Aurantosides D, E, and F: New Antifungal Tetramic Acid Glycosides from the Marine Sponge *Siliquariaspongia japonica*[1]

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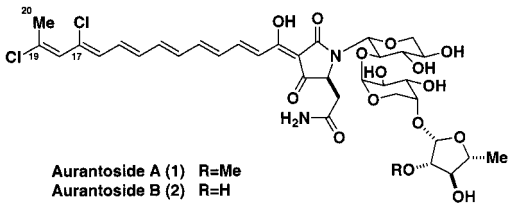
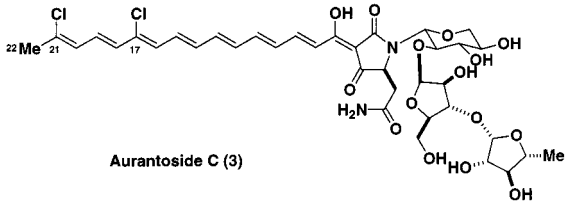
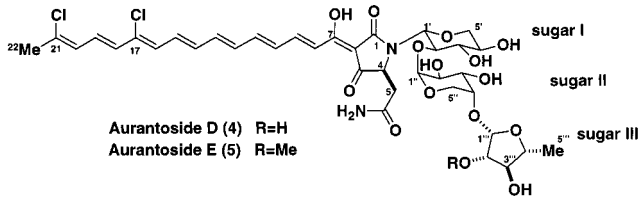
Received January 6, 1999

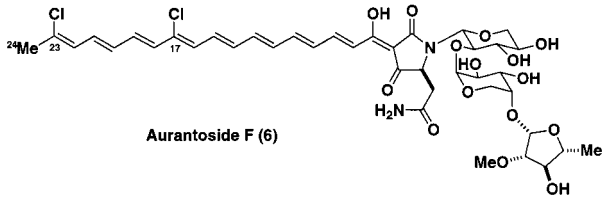
Aurantosides D−F (**4**−**6**), new polyene tetramic acids comprising an *N*-trisaccharide unit, have been isolated from the marine sponge *Siliquariaspongia japonica*. Their structures were determined by spectral and chemical methods. A reinvestigation of NMR data of the previously isolated aurantosides A and B led to revision of the geometry of the terminal double bond. Aurantosides exhibit potent antifungal activity against *Aspergillus fumigatus* and *Candida albicans*.

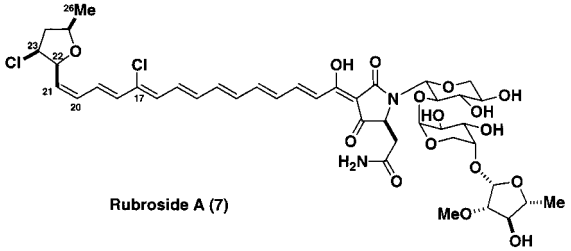
Aurantosides A (**1**) and B (**2**) are polyketide metabolites isolated from the marine sponge *Theonella swinhoei*;[2] they are composed of a dichlorohexaene, a tetramic acid, and a trisaccharide unit; these features resemble erythroskyrin[3] and lipomycins.[4] The aurantosides were originally obtained as antifungal and cytotoxic constituents and later found to inhibit binding of interleukin-6 to its receptors (unpublished data). Quite recently, Schmitz and co-workers[5] reported aurantoside C (**3**), which is lethal to brine shrimp, from the Philippine sponge *Homophymia conferta* (Theonellidae). In our continuing search for potential drugs from Japanese benthic invertebrates, the extract of the marine sponge *Siliquariaspongia japonica* [6] collected off Hachijo-jima Island showed antifungal activity against *Aspergillus fumigatus* and *Candida albicans*. Bioassay-guided isolation afforded three new aurantosides D (**4**), E (**5**), and F (**6**). This paper describes the isolation, structure elucidation, and biological activities of these compounds as well as revision of the stereochemistry of aurantosides A (**1**) and B (**2**).











Results and Discussion

The EtOH extract of the sponge (400 g wet wt) was separated by a series of solvent partitionings. The active *n*-BuOH and 90%MeOH layers were fractionated by flash chromatography on ODS followed by reversed-phase HPLC to afford aurantoside D (**4**, 1.4 mg, 3.5×10^{-4} % yield, based on wet wt), aurantoside E (**5**, 57.9 mg, 1.4×10^{-2} %), and aurantoside F (**6**, 2.9 mg, 7.3×10^{-4} %).

The major antifungal metabolite aurantoside E (**5**) had a molecular formula of $C_{38}H_{48}Cl_2N_2O_{15}$ as established by HRFABMS and ^{13}C NMR data. The ^1H NMR spectrum of aurantoside E[7] was similar to that of aurantoside A (**1**)[2] except for the presence of two additional olefinic methine protons (Table 1). Interpretation of the COSY spectrum disclosed that aurantoside E had the polyene chain from H-8 to H-16, the tetramic acid core, and the trisaccharide unit found in aurantoside A. Also indicated were three contiguous olefinic protons (H-18−H-20); of these H-18 was long-range coupled to H-16, while H-20 was coupled to an olefinic methyl (C-22) at 2.22 ppm. HMBC data connected C-16 and C-18 through a carbon at 134.7 ppm and C-20 and C-22 via a carbon at 135.5 ppm. The ^{13}C chemical shifts and the molecular formula of aurantoside E indicated that both C-17 and C-21 were chlorinated. Therefore, auranto-

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10.1021/np9900021 CCC: $18.00
Published on Web 05/29/1999

Table 1. NMR Data for Aurantosides D (**4**), E (**5**), and F (**6**) in CD_3OD

	4		**5**	**6**
position	^{1}H mult (*J*, Hz)	^{13}C mult	^{1}H mult (*J*, Hz)	^{1}H mult (*J*, Hz)
1		176.2 s		
2		102.0 s		
3		194.9 s		
4	4.15 br	65.6 d	4.30 br s	4.26 br
5α	2.50 m	38.1 t	2.66 br	2.61 m
β	2.77 dd (3.4,17.0)		2.78 dd (3.6,16.2)	2.79 dd (4.0,16.2)
6		174.3 s		
7		174.8 s		
8	7.47 br	121.9 d	7.24 d (14.4)	7.30 br
9	7.34 br	146.4 d	7.59 dd (11.2,14.4)	7.54 br
10	6.60 m	133.3 d	6.62 m	6.60 m
11	6.75 m	145.2 d	6.89 m	6.85 br
12	6.55 m	135.6 d	6.57 m	6.55 m
13	6.75 m	140.3	6.70 dd (11.9,14.6)	6.70 m
14	6.55 m	137.4 d	6.59 m	6.61 m
15	6.78 m	132.8 d	6.87 dd (11.9,14.2)	6.85 m
16	6.55 m	131.1 d	6.56 m	6.55 m
17		134.7 s		
18	6.46 d (15.0)	132.1 d	6.48 d (14.6)	6.49 d (14.0)
19	6.87 dd (10.4,15.0)	128.9 d	6.93 dd (10.6,14.6)	6.76 dd (14.0,11.1)
20	6.29 d (10.4)	126.1 d	6.33 d (10.6)	6.43 dd (11.1,15.0)
21		135.5 s		6.63 dd (10.4,15.0)
22	2.20 br s	26.7 q	2.22 br s	6.26 d (10.4)
23				
24				2.21 br s
1′		86.2 d	4.50 br s	
2′		81.2 d	3.63 m	
3′	3.44 m	79.2 d	3.48 t (8.9)	3.44 t (9.2)
4′	3.58 m	70.4 d	3.62 m	3.60 m
5′α	3.15 dd (9.5,11.0)	69.2 t	3.20 t (11.3)	3.19 t (11.0)
β	3.82 m		3.86 dd (6.0,11.3)	3.84 m
1″	5.08 br s	103.8 d	5.02 br s	5.04 br s
2″	3.73 m	71.6 d	3.79 dd (2.3,10.6)	3.77 m
3″	3.73 m	70.7 d	3.75 m	3.75 m
4″	3.89 m	76.1 d	3.90 dd (3.0,4.2)	3.89 m
5″α	3.61 m	61.5 t	3.58 dd (3.0,12.4)	3.57 m
β	3.76 m		3.70 br d (12.4)	3.71 m
1‴	4.91 d (3.4)	99.0 d	5.08 d (4.2)	5.08 d (4.6)
2‴	3.85 m	87.3 d	3.68 dd (4.2,8.1)	3.65 dd (4.6,8.2)
3‴	3.73 m	79.7 d	3.89 m	3.88 m
4‴	3.71 m	79.5 d	3.74 m	3.74 m
5‴	1.28 d (5.8)	20.8 q	1.32 d (6.2)	1.30 d (6.4)
OMe		58.3 q	3.34 s	3.34 s

side E has a vinyl unit inserted between C-17 and C-18 of aurantoside A (**1**). The geometry of the olefins was assigned on the basis of ^{1}H−^{1}H coupling constants and NOESY data. Analysis of HMBC data disclosed that the eastern part of aurantoside E (**5**) was identical with that of aurantoside A (**1**). Chiral GC analysis of the acid hydrolysate showed that both xylose and arabinose were in the D-form, whereas the acid hydrolysate of the Lemieux oxidation product of **5** afforded L-aspartic acid, thereby determining 4*S*-stereochemistry. Because aurantosides A and E exhibited almost superimposable NMR signals for the trisaccharide portions, the remaining 5-deoxy-2-*O*-methylarabinofuranose was most likely to have D-stereochemistry.

After completion of the structural study of aurantoside E (**5**), we noticed a significant discrepancy in the chemical shifts of the terminal olefinic methyls in aurantoside E and those of aurantoside A;[2] Me-22 resonated at δ_H 2.22 and δ_C 26.5 in aurantoside E, while Me-20 in aurantoside A appeared at δ_H 2.38 and δ_C 23.6. The stereochemistry for the Δ^{20} olefin in aurantoside E was assigned *Z* on the basis of a NOESY cross-peak between H-20 and Me-22. However, the geometry of the Δ^{18}-olefin in aurantoside A had not been rigorously determined. A NOESY spectrum of aurantoside A measured under the same condition, revealed a cross-peak between H-16 and H-18, but not between H-18 and Me-20. Therefore, the Δ^{18} double bond of aurantoside A has *E* geometry.[8]

The ^{1}H and ^{13}C NMR spectra of aurantoside D (**4**), which is more polar than **5**, were almost superimposable on those of aurantoside E (**5**), except for the absence of the C-2‴-methoxy group, which is replaceable by a hydroxyl group by interpretation of 2D NMR spectra. This was confirmed by FABMS data. Therefore, **4** is the 2‴-des-*O*-methyl derivative of aurantoside E.

The molecular formula of aurantoside F (**6**) was larger by a C_2H_2 unit than that of aurantoside E. Interpretation of the COSY spectrum readily implied the presence of an additional vinyl group between C-17 and C-18 of aurantoside E. Detailed analysis of 2D NMR spectra led to the structure of aurantoside F (**6**) as shown.

The aurantosides are cytotoxic against P-388 murine leukemia cells and antifungal against *A. fumigatus* and *C. albicans* as shown in Table 2. Interestingly, aurantoside E was significantly more potent against both fungi than aurantosides A and B. It is also noted that aurantoside F was 10 times more cytotoxic against P-388 murine leukemia cells than aurantosides D or E.

Experimental Section

General Experimental Procedures. NMR spectra were recorded either on a JEOL α-500 or α-600 spectrometer.

Table 2. Biological Activities of Aurantosides

compound	cytotoxicity[a]	antifungal activity[b]	
		C. albicans	*A. fumigatus*
aurantoside A (**1**)	>5.0	11.3 (1.25)	18.0 (0.16)
aurantoside B (**2**)	>5.0	11.8 (0.63)	17.2 (0.16)
aurantoside D (**4**)	0.2	9.5	11.0
aurantoside E (**5**)	0.2	9.7 (0.16)	13.6 (0.04)
aurantoside F (**6**)	0.05	inactive	inactive

[a] IC_{50} μg/mL against P-388 murine leukemia cells. [b] Inhibitory zone (mm) at 2 μg/disk (8 mm ϕ) and MIC value (μg/mL) in parenthesis.

Chemical shifts were referenced to the solvent (δ_C 49.0; δ_H 3.30 in CD_3OD). Standard pulse sequences were employed for the 2D NMR experiments. NOESY spectra were measured with a mixing time of 500 ms. FABMS were obtained on a JEOL SX102 spectrometer. Optical rotations were measured on a JASCO DIP-371 digital polarimeter.

Biological Material. The vermilion sponge *Siliquariaspongia japonica* (family Theonellidae, order Lithistida) was collected at a depth of 15 m off Hachijo-jima Island, 300 km south of Tokyo. The main skeleton was an interlocked mass of small tetracrepid desmas of 150-μm diameter, which were caltroplike in having the cladi more or less equal in length and shape. These desmas have characteristic conical spines. At the surface there was a thin discontinuous layer of discotriaenes with diameters of 100−140 μm, of rounded or slightly irregular outline and irregular margins, and they had very short rhabds. Microrhabds formed a thick cover at the surface and were dispersed in the interior. They were of three sorts: small thin centrotylote, ca. 18 × 0.5 μm; long, profusely spined oxeotes, ca. 35 × 4 μm; and thick, almost smooth spindles, ca. 25 × 6 μm. A voucher specimen (ZMAPOR.13013) was deposited at the Zoological Museum of the University of Amsterdam, The Netherlands.

Extraction and Isolation. The frozen sponge (400 g wet wt) was extracted with EtOH (3 × 1 L), and the concentrated extract was partitioned between H_2O (500 mL) and Et_2O (3 × 500 mL). The Et_2O phase was partitioned between *n*-hexane and MeOH−H_2O (9:1), while the H_2O layer was partitioned between H_2O and *n*-BuOH. The active *n*-BuOH and 90%MeOH-soluble portions were combined (2.5 g) and flash chromatographed on ODS with aqueous MeOH. The 90% MeOH eluate was fractionated by MPLC on ODS with CH_3CN−H_2O (55:45) containing 0.05% TFA to yield nine fractions. The second fraction of the ODS MPLC was separated by HPLC on ODS with CH_3CN−H_2O−TFA (55:45:0.05) followed by ODS HPLC with MeOH−H_2O−TFA (85:15:0.05) to yield aurantoside D (**4**, 1.4 mg, 3.5×10^{-6} %). The third fraction from the ODS MPLC was purified in the same way to afford aurantoside E (**5**, 57.9 mg, 1.4×10^{-4} %). The fifth fraction was repeatedly purified by ODS HPLC with (a) CH_3CN−H_2O−TFA (55:45:0.05), (b) MeOH−H_2O−TFA (90:10:0.05), and (c) CH_3CN−H_2O−TFA (60:40:0.05) to furnish aurantoside F (**6**, 2.9 mg, 7.3×10^{-6} %).

Aurantoside D (4): red amorphous solid, $[\alpha]^{24}_D$ −536° (*c* 0.001, MeOH); UV(MeOH) λ_{max} (log ϵ) 244 (4.30), 433 (4.83) nm; HRFABMS *m/z* 827.2150 (calcd for $C_{37}H_{45}{}^{35}Cl_2N_2O_{15}$, 827.2197); ^{1}H NMR data, see Table 1.

Aurantoside E (5): red amorphous solid, $[\alpha]^{24}_D$ −1038° (*c* 0.001, MeOH); UV(MeOH) λ_{max} (log ϵ) 250 (4.12), 423 (4.99) nm; UV (0.01 N HCl in MeOH) λ_{max} (log ϵ) 342 (4.30), 472 (4.97) nm; UV (0.01 N NaOH in MeOH) λ_{max} (log ϵ) 250 (4.34), 423 (5.04), 448 (5.01) nm; HRFABMS *m/z* 841.2279 (calcd for $C_{38}H_{47}{}^{35}Cl_2N_2O_{15}$, 841.2353); ^{1}H and ^{13}C NMR data, see Table 1.

Aurantoside F (6): red amorphous solid, $[\alpha]^{24}_D$ −1012° (*c* 0.001, MeOH); UV(MeOH) λ_{max} (log ϵ) 440 (4.79), 465 (4.85) nm; HRFABMS *m/z* 868.2622 (calcd for $C_{40}H_{50}{}^{35}Cl_2N_2O_{15}$, 868.2588); ^{1}H NMR data, see Table 1.

Determination of the Absolute Stereochemistry of Xylose and Arabinose Residues in Aurantoside E (5). Aurantoside E (**5**) (1.5 mg) in 10% HCl−MeOH (1.0 mL) was heated at 100 °C for 2 h. After evaporation of the solvent, the residue was chromatographed on ODS with H_2O and MeOH. The H_2O fraction was evaporated and treated with trifluoroacetic anhydride (0.2 mL) in CH_2Cl_2 (0.2 mL) at 100 °C for 5 min in a screw-capped vial. The reaction mixture was dried in a stream of N_2 and dissolved in CH_2Cl_2 (0.1 mL); a 2-μL portion of the solution was subjected to GC analysis on a Chirasil-L-Val capillary column (25 m × 0.25 mm, i.d.); detection, FID; initial temperature 50 °C for 6 min; final temperature 160 °C for 1 min; temperature was raised at 4 °C min^{-1}. Retention times: L-Xyl (16.785, 20.283 min), D-Xyl (16.427, 19.963 min), L-Ara (17.365, 20.325 min), D-Ara (17.385, 20.740 min): products from aurantoside E, 15.670, 16.927, 19.403, and 20.478 min. Because the retention times fluctuated, identity of the peaks was examined by co-injection with the standards.

Determination of the Absolute Stereochemistry at C-4 of Aurantoside E (5). To a solution of aurantoside E (**5**) (1.0 mg) in H_2O (0.1 mL) was added $KMnO_4$ (0.25 mL of 10 mg/mL solution in H_2O) and $NaIO_4$ (0.3 mL of 10 mg/mL solution in H_2O) and the mixture stirred at room temperature for 10 min. The reaction mixture was centrifuged for 10 min. The supernatant was evaporated to afford a residue that was dissolved in 6N HCl (1.0 mL); the mixture was heated at 105 °C for 2 h. After evaporation of the solvent, the residue was chromatographed on ODS with H_2O and MeOH. The H_2O fraction was dissolved in 10% HCl in MeOH (0.5 mL) and heated at 100 °C for 2 h. After removal of the solvent in a stream of N_2, CH_2Cl_2 (0.2 mL) and trifluoroacetic anhydride (0.2 mL) were added to the residue, and the mixture heated at 100 °C for 5 min in a screw-capped vial. The solvents were removed in a stream of N_2, and the residue was dissolved in CH_2Cl_2 (0.1 mL); a 2-μL portion was subjected to GC analysis on a Chirasil-L-Val capillary column (25 m × 0.25 mm, i.d.); detection, FID; initial temperature 80 °C for 5 min; final temperature 200 °C for 10 min; temperature was raised at 4 °C min^{-1}. Retention times: L-Asp (12.600 min), D-Asp (12.940 min); product from aurantoside E (12.785 min). Because the retention times fluctuated, identity of the peaks was confirmed by co-injection with the standards.

Acknowledgment. We are grateful to Professor P. J. Scheuer of University of Hawaii for reading the manuscript. We also thank Dr. K. Suzuki, Yamanouchi Pharmaceutical Co., Ltd., for antifungal tests. This work was partly supported by Grant-in-Aids for Scientific Research from the Ministry of Education, Science, Sport, and Culture of Japan. A JSPS Research Fellowship for Young Scientists to N. U. S. is also acknowledged.

Supporting Information Available: Copies of the ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC, NOESY and mass spectral data for aurantosides D−F. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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(7) Aurantosides gave better NMR spectra in CD_3OD than in C_5D_5N.
(8) One of the referees pointed out that the lack of a NOESY cross-peak is not sufficient to revise the olefin geometry, which led us to determine the $^3J_{CH}$ values for **1** and **5**: the values of 6.2 Hz between H-18 and C-20 in aurantoside A and 3.8 Hz between H-20 and C-22 in aurantoside E were in agreement with the tendency that the value for the trans-isomer is larger than that of the cis-isomer (Marshall, J. L. *Carbon−Carbon and Carbon-Proton NMR Couplings*; Verlag Chemie International: Deerfield Beach, FL, 1983; pp 33−38).

NP9900021