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NOTE

Two new sacrolide-class oxylipins from the edible cyanobacterium *Aphanothece sacrum*

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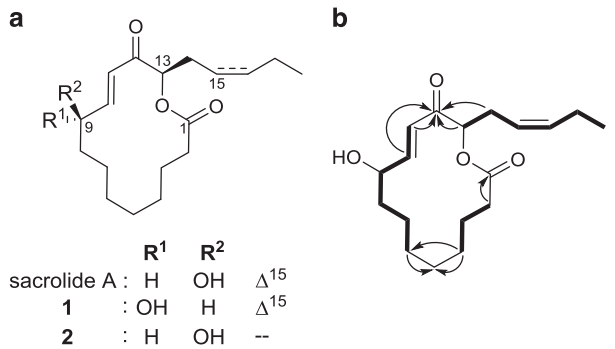
Tradition of eating cyanobacteria is seen in the coastal area of Lake Chad, east and southeast Asian countries, and among Peruvian highlanders.[1] In Japan, consumption of *Nostoc commune*, *N. verrucosum* and *Aphanothece sacrum* ('suizenji-nori' in Japanese) has been reported, only the last of which continues on a commercial basis.[2] Because cyanobacteria are known as a prolific source of bioactive secondary metabolites,[3] edible but less investigated species hold promise for new drug leads.

As part of our program to evaluate the biomedical potential of edible cyanobacteria, we examined the ethanolic extract of *A. sacrum* and discovered sacrolide A as an antimicrobial principle.[2] It belongs to the oxylipin class metabolites, which are enzymatically or chemically derived fatty acid peroxidation products,[4] and from cyanobacteria only four precede it which are as follows: malyngic acid,[5] mueggelone,[6] (9*R*, 10*E*, 12*Z*, 15*Z*)-octadecatrienoic acid[7] and (9*R*, 10*E*, 12*Z*)-octadecadienoic acid.[7] Although oxylipins are known to function as intra- and interspecific signaling molecules or as microbicides in plants,[4] their role in prokaryotes is practically unknown. To gain further insights into the function and structural diversity of prokaryotic oxylipins, congeners of sacrolide A (Figure 1) were pursued in *A. sacrum*, which resulted in the isolation of two new congeners, 9-*epi*-sacrolide A (**1**) and 15,16-dihydrosacrolide A (**2**).



	R1	R2	
sacrolide A :	H	OH	Δ^{15}
1 :	OH	H	Δ^{15}
2 :	H	OH	--

Figure 1 (**a**) Structure of sacrolide A, 9-*epi*-sacrolide A (**1**) and 15,16-dihydrosacrolide A (**2**). (**b**) Selected COSY/TOCSY (bold lines) and HMBC (arrows) correlations for **1**.

The ethanolic extract of the algal colonies was partitioned between dichloromethane and 60% aqueous MeOH, and the former between *n*-hexane and 90% aqueous MeOH. The antimicrobial principles in the latter layer were purified by open column chromatography on silica gel, gel filtration on Sephadex LH-20, normal phase HPLC on silica gel to yield crude sacrolide A, as previously described. NMR-based examination of the peaks eluting before and after sacrolide A revealed the presence of its congeners, which prompted further purification of both fractions by reversed-phase HPLC to yield **1** and **2**, respectively.

Compound **1** has the same molecular formula as sacrolide A ($C_{18}H_{30}O_4$) as evidenced by a molecular ion peak at *m/z* 331.1864 ($[M+Na]^+$, $C_{18}H_{28}NaO_4$, Δ-1.6 mmu) in the HRESITOF-MS analysis.[2] The ^{1}H NMR spectrum of **2** (Supplementary Table S4) assorted all signals from sacrolide A,[2] some of which though shifted upfield and changed shape, indicating that **1** is either a diastereomer or transesterified isomer of sacrolide A. Indeed, interpretation of a full set of NMR spectra supported this idea: the presence of spinsystems $H_2$2/$H_2$3/$H_2$4, $H_2$6/$H_2$7/$H_2$8/H9/H10/H11 and H13/$H_2$14/H15/H16/$H_2$17/$H_3$18 was evident from the sequence of COSY and TOCSY crosspeaks (Figure 1); the 10*E* and 15*Z* geometries from $^3J_{H10\text{-}H11} = 15.8$ Hz and $^3J_{H15\text{-}H16} = \sim 10$ Hz; placement of carboxylate at C1 from HMBC correlations H2/C1 (δ_H 2.56/δ_C 172.8 Figure 1); connectivity between C4 and C6 through C5 from HMBC correlations H4/C5 (δ_H 1.32/δ_C 27.0), H4/C6 (δ_H 1.32/δ_C 25.4) and H6/C5 (δ_H 1.36/δ_C 27.0); interruption of ketone between C11 and C13 from HMBC correlations H11/C12 (δ_H 6.47/δ_C 196.8) and H13/C12 (δ_H 5.13/δ_C 196.8). Thus, an acyloxy backbone with an α,β-unsaturated-α',γ-ketodiol motif, present in sacrolide A, was assembled. Formation of an ester linkage between C1 and C9 was denied by the fact that **1** and sacrolide A both gave the same oxidation product (see below), leaving

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diastereomeric structure as the only possibility. Difference in a coupling constant between H9 and H10 (**1**, 7.6 Hz; sacrolide A,[2] 4.7 Hz) and an inverted intensity of a pair of NOESY correlations H9/H10 and H9/H11 (**1**, weak and strong; sacrolide A, strong and weak: see S14 and S15) endorsed this assignment.

To specify which of the two stereocenters (C9 or C13) is epimerized, **1** and sacrolide A were separately oxidized with MnO_2 and CD spectra of the resulting diketone products **3** was compared, which retain the configuration of C13 from the mother compounds (Supplementary Figure S5). Because both **3** exhibited a weak but obvious negative CD Cotton effect, they were enantiomerically identical. Considering the (9*R*,13*R*) configuration of sacrolide A, **1** was concluded to have a (9*S*, 13*R*) configuration.

Compound **2** gave a molecular ion at *m/z* 333.2002 $[M+Na]^+$ ($C_{18}H_{30}O_4$, $\Delta -3.4$ mmu) in an ESITOFMS measurement, which is larger by two hydrogens than sacrolide A. The ^1H NMR spectrum of **2** was reminiscent of sacrolide A, as represented by the keto-conjugated *E*-olefin (H10: δ_H 6.95 dd, $J=4.7$ and 15.8 Hz, H11: δ_H 6.57 dd, $J=1.4$ and 15.9 Hz, Supplementary Table S6) and adjacent two oxymethine (H9: δ_H 4.50 m, H13: δ_H 5.13 dd, $J=6.7$ and 6.9 Hz) signals. However, signals for two olefinic methines (δ_H 5.30 and δ_H 5.54) and allylic methylenes (δ_H 2.61, 2.56, and 2.06) were missing, implying the saturation of the side chain in **2**. This assignment was confirmed by HMBC correlations from a methyl proton (H18: δ_H 0.89) to two aliphatic carbons (C16: δ_C 31.4 and C17: δ_C 22.4). A closer similarity of the ^1H NMR chemical shift values and coupling constants to those of sacrolide A, rather than of **1**, verified the same conformation and relative configuration to sacrolide A. The positive sign of the optical rotation was also the same (**2**: $[\alpha]_D^{24.1}$ +4.9, c 0.038: sacrolide A: $[\alpha]_D^{24.1}$ +24.5, c 0.392, both in MeOH), supporting the (9*R*,13*R*)-absolute stereochemistry. Thus, the structure of **2** was concluded to be a 15,16-dihydro derivative of sacrolide A.

Compound **1** was inactive against four microorganisms sensitive to sacrolide A (*Staphylococcus aureus*, *Streptomyces lividans*, *Saccharomyces cerevisiae* and *Penicillium chrysogenum*) at the tested concentration range, while **2** was marginally active against eumycetes (Table 1). These results demonstrate that the 9*S*-hydroxy group is crucial for the antimicrobial activity of sacrolide A but unsaturation at the aliphatic tail is less so against eumycetes. A substantial number of oxylipins bear an α,β-unsaturated ketone motif and are known to disable a certain set of redox-controlled proteins by modifying the active site sulfhydryl groups.[8,9] Sacrolide A belongs to the same oxylipin class, but loss of the activity upon epimerization of C9 suggests that it is more of a target-specific inhibitor than promiscuous Michael acceptor.[10]

Conservation of an *S*-configuration at C13 among sacrolide A, **1** and **2** implies that hydroxylation of this carbon is an enzymatic process. In contrast, variation in the chirality of C9 carbinol and the degree of unsaturation at C15 is allowed, underpinning a non-enzymatic formation of α,β-unsaturated-γ-ketol from 12,13-allene oxide precursors (Supplementary Scheme S7).[11]

Table 1 Comparison of the antimicrobial activity among sacrolide A congeners (MIC μg ml^{-1})

Microbe	*1*	*2*	*Sacrolide A*
Gram-positive			
Staphylococcus aureus	8.0≦	8.0≦	0.5
Streptomyces lividans	8.0≦	8.0≦	1.0
Yeast			
Saccharomyces cerevisiae	8.0≦	8.0	8.0
Fungus			
Penicillium chrysogenum	8.0≦	8.0	1.0

Abbreviation: MIC, minimum inhibitory concentration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Oku, N., Yonejima, K., Sugawa, T. & Igarashi, Y. Identification of the n-1 fatty acid as an antibacterial constituent from the edible freshwater cyanobacterium *Nostoc verrucosum*. *Biosci. Biotechnol. Biochem.* **78**, 1147–1150 (2014).
2 Oku, N., Matsumoto, M., Yonejima, K., Tansei, K. & Igarashi, Y. Sacrolide A, a new antimicrobial and cytotoxic oxylipin macrolide from the edible cyanobacterium *Aphanothece sacrum*. *Beilstein J. Org. Chem.* **10**, 1808–1816 (2014).
3 Leão, P. N., Engene, N., Antunes, A., Gerwick, W. H. & Vasconcelos, V. The chemical ecology of cyanobacteria. *Nat. Prod. Rep.* **29**, 372–391 (2012).
4 Mosblech, A., Feussner, I. & Heilmann, I. Oxylipins: structurally diverse metabolites from fatty acid oxidation. *Plant Physiol. Biochem.* **47**, 511–517 (2009).
5 Cardellina, J. H. II & Moore, R. E. Malyngic acid, a new fatty acid from *Lyngbya majuscula*. *Tetrahedron* **36**, 993–996 (1980).
6 Papendorf, O., König, G. M., Wright, A. D., Chorus, I. & Oberemm, A. Mueggelone, a novel inhibitor of fish development from the fresh water cyanobacterium *Aphanizomenon flos-aquae*. *J. Nat. Prod.* **60**, 1298–1300 (1997).
7 Murakami, N., Shirahashi, H., Nagatsu, A. & Sakakibara, J. Two unsaturated 9*R*-hydroxy fatty acids from the cyanobacterium *Anabaena flos-aquae f. flos-aquae*. *Lipids* **27**, 776–778 (1992).
8 Farmer, E. F. & Davoine, C. Reactive electrophile species. *Curr. Opin. Plant Biol.* **10**, 380–386 (2007).
9 Mueller, M. J. & Berger, S. Reactive electrophilic oxylipins: pattern recognition and signaling. *Phytochemistry* **2009**, 1511–1512 (2009).
10 Robert F. Bruns, R. F. & Watson, I. A. Rules for identifying potentially reactive or promiscuous compounds. *J. Med. Chem.* **55**, 9763–9772 (2012).
11 Andreou, A., Brodhun, F. & Feussner, I. Biosynthesis of oxylipins in non-mammals. *Prog. Lipid Res.* **48**, 148–170 (2009).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)