1			173.2		
19	36.1	2.37, m, 2H	18, 20, 21	36.1	2.37, m, 2H	18, 20, 21
20	21.63	2.02, m, 2H	18, 19, 21, 22	21.62	2.02, m, 2H	18, 19, 21, 22
21	38.07	3.00, m, 2H	19, 20, 22	38.11	3.00, m, 2H	19, 20, 22
22	200.81			200.80		
23	131.6			131.6		
24	115.6	7.41, dd, 2.3, 3.0, 1H	22, 23, 25, 26, 28	115.6	7.41, dd, 2.3, 3.0, 1H	22, 23, 25, 26, 28
25	147.7			147.7		
25-O*H*		(7.91, br)[d]			(7.91, br)[d]	
26	153.7			153.7		
26-CH_3	56.5	3.92, s, 1H	26, 27	56.5	3.92, s, 1H	26, 27
27	111.8	6.98, dd, 8.3, 2.0, 2H	22, 23, 24, 25, 26	111.8	6.98, dd, 8.3, 2.0, 2H	22, 23, 24, 25, 26
28	122.7	7.53, dd, 8.5, 2.3, 2H	22, 24, 25, 26, 27	122.7	7.53, dd, 8.5, 2.3, 2H	22, 24, 25, 26, 27

[a] 750 MHz. [b] 125 MHz. [c] Correlation from ^1H to ^{13}C. [d] Recorded in acetone-d_6.

a hydroxamate siderophore from *Tenacibaculum* sp.,[8] and an AChE inhibitor marinoquinoline from *Rapidithrix thailandica*.[9]

As part of our continuing effort to discover new bacterial or fungal taxa with biomedical potential from marine environments, the fermentation extract of isolate HC35 that belongs to a recently described *Bacteroidetes* genus *Rapidithrix*[10] was found to exhibit potent antibacterial activity against *Staphylococcus aureus* and the marine *Bacteroidetes* bacterium *Cytophaga marinoflava*. Fractionation of the extract guided by antistaphylococcal activity resulted in the isolation of a pair of novel polyketide-peptides designated ariakemicins A (**1**) and B (**2**).

Strain HC35 was isolated from a sample of subsurface silt collected on the muddy land alongside the Ariake Inland Sea in southwest Japan by the so-called "baiting method" using *E. coli* as bait (Supporting Information). Strain HC35 cultivated at 30 °C for 4 days in modified Marine Broth 2216 (3 L) was lyophilized and then extracted with aqueous EtOH. The extract was concentrated to an aqueous suspension and partitioned between dichloromethane and 60% MeOH and then between 90% MeOH and *n*-hexane. The 90% MeOH layer, in which the antistaphylococcal activity was concentrated, was successively fractionated by gel filtration on Sephadex LH-20 and by flash chromatography on ODS. Purification of the active fraction by two-step HPLC on different reversed-phase supports yielded **1** and **2** as an inseparable mixture (3.1 mg).

The purified material gave a single molecular ion at *m*/*z* 591.2797 $[M + H]^+$ in a FABMS measurement. However,

(6) Spyere, A.; Rowley, D. C.; Jensen, P. R.; Fenical, W. *J. Nat. Prod.* **2003**, *66*, 818–822.
(7) Matsuo, Y.; Imagawa, H.; Nishizawa, M.; Shizuri, Y. *Science* **2005**, *307*, 1598.
(8) Jang, J. H.; Kanoh, K.; Adachi, K.; Matsuda, S.; Shizuri, Y. *J. Nat. Prod.* **2007**, *70*, 563–566.
(9) Kanjana-opas, A.; Panphon, S.; Fun, H.-K.; Chantrapromma, S. *Acta Crystallogr., Sect. E* **2006**, *62*, o2728–o2730.
(10) Srisukchayakul, P.; Suwanachart, C.; Sangnoi, Y.; Kanjana-Opas, A.; Hosoya, S.; Yokota, A.; Arunpairojana, V. *Int. J. Syst. Evol. Microbiol.* **2007**, *57*, 2275–2279.

its ^{1}H and ^{13}C NMR spectra (Table 1) contained several minor signals, which would not have originated from conformational interchange, because they were not simple duplications of the major signals. Unlikely also was tautomerism, because there was no indication of H−D exchange during overweekend acquisition of a set of NMR spectra. Considering both of these points, we concluded that the material was a mixture of two structural isomers having the molecular forumula $C_{32}H_{38}N_4O_7$. The ratio of the components was estimated to be 2:1 on the basis of the signal intensity of H16 (δ_H **1**:6.45/**2**:6.23). Consistent with the index of hydrogen deficiency of 16 and the UV absorption data (228, 278, and 302 nm), the 1D spectra demonstrated a highly unsaturated nature of **1** and **2**: there were a number of olefinic and aromatic methines (δ_H 5.27−7.53; δ_C 111.4−146.0), three amide groups (δ_C **1**:173.1/**2**:173.2; **1**:171.6/**2**:165.8, and 166.6), one each of ketone (δ_C **1**:200.81/**2**:200.80), methoxy (δ_H 3.92s; δ_C 56.5), and methyl (δ_H 2.55s) groups substituted on aromatic rings, two olefinic methyl groups (δ_H **1**:1.87s/**2**:1.88s and 1.89s), and six sp^2 quaternary carbons (δ_C 162.3, 155.0, 153.7, 147.7, 131.6, and 129.9). The relay COSY spectrum contained six allylic crosspeaks δ_H 1.89 (7-C*H*$_3$)/5.43 (H6), 5.43 (H6)/6.36 (H8), 1.87 (15-C*H*$_3$, **1**)/5.86 (H14, **1**), 5.86 (H14, **1**)/6.45 (H16, **1**), 1.88 (15-C*H*$_3$, **2**)/5.27 (H14, **2**), 7.41 (H24)/7.53 (H28) and two other long-range crosspeaks δ_H 2.55 (3-C*H*$_3$)/3.59 (H-5), 3.92 (26-OC*H*$_3$)/6.98 (H27), which when combined with the HSQC and HMBC data helped establish the following six partial structures: 2,4-disubstituted anisolyl group **A**, three consecutive methylene unit **B**, pair of positionally isomeric triene units **C** and **C′**, diene unit **D**, and methyl-bearing unsaturated C$_4$ moiety **E** (Figure 1). The geometry of the disubstituted olefins was

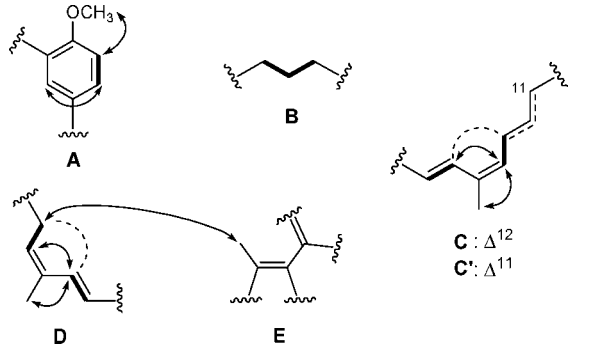


Figure 1. Structural fragments **A**−**E**, key long-range homonuclear couplings (arrows), and NOE correlations (dotted lines) for ariakemicins.

assigned as all *E* from the large coupling constants (J = 14.3−15.1 Hz, Table 1) between each olefinic proton pair, whereas that for the trisubstituted olefins was determined to be all *Z* on the basis of NOESY correlations between the allylic protons across the double bonds (Figure 1).

The connection between units **A** and **B** through a ketone functionality was evident from the HMBC correlations from H28, H27, H21 (δ_H 3.00), and H20 (2.02) to δ_C 200.81 (C22) (Figure 2). The presence of amide linkages between units **B** and **C**, **B** and **C′**, **C** and **D**, and **C′** and **D** was implied by

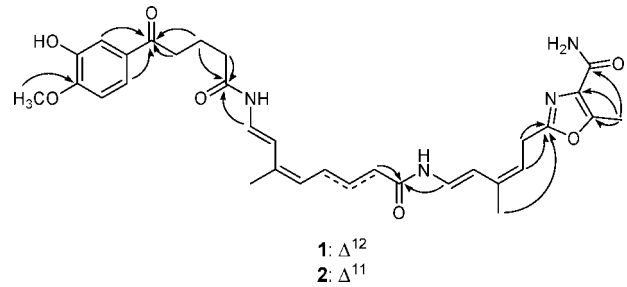


Figure 2. Key HMBC correlations (arrows) for **1** and **2**.

correlations from H20, H19 (δ_H 2.37), and H17 (**1**:6.97/**2**:7.00) to C18 (δ_C **1**:173.1/**2**:173.2) and from H12 (δ_H **1**:5.68/**2**:6.91), H11 (**1**:3.11/**2**:5.94), and H9 (**1**:7.03/**2**:7.10) to C10 (δ_C **1**:171.6/**2**:165.8). This was confirmed by additional TOCSY correlations that emerged in acetone-d_6[11] between exchangeable proton δ_H **1**:9.16/**2**:9.33 (9-N*H*) and H9 and between another exchangeable proton 9.19 (17-N*H*) and H17. Thus, units **C** and **C′** were respectively identified as parts of **1** and **2**. The remaining unit **E** would have been attached at the unclosed terminus of the so-far assembled structure (**A**-**B**-**C**/**C′**-**D**-) in the form of an oxazole, which was in good agreement with the chemical shift values for C1−C4 and aromatic methyl 3-CH$_3$.[12] Although no intra-ring HMBC correlations were available to support this assignment, the presence of long-range homonuclear coupling between 3-CH$_3$ and H5 as well as the structural validity from a biosynthetic point of view justified the proposed structure. Finally, the remaining mass of 17 was accounted for by a phenolic proton (25-OH, δ_H 7.91 br in acetone-d_6) and a primary amide blockage at C1 (1-NH$_2$, δ_H 6.96 br and 6.46 br in acetone-d_6), the latter being suggested by the IR spectral absorption at 1632 cm^{-1} and lack of absorption at 2700−2600 cm^{-1} that corresponds to free carboxylic acid. Thus, the structure of **1** and **2** was completed as shown.

The crude ariakemicins lost their antibacterial activity after a 3-day preservation in basic (0.5% Et$_3$N) and acidic (0.05% TFA) media, and the pure ariakemicin mixture decomposed during NMR experiments in DMSO-d_6. Such instability of the antibiotics would have been conferred by the unusually activated amide bonds, of which the equilibrium of amide−imidic acid tautomerization leans to the latter by participating in the neighboring conjugation systems.[13]

(11) No diagnostic COSY correlations were observed in CD$_3$OH because of the fast exchange of the amide protons (signals broadened), and the experiment in DMSO-d_6 was not successful because of the decomposition of the compounds. Although the solubility of the compounds in acetone-d_6 was poor, it was enough for the homonuclear experiments.

(12) Typical chemical shift values for 2-substituted-5-methyloxazole-4-carboxamides: δ_c 161−163 (C2), 127−130 (C4), 163−167 (4-*C*ONH$_2$), 154−156 (C5), 11−14 (5-*C*H$_3$), and δ_H 2.6 (5-C*H*$_3$). (a) David, J. R.; Kane, P. D.; Moody, C. J. *J. Org. Chem.* **2005**, *70*, 7305–7316. (b) Herrmann, M.; Ehrler, J.; Kayser, H.; Rindlisbacher, A.; Höfle, G. *Eur. J. Org. Chem.* **1999**, 3381–3392. (c) Bagley, M. C.; Buck, R. T.; Hind, S. L.; Moody, C. J. *J. Chem. Soc., Perkin Trans. 1* **1998**, 591–600.

The ariakemicins were tested against a panel of microbial strains constituted by three Gram-positive bacteria (*Brevibacterium* sp., *S. aureus*, and *Bacillus subtilis*), four Gram-negative bacteria (*Cytophaga marinoflava*, *Pseudovibrio* sp., *E. coli*, and *Pseudomonas aeruginosa*), and a yeast (*Candida albicans*).[14] The antibiotic mixture selectively inhibited the growth of Gram-positive bacteria (Table 2), among which

Table 2. Antibacterial Activity of the Ariakemicin Mixture (μg/mL)

strain	2.6[a]	0.26[a]	MIC[b]
Brevibacterium sp. JCM6894	12 (23)[c]	(12)	83
Staphylococcus aureus IFO12732	16	8	0.46
Bacillus subtilis IFO3134	15		83
Cytophaga marinoflava IFO14170			>700
Pseudovibrio sp. MBIC3368			>700
Escherichia coli IFO3301			>700
Pseudomonas aeruginosa IFO3446			>700
Candida albicans IFO1060			>700

[a] Amount of compound (μg) applied to a paper disk. [b] Minimum inhibitory concentration. [c] Diameter of inhibition zone (mm). Values in parentheses denote obscure inhibition zones.

S. aureus was the most affected. The antibiotics had slight cytotoxicity against A549 human lung cancer cells and BHK baby hamster kidney cells with IC_{50} values of 25 and 15 μg/mL, respectively.

The linear and achiral architecture of **1** and **2** that incorporates 3-hydroxy-4-methoxyphenyl and 5-methyloxazole rings along with unsaturated polyketide chain(s) is reminiscent of siphonazoles from *Herpetosiphon* sp.,[15] a marine gliding bacterium belonging to phylum *Chloroflexi*. Production of the related metabolites by two phylogenetically distant taxa may imply the evolutionary diversification of these metabolites from the same molecular ancestry or their common physiological function in marine ecosystems. Either or both, the present study revealed that *Bacteroidetes* bacteria are novel producers of hybrid polyketide-peptides and further encourages extensive investigation of their chemistry. We have found another antibiotic-producing strain from the same phylum, and the responsible ingredient was identified as a novel peptide-alkaloid. The details of the study will be presented in due course.

Acknowledgment. We thank Prof. Ryuichi Sakai (Kitasato University) for assistance with HRFABMS measurements, Sachiko Kawasaki (MBI) for fermentation of HC35 and purification of ariakemicins by HPLC, Tomoe Sasaki (MBI) for cytotoxicity evaluation, and Dr. Sunji Takahashi (RIKEN) for helpful discussion. This study was conducted as part of the project entitled "Construction of a Genetic Resource Library of Unidentified Microorganisms" supported by New Energy Industrial Technology Development Organization (NEDO).

Supporting Information Available: General experimental procedures; isolation, taxonomy, and cultivation of strain HC35; isolation of **1** and **2**; NMR, IR, and UV spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL8007292

(13) The behavior of the compounds described in ref 11 in part demonstrates this speculation.

(14) Oku, N.; Kawabata, K.; Adachi; Katsuta, A.; Shizuri, Y. *J. Antibiot.* **2008**, *61*, 11–17.

(15) Nett, M.; Erol, Ö.; Kehraus, S.; Köck, M.; Krick, A.; Eguereva, E.; Neu, E.; König, G. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 3863–3867.