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Akaeolide, a Carbocyclic Polyketide from Marine-Derived *Streptomyces*

Yasuhiro Igarashi,*[,†] Tao Zhou,[†] Seizo Sato,[‡] Takashi Matsumoto,[§] Linkai Yu,[†] and Naoya Oku[†]

Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan, Central Research Laboratory, Nippon Suisan Kaisha, Ltd., Tokyo Innovation Center, 1-32-3 Nanakuni, Hachioji, Tokyo 192-0991, Japan, and Application Laboratories, Rigaku Corporation, 3-9-12 Matsubara-cho, Akishima, Tokyo 196-8666, Japan

yas@pu-toyama.ac.jp

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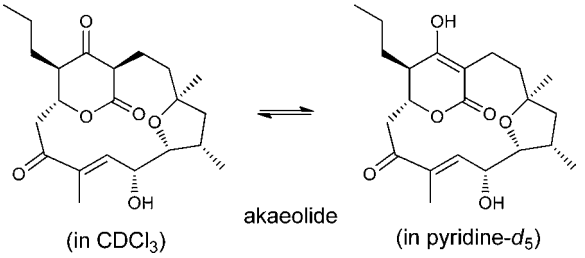
ABSTRACT



Akaeolide, a novel polycyclic polyketide, was isolated from the culture extract of a marine-derived actinomycete belonging to the genus *Streptomyces*. The planar structure of the new compound was elucidated by spectroscopic analysis including NMR and MS, and the absolute configuration was determined by X-ray crystallographic analysis of its chlorinated derivative. Akaeolide possesses a 15-membered carbocyclic framework, apparently derived from the malonate pathway, with a tetrahydrofuran ring and a β-keto-δ-lactone unit.

Polyketides are the most chemically diverse secondary metabolites widely distributed from prokaryotic to eukaryotic microorganisms.[1] Among this group, products of bacterial type I polyketide synthases (PKSs) are especially attractive as a source of drug discovery because these enzymes utilize various starter and extender units to build highly branched and substituted backbones. In contrast, type II or type III PKSs incorporate malonate as a sole substrate for chain elongation, which essentially finalize the formation of polyaromatic cores.[2] Most of the metabolites produced by type I PKS, except for polyenes, contain relatively small conjugated systems that display UV absorption bands around 230−300 nm, distinguishable from metabolites containing highly conjugated systems. In our screening program for structurally rare type I PKS products from bacterial species using HPLC/UV-based chemical analysis,[3] production of an unknown compound with an absorption maximum at 235 nm was observed in the culture extract of *Streptomyces* sp. NPS554, which was isolated from a sediment sample collected at a depth −38 m near Miyazaki Harbor (previously known as Akae Harbor), Miyazaki, Japan.[4] UV-guided isolation from the 1-butanol extract of strain NPS554 cultured in A16 seawater medium resulted in the discovery of akaeolide (**1**), a novel polyketide possessing an unprecedented 15-membered carbocyclic framework. Herein, we report the structure determination and biological properties of **1** (Figure 1).

Akaeolide (**1**) was obtained as an optically active colorless solid ($[\alpha]_D$ +33, *c* 0.10, $CHCl_3$) from the culture extract

[†] Toyama Prefectural University.
[‡] Nippon Suisan Kaisha, Ltd.
[§] Rigaku Corporation.

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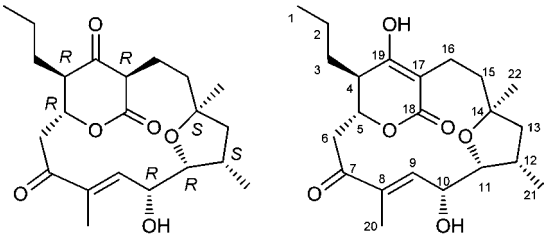

Figure 1. Structures of dominant tautomers of akaeolide (**1**) in $CDCl_3$ and pyridine-d_5.

of strain NPS554 (9.7 mg from 2.3 L of culture). A molecular formula of $C_{22}H_{32}O_6$ was determined by high-resolution ESITOFMS analysis that showed a pseudomolecular ion at m/z 391.2127 $[M - H]^-$ (Δ +0.1 mmu, calcd for $C_{22}H_{31}O_6$). The IR spectrum displayed the absorption bands at 1712 and 1660 cm^{-1}, indicating the presence of carbonyl groups. The UV spectrum measured in alkaline solution showed an absorption band of bathochromic shift at 393 nm that was not observed in acidic or neutral solution, suggesting the presence of a phenolic or enolic functional group.[5] The ^1H NMR spectrum of **1** in $CDCl_3$ displayed the resonances for at least three distinct isomers. However, during the overnight NMR measurement at 27 °C, these isomers converged into one dominant isomer although small peaks for minor isomers were still remaining (Figure S13, Supporting Information). The structure analysis of **1** was thus started with this major isomer present in $CDCl_3$.

^1H and ^{13}C NMR data in combination with the HSQC analysis revealed the presence of 22 carbons attributable to three oxygen-bearing quaternary sp^2 carbons, one quaternary sp^2 carbon, one sp^2 methine, one oxygenated quaternary sp^3 carbon, six sp^3 methylene, six sp^3 methine (three are oxygenenated), and four methyl groups. Since three carbonyls and one C−C double bond accounted four of the seven double-bond equivalents, **1** must possess three rings to satisfy the molecular formula.

Interpretation of 2D NMR spectroscopic data allowed three partial structures to be assembled: a β-keto-δ-lactone, an α,β-unsaturated ketone, and a tetrahydrofuran unit (Figure 2, Table 1). A four-carbon fragment, deduced from a series of COSY correlations from the methyl proton triplet H-1 to a methine H-4, was joined with an another COSY-defined fragment H-5/H-6 by HMBC correlations from H-5 and H-6 to C-4, H-4 to C-6, and H-3 to C-5 to provide a six-carbon fragment with an oxygen substitution at C-5. A COSY-cross peak was observed between H-15 and H-16, and the latter protons were further correlated to H-17. This three-carbon fragment was expanded to include two carbonyl carbons C-18 (δ_C 169.7) and C-19 (δ_C 203.7) each connecting at C-17 on the basis of HMBC correlations from H-16 and H-17 to these carbons. This fragment and the aforementioned six-carbon fragment were joined on the basis of HMBC correlations from H-5 to C-18 and H-5 and H-3 to C-19, establishing a β-keto-δ-lactone substructure bearing three aliphatic substituents.

Table 1. ^1H and ^{13}C NMR Data for Akaeolide (**1**) in $CDCl_3$

position	δ_C[a]	δ_H mult (J in Hz)[b]	HMBC[b,c]
1	13.6, CH_3	0.91, t (7.0)	2, 3
2	19.9, CH_2	1.38[d–f], m	
3	32.2, CH_2	1.56, m	1, 2, 4, 5, 19
		1.53, m	1, 2, 4, 5, 19
4	50.3, CH	2.49[e], m	3, 6, 19
5	76.7, CH	4.85, dd (12.0, 5.5)	3, 4, 6, 18, 19
6	41.5, CH_2	3.77, dd (12.0, 12.0)	4, 5, 7
		2.43, dd (12.0, 5.5)	5, 7, 8
7	200.1, qC		
8	136.2, qC		
9	146.4, CH	6.51, d (8.5)	7, 8, 10, 11, 20
10	68.3, CH	4.64, d (8.5)	8, 9
11	82.2, CH	3.66, d (7.0)	9, 10, 14, 21
12	35.8, CH	2.53, m	13, 21
13	49.3, CH_2	2.14, dd (12.5, 9.0)	11, 12, 15, 21, 22
		1.42[d–f], m	
14	83.0, qC		
15	31.8, CH_2	2.49[d–f], m	22
		1.37[d–f], m	
16	20.2, CH_2	2.50[d–f], m	14, 15, 17, 18, 19
		1.71, m	14, 17, 18
17	54.2, CH	4.59, dd (9.5, 1.5)	15, 16, 18, 19
18	169.7, qC		
19	203.7, qC		
20	11.8, CH_3	1.83, s	7, 8, 9
21	18.0, CH_3	1.19, d (7.5)	11, 12, 13
22	25.3, CH_3	1.43, s	13, 14, 15

[a] Recorded at 125 MHz. [b] Recorded at 500 MHz. [c] HMBC correlations are from proton(s) stated to the indicated carbon. [d–f] Overlapping signals.

Structure elucidation of the second partial structure was started from the vinylic methyl proton singlet H-20 that showed HMBC correlations to the carbonyl carbon C-7 (δ_C 200.1) and olefinic carbons C-8 and C-9. The proton attached to this latter carbon C-9 showed a COSY correlation to the oxymethine proton H-10, establishing this partial structure as an α,β-unsaturated ketone with a methyl and an oxygenated methine substitutions at the α- and β-positions, respectively. An HMBC correlation from H-6 to C-7 allowed this fragment to be connected to the δ-lactone unit through C-6.

The tetrahydrofuran unit was elucidated starting from the tertiary methyl proton singlet (H-22) that showed HMBC correlations to C-13, C-14, and C-15. The proton attached to C-13 showed a COSY correlation to a methine proton H-12, which was in turn correlated to a methyl proton doublet (H-21) and an oxygenated methine (H-11). Finally, an HMBC correlation from H-11 to C-14 established a tetrahydrofuran ring being connected to the δ-lactone unit at C-15. Further HMBC correlations observed from H-10

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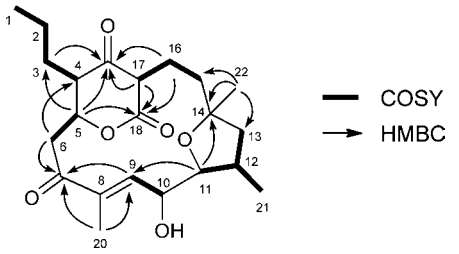

Figure 2. COSY and key HMBC correlations of **1** in $CDCl_3$.

and H-11 to C-9 and from the olefinic proton attached to this carbon back to C-10 and C-11 completed the structure assignment of **1**. The methine proton H-17 flanked by two carbonyl carbons can be dissociated giving the enolized isomer. Further detailed analysis on tautomerizaion using NMR revealed that the enol-form was the major isomer in pyridine-d_5 (Figure 1).[6]

In order to establish the relative configuration of **1**, crystallization was attempted in various solvents but failed to give crystalline solid. As the tautomeric characteristic of this molecule was likely affecting negatively the crystal formation, the configuration of the enolizable carbon C-17 was fixed by chlorinating this carbon by the treatment with *N*-chlorosuccinimide in CH_2Cl_2.[7] The reaction completed in 10 min at room temperature to give a derivative (**2**) selectively chlorinated at C-17 as a single product. After purification by flash chromatography, **2** was crystallized from a mixture of diisopropyl ether and methanol to give plate crystals suitable to X-ray crystallographic analysis. On the basis of the diffraction anisotropy of the chlorine atom the absolute configurations of all seven asymmetric centers in **2** were determined as 4*R*,5*R*,10*R*,11*R*,12*S*,14*S*,17*R*, defining the absolute configuration of **1** except for the chlorinated carbon C-17 (Figures 3 and 4, CCDC accession no. 949379). The relative configuration at C-17 of **1** in $CDCl_3$ was determined by analyzing NOESY spectrum that gave cross peaks for H-17/H-6 (δ_H 3.77), H-17/H-9, H-6 (δ_H 3.77)/H-9. These correlations allowed the placement of the H-17 methine proton and the carbon chain branched at C-5 on the same side of the δ-lactone ring, establishing the C-17 configuration of **1** as *R* (Figure 5).

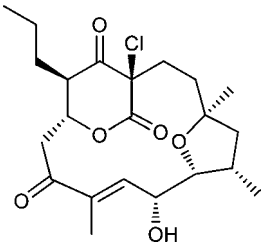

Figure 3. Structure of 17-chloroakaeolide (**2**).

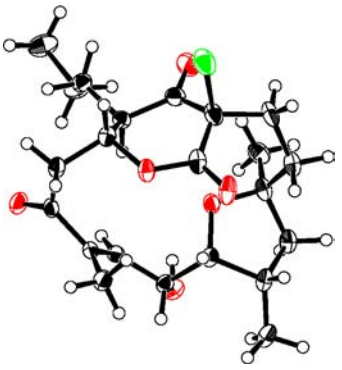

Figure 4. ORTEP drawing of crystal structure of **2**.

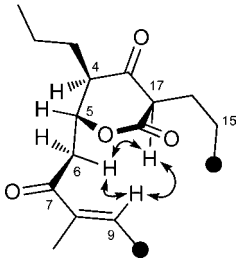

Figure 5. Key NOEs observed for **1** in $CDCl_3$.

Limited biological testing has shown that **1** is active against *Micrococcus luteus* with an MIC value of 25 μg/mL but is inactive to *Escherichia coli* and *Candida albicans*. In addition, **1** displayed modest cytotoxicity to 3Y1 rat fibroblasts with an IC_{50} value of 8.5 $\pm$ 1.5 μM. **1** was not active in adipocyte differentiation assay[8] and also did not show inhibitory effects on *Staphylococcus aureus* and *Enterococcus faecalis* quorum sensing signaling.[9]

Akaeolide (**1**) is featured by its 15-membered carbocyclic structure functionalized with a five-membered cyclic ether and a β-keto-δ-lactone unit. Mangromicin B was described to have the same planar structure as **1**, but the NMR data of these compounds are different and also the sign of the specific rotation is opposite (mangromicin B: $[\alpha]_D^{25.3}$ -24.08, *c* 0.1, MeOH; **1**: $[\alpha]_D^{23}$ $+35$, *c* 0.10, MeOH).[10] It is likely that mangromicin B and **1** are in relationship of stereoisomers. Several compounds exist that might

(6) More than three isomers were recognized in the ^1H and ^{13}C NMR spectra of **1**. In addition to the two keto−enol tautomers defined in this study, other isomeric forms possibly including conformational isomers may exist.

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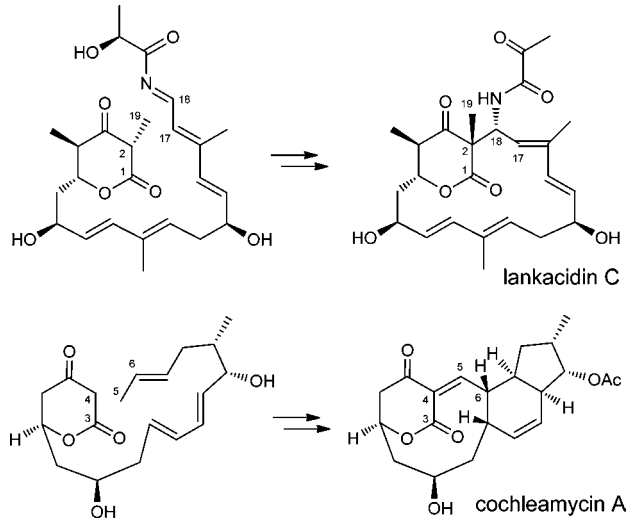

Figure 6. Proposed biosynthetic precursors for carbocyclic formation in lankacidin and cochleamycin.

have a close biosynthetic relationship to **1**. Lankacidin,[11] cochleamycin,[12] and macquarimicin,[13] all of which are the metabolites of actinomycetes, are carbocyclic polyketides bearing a β-keto-δ-lactone unit. In lankacidin biosynthesis, the polyketide chain extension begins from a glycine-derived C-18/C-17 unit and ends with the terminating propionate unit C-19/C-2/C-1 (Figure 6). The C−C bond between C-2 and C-18 is thought to be formed by the nucleophilic addition of the activated methine carbon C-2 to the *N*-acyl imine carbon C-18 with the catalysis of an amine oxidase.[14] Biosynthetic origin of cochleamycin has been elucidated by incorporation of ^{13}C-labeled precursors.[15] Starting from the C-5/C-6 acetate unit, the chain extension terminates with a C-4/C-3 acetate unit accompanied by the δ-lactone formation. The additional C−C bond formation is proposed to arise from the oxidation of C-5 methyl carbon to the aldehyde and the following intramolecular Aldol reaction (Figure 6). Similar to these biosynthetic pathways, an extra C−C bond formation must take place for the construction of macrocyclic structure of **1** in addition to the regular C−C bond formation by PKS. In our preliminary incorporation experiments of ^{13}C-labeled precursors, [1-^{13}C]acetate feeding labeled C-15 and C-18 but not C-16 and C-17. This labeling pattern is consistent with those for lankacidin and cochleamycin, suggesting that **1** is a new member of this minor family of carbocyclic polyketides. Results on our ongoing biosynthetic study of **1** will be reported in a forthcoming paper.

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Supporting Information Available. Experimental details; NMR data and 1D/2D NMR spectra of **1** and **2**; UV and IR spectra of **1**; X-ray crystallographic file in CIF format for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Publication. This manuscript was published ASAP on October 17, 2013. The description of mangromicin B beneath Figure 5 and ref 10 have been updated, and a revised Supporting Information file is available. The revised version was reposted on November 1, 2013.

(10) The ^{1}H NMR spectrum of akaeolide in CD_3OD displayed a different feature from the data described for mangromicin B. The spectrum showed the resonances for at least four isomers which could not be deciphered into any specific structures because of the signal overlapping (Figure S14). This isomeric property was not described for mangromicin B and its NMR assignment was completely accomplished in CD_3OD. Omura, S.; Takahashi, Y.; etc. Novel Mangromicin Compound and Production Method Thereof. WO2013031239, 2013; Japan Patent 2011-191404.

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The authors declare no competing financial interest.