Aurantoside C, a New Tetramic Acid Glycoside from the Sponge *Homophymia conferta*

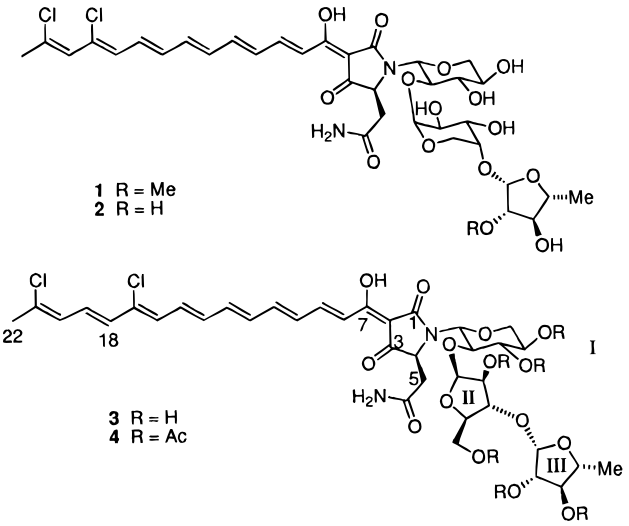
Dietmar Wolf,[†] Francis J. Schmitz,*[,†] Feng Qiu,[‡] and Michelle Kelly-Borges[§]

Department of Chemistry and Biochemistry, The University of Oklahoma, Norman, Oklahoma 73019-0370, Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma, 74078-3071, and Faculty of Health, Science and Technology, UNITEC Institute of Technology, Private Bag 92025, Auckland, New Zealand

Received June 26, 1998

A new tetramic acid glycoside, aurantoside C (**3**), was isolated from the sponge *Homophymia conferta* and the structure was determined by detailed 1D and 2D NMR analysis of the natural product and a hexaacetate derivative.

Sponges of the family Theonellidae have been the subject of extensive chemical studies and have yielded a wide range of structurally novel and bioactive compounds, e.g. terpenoids,[1] secosteroids,[2] polyketides,[3] cytotoxic[4] and antitumor[5] macrolides, cytotoxic cyclic peptides,[6] and antifungal glycopeptides.[7] Among the cytotoxic metabolites reported are aurantosides A (**1**)[8,9] and B (**2**),[8] orange pigments consisting of a chlorinated polyene chain attached to a tetramic acid bearing a trisaccharide moiety. In routine pre-screening of sponge extracts for potential anticancer drug leads we found that the extracts of a sponge from the Philippines, *Homophymia conferta* Pulitzer-Finali 1982 (Order Astrophorida, lithistid, Family Theonellidae) were toxic to brine shrimp.[10] Fractionation of these extracts resulted in the isolation of a new tetramic acid glycoside, aurantoside C (**3**), which differs from aurantoside A and B in polyene chain length and in the trisaccharide structure. Herein we describe the structure elucidation of **3** by spectroscopic methods and also its conversion to a hexaacetate (**4**).



Methanol and MeOH/CH_2Cl_2 extracts of the sponge were combined and subjected to solvent partitioning[11] to afford hexane, CH_2Cl_2 and *n*-butanol soluble materials. The *n*-butanol soluble fraction was toxic to brine shrimp, LC_{50} ~90 μg/mL. Flash chromatography on silica gel followed by C_{18} RP-HPLC yielded **3** as a red-colored amorphous powder. Aurantosides A and B were not detected. An electrospray mass spectrum of **3** showed clusters of ions at *m/z* 855 (106), 853 (82), 851 (21) $(M + Na)^+$ and 833, 831, 829 $(M + H)^+$ and MALDI-TOF MS produced ion clusters at *m/z* 877, 875, 873 $(M - H + 2Na)^+$ and 855, 853, 851 $(M + Na)^+$. The isotopic pattern in these mass spectra indicated the presence of two chlorines and this low resolution mass data considered together with ^1H and ^{13}C NMR data pointed to a molecular formula of $C_{37}H_{46}Cl_2N_2O_{15}$. FABMS analysis of **3** was unsuccessful but HRFABMS analysis of the acetylated product **4** was obtained and confirmed this formula (see experimental). The UV−visible spectrum (H_2O) showed absorptions characteristic of a polyene system (422, 252 cm^{-1}) like that of aurantosides A and B and also exhibited the same type of pH dependent shifts. NMR analysis was complicated by limited solubility (solutions of < 3 mg/mL at elevated temperatures gave well-resolved ^1H and ^{13}C NMR spectra) and sample decomposition. After experimentation with polar and nonpolar solvents it was found that the optimum solvent system was CD_2Cl_2: CD_3OD (10:1).

The ^1H NMR spectrum (600 MHz, 45 °C) showed 12 resolved downfield signals (7.6−6.2 ppm), 17 resolved resonances in range from 5.1 to 3.2 ppm (typical for saccharides), one vinylic methyl (δ 2.22, s) and one aliphatic methyl group signal (δ 1.35, d, J = 6.5 Hz).

Analysis of the COSY and TOCSY spectra confirmed the coupled spin system corresponding to H-8 to H-16 and the chemical shifts and coupling constants were nearly identical to those found for aurantosides A (**1**) and B (**2**). The singlet proton signal for H-18 in **1** and **2** was missing in **3** and in its place were signals for three coupled olefinic signals (COSY) consistent with an extended polyene chain as in **3** with the 3-proton spin system flanked by quaternary carbons. The C-18 to C-22 unit connection to the remainder of the chain was confirmed by results of COSY and TOCSY experiments and an HMBC experiment, see Table 1. The ^{13}C NMR data assigned by HMQC and HMBC were also consistent with the polyene side chain. The stereochemistry of the terminal portion of the polyene chain could be assigned as 16Z, 18E, 20Z based on a large coupling constant (14.6 Hz) for H-18 and NOEs of H-16 with H-18, and H-20 with H-22. The tetramic acid unit with its pendant acetamide group was deduced from COSY data (−CH−CH_2), HMQC, HMBC data (H-5 to C-6; H-9 to C-7)

* To whom correspondence should be addressed. Tel: (405)325−5581. Fax: (405) 325-6111. E-mail: fjschmitz@chemdept.chem.ou.edu.
[†] University of Oklahoma.
[‡] Oklahoma State University.
[§] UNITEC Institute of Technology.

10.1021/np980283x CCC: $18.00
Published on Web 11/19/1998

Table 1. ^{1}H NMR Data of Aurantoside C (**3**)[a,b]

no.	δ_H m	*J* in Hz	HMBC ^{13}C correl	no.	δ_H m	*J* in Hz	HMBC ^{13}C correl
4	4.41 br			1′	4.60 br		
5a	2.67 br d	16		2′	4.22 br		
5b	2.78 dd	4.0, 16.1	6	3′	3.48 t	9	2′
8	7.22 br d	15.0		4′	3.67 ddd	5, 9, 10	2′
9	7.58 dd	12.0, 15.0	7, 11	5′a	3.23 t	10	1′, 3′, 4′
10	6.59 dd	12.0, 14.6	9	5′b	3.93 dd	5, 10	
11	6.81 dd	11.4, 14.6		1″	5.10 d	4	4″
12	6.52 dd	11.4, 14.6	13, 14	2″	4.14 dd	4, 7.3	3″
13	6.65 dd	11.3, 14.6	11, 15	3″	4.06 t	7.3	2″, 5″, 1‴
14	6.54 dd	11.3, 14.6		4″	3.83 m		
15	6.88 dd	11.0, 14.6	13, 17	5″a	3.51 dd	4, 12	3″
16	6.45 d	11.0	14, 17, 18	5″b	3.56 dd	3, 12	3″
17				1‴	4.96 d	4.7	3″, 3‴
18	6.38 d	14.6	16, 17, 20	2‴	3.95 dd	4.7, 7.5	3‴
19	6.97 dd	10.6, 14.6	17	3‴	3.76 t	7.5	2‴, 4‴
20	6.23 d	10.6	18, 21	4‴	3.84 m		1‴, 3‴
21				5‴	1.35 d	6.5	3‴
22	2.22 br s			OMe			

[a] Assignments based on interpretation of COSY, TOCSY, NOESY, HMQC, and HMBC experiments. [b] $CD_2Cl_2CD_3OD$ (10:1), 45 °C.

Table 2. ^{13}C NMR Data **3** and **4**

no.	**3**[a,b]	**4**[c,d]	no.	**3**[a,b]	**4**[c,d]
1	*e*	174.8	20	125.2 d	125.2
2	*e*	100.3 br	21	135.2 s	135.3
3	197.7 br s	193.2	22	26.8 q	27.0
4	63.1 d	63.0 br	1′	86.0 d[f]	83.9
5	37.2 br t	37.9	2′	80.0 d[f]	76.1
6	173.3 br s	173.4 br	3′	77.9 d	75.2
7	175.0 br s[f]	175.7	4′	69.3 d	69.4
8	121.2 br d	121.2	5′	68.4 t	65.0
9	146.0 br d	145.9	1″	104.9 br d	102.2
10	132.5 d	132.4	2″	77.3 d	75.8
11	144.1 d	144.3	3″	82.9 d	80.2
12	134.5 d	134.4	4″	81.4 br d	77.7
13	139.4 d	139.5	5″	63.1 br t	64.7
14	136.1 d	136.1	1‴	102.0 d	100.8
15	132.2 d	132.2	2‴	77.6 d	77.5
16	130.0 d	130.0	3‴	80.3 d	79.8
17	134.3 s	134.2	4‴	78.8 d	77.7
18	131.0 d	130.9	5‴	20.3 q	20.6
19	128.5 d	128.5	OMe		*g*

[a] Assignments and multiplicity based on HMQC and HMBC. [b] $CD_2Cl_2CD_3OD$ (10:1). [c] CD_2Cl_2. [d] Assignment aided by HMQC and analogy. [e] Not observed. [f] Not observed in ^{13}C NMR, but by correlation in HMBC. [g] Six acetyl groups: 170.9 s, 170.64 s, 170.60 s, 170.57 s, 170.57 s, 170.2 s, 21.3 q, 21.1 q, 21.0 q, 20.84 q, 20.80 q, 20.78.

and comparison of NMR data with the corresponding portion of aurantoside A (**1**). Although the resonances of C-1 and C-2 were not observed in the ^{13}C NMR spectrum of **3** (likely due by tautomerization), signals for these carbons were observed in the spectrum of the more stable acetylated compound **4** (δ_{C-1} = 174 s, δ_{C-2} = 100.3 s). Attempts to trap **3** in one tautomeric form as an enol ether by treatment with CH_2N_2 failed.

From a combination of COSY, TOCSY, HMQC and HMBC data the presence of the three sugar units was confirmed. All of the NMR data, including proton coupling constants, for sugar units I and III were nearly identical to the corresponding sugars in aurantoside B except for a slight upfield shift of the H-2′ resonance of sugar I. Hence sugars I and III were assigned the structures shown. Starting with the anomeric proton signal of sugar II at δ 5.10 (δ_c 104.9) a five carbon sugar was deduced. The coupling constants in this spin system were similar to those of the furanose ring in aurantoside A as were the ^{13}C NMR chemical shifts of the ring carbons. The furanose nature of sugar II was established by the HMBC correlation between H-1″ and C-4″. The proton coupling constants for the ring protons were nearly identical to those of the furanose ring of aurantoside A and hence the same relative stereochemistry was assigned. A NOESY correlation was also observed between H-1″ and H-2″. The connection between sugars I and II was assigned on the basis of a NOESY correlation between H-2′ and H-1″. The HMBC correlations between H-3″/C-1‴ and H-1‴/C-3″ established the site of connection between sugars II and III. NOE's were also detected between H-2″/H-1‴, H-5‴ and between H-1‴/H-3.″

Electrospray MS/MS analysis of **3** produced fragment ions at *m*/*z* 581 (loss of sugars II and III) and 449 (loss of all sugars) from the psuedomolecular ion at *m*/*z* 829, thus giving some additional evidence for the trisaccharide sequence. Connection of the trisaccharide unit to the tetramic acid nitrogen was based on analogy with structures **1** and **2** and on the fact that the carbon chemical shift for C-6 in **3** is virtually the same (± 1 ppm) as found for **1** and **2**. Likewise the proton and carbon chemical shifts for H/C-4,5 are very nearly the same in **1** − **3**. Changing the connection site of the trisaccharide residues to the pendant amide group of the tetramic acid would be expected to bring about noticeable chemical shifts for C-6 and C-4.

Since the sign and magnitude of the optical rotation of **3** is nearly the same as those of **1** and **2**, we assume that the absolute stereochemistry in all three compounds is identical (4S, D-saccharides). Likewise, we assume that the tautomeric form of the tetramic acid depicted is the predominant one as was argued[8] for **1** and **3** based on results of an X-ray study of a tetramic acid.[12] Aurantoside C (**3**) was mildly toxic to brine shrimp, LC_{50} ~50 μg/mL.

Experimental Section

General Experimental Procedures. All solvents were redistilled. Chromatography was performed with Merck Si gel 60 (230−240 mesh) for vacuum flash chromatography. Reversed-phase-HPLC (RP-HPLC) was conducted using a UV detector and a Phenomenex C_{18} column (250 × 10 mm). IR and UV spectra were obtained on Bio-Rad 3240-SPC FT and Hewlett-Packard spectrophotometers, respectively. MALDI-TOF mass spectra were taken on a PerSeptive Biosystems Voyages Elite instrument and electrospray data were obtained with a Sciex API III$^+$ triple-quadrupole mass spectrometer (Perkin-Elmer-Sciex, Inc.). NMR experiments were conducted with a Varian VXR-500 instrument equipped with a Nalorac 3 mm ^{1}H/^{13}C switchable gradient microprobe (MDG-500−3) and a pulsed field gradient driver and with a Varian Inova-600 instrument; signals are reported in parts per million (ppm) referenced to the solvent used.

Animal Material. The sponge was collected from Davao, Philippines, at a depth of 21 m, in 1996, and forms erect fused columns 7−12 cm high, each column 3−10 cm diameter, 18−25 cm total diameter. The surface is smooth and wrinkled at the apex of each column, the texture stony except on the apex of the columns which are softer. Color in life externally and internally is bright orange red. The skeleton consists of curved tracts of strongyloxea 700−900 μm long, perpendicular to the surface of the sponge, the desma skeleton is very dense and individual desmas are heavily knobbed, and the surface is lined with dichotriaenose phyllotriaenes with short rhads. The surface and inner regions are densely crowded with microscleres 9−12 μm long that grade between oxyspherasters or spinorhabds (see Kelly-Borges and Vacelet, 1995). The sponge is *Homophymia conferta* Pulitzer-Finali 1982 (Order Astrophorida, lithistid, Family Theonellidae). A voucher specimen has been deposited at the Natural history Museum, London, United Kingdom (BMNH 1998.1.27.2) and another is kept at the University of Oklahoma (26-PH-96).

Extraction and Isolation. Freshly thawed specimens of the sponge (940 g wet wt., 174 g dry wt. after extraction) were minced and soaked in MeOH (2 × 1000 mL) followed by MeOH−CH_2Cl_2 (1:1, 2 × 1000 mL). All extracts were combined, the solvents removed in vacuo, and the residue subjected to solvent partitioning as described previously[11] to afford hexane (2.0 g), CH_2Cl_2 (1.7 g), and *n*-BuOH (8.6 g) soluble fractions. The *n*-BuOH solubles were fractionated by vacuum flash chromatography over silica gel (SiO_2) using increasing amounts of MeOH in $CHCl_3$ as eluent (30% MeOH to 100% MeOH) to yield four fractions. The third fraction (0.22 g) was further purified by reverse-phase HPLC using 50% H_2O-MeCN containing 0.1% TFA as eluent. The HPLC fraction containing pure aurantoside C (**3**) was partitioned between MeCN/CH_2Cl_2/H_2O to remove TFA. Evaporation of the organic phase yielded pure aurantoside C (**3**).

Aurantoside C (3): $[\alpha]^{D}_{20} = -480$ [*c* 0.2, CH_2Cl_2:MeOH (10:1)]; IR (film) ν_{max} 3360 (br, OH, −CO−NH_2), 2925, 1684 (br), 1140 (C−O); UV−vis (H_2O) λ_{max} 426 (ϵ 41600), 252 (ϵ 9700); UV−vis (0.01 N HCl) λ_{max} 448 (ϵ 31800), 324 (ϵ 11200); UV−vis (0.01 N NaOH) λ_{max} 430 (ϵ 47000), 252 (ϵ 10600); ^{1}H and ^{13}C NMR, see Table 1; ESMS: *m*/*z* 855 (100), 853 (82), 851 (21) $[M+Na]^+$, 833 (100), 831 (86), 829 (28) $[M+H]^+$; MALDI-TOF-MS *m*/*z* 877 (100), 875 (79), 873 (27) $[M+2Na-H]^+$, 855 (100), 853 (80), 851 (28) $[M+Na]^+$.

Acetylation of aurantoside C (3). To 78 mg of a fraction containing aurantoside C (^{1}H NMR analysis) and substantial amounts of salt was added 5 mL Ac_2O, 5 mL pyridine and ∼1 mg 4-(dimethylamino)pyridine. After standing at room temperature overnight the reaction was worked up by addition of water followed by extraction with CH_2Cl_2. After evaporation of the solvent, the residue was subjected to C_{18} RP-HPLC separation using 13% H_2O in MeOH with 0.1% TFA as eluent. The orange-colored fraction containing the acetylated product was partitioned vs CH_2Cl_2. Evaporation of the CH_2Cl_2 layer left 7.3 mg of aurantoside C hexacetate (**4**). ^{1}H NMR (CD_2Cl_2) δ 7.60 (1H, dd, J = 14.8, 11.8 Hz, 9-H), 7.17 (1H, br d, J = 14.8 Hz, 8-H), 6.97 (1H, dd, J = 14.6, 10.8 Hz, 19-H), 6.89 (1H, dd, J = 14.6, 11.2 Hz, 15-H), 6.83 (1H, dd, J = 14.6, 11.6 Hz, 11-H), 6.67 (1H, dd, J = 14.5, 11.4 Hz, 13-H), 6.58 (1H, dd, J = 14.6, 11.8 Hz, 10-H), 6.54 (1H, dd, J = 14.6, 11.4 Hz, 14-H), 6.52 (1H, dd, J = 14.5, 11.6 Hz, 12-H), 6.46 (1H, d, J = 11.2 Hz, 16-H), 6.40 (1H, d, J = 14.6 Hz, 18-H), 6.24 (1H, d, J = 10.8 Hz, 20-H), 5.20 (2H, m, 1‴−H, 1″−H), 5.13 (1H, dd, J = 7.2, 5.1 Hz, 2″−H), 5.08 (2H, m, 3′−H, 3‴−H), 4.89 (2H, m, 4′−H, 2‴−H), 4.79 (1H, br s, 1′−H), 4.74 (1H, br s, 2′−H), 4.45 (1H, br s, 4-H), 4.08 (1H, br d, J = 10.7 Hz, 5″−H), 4.01 (1H, dd, J = 10.7, 5.6 Hz, 5′α-H), 3.98 (1H, t, J = 7.2 Hz, 3″), 3.93 (1H, qd, J = 6.5, 6.5, 4‴−H), 3.81 (1H, m, 4″), 3.67 (1H, dd, J = 10.7, 7.0 Hz, 5″α-H), 3.33 (1H, t, J = 10.7 Hz, 5′β-H), 2.85 (1H, m, 5β-H), 2.76 (1H, m, 5α-H), 2.23 (3H, br s, 21-Me), 2.06 (3H, s), 2.04 (3H, s), 2.023 (3H, s), 2.018 (3H, s), 2.00 (3H, s), 1.98 (3H, s), 1.31 (3H, d, J = 6.5 Hz, 4‴-Me); ES-MS *m*/*z* 1107, 1105, 1103 $[M+Na]^+$, 1085, 1083, 1081 $[M+H]^+$; MALDI-TOF-MS *m*/*z* 1129, 1127, 1125 $[M+2Na-H]^+$, 1107, 1105, 1103 $[M+Na]^+$; HR−FABMS *m*/*z* 1081.3023 $(M + H)^+$ (calcd for $C_{49}H_{58}Cl_2N_2O_{21}$, 1081.2987).

Acknowledgment. This work was supported in part by grants from the Department of Commerce, NOAA, Sea Grant Project NA66RG0172, and National Cancer Institute Grant CA52955. We thank the Coral Reef Research Foundation for specimen collection and the Government of the Philippines for permission to collect specimens from their waters through the National Cancer Institute marine collections program. MALDI-TOF mass spectra were performed by the NSF EPSCOR Laser Mass facility at the University of Oklahoma Health Sciences Center and ES mass spectra were performed by the Molecular Biology Resource Facility of the William K. Warren Medical Research Institute, Oklahoma City, OK. We gratefully acknowledge the National Science Foundation (BIR-9512269), the Oklahoma State Regents for Higher Education, the W. M. Keck Foundation and Conoco, Inc. for funds to purchase the Oklahoma Statewide Shared 600 MHz NMR spectrometer.

References and Notes

(1) Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1984**, *25*, 5401−5404.
(2) Umeyama, A.; Shoji, N.; Enoki, M.; Arihara, S. *J. Nat. Prod.* **1997**, *60*, 296- 298.
(3) Kobayashi, J.; Kondo, K.; Ishibashi, M.; Walchli, M. R. *J. Am. Chem. Soc.* **1993**, *115*, 6661−6665.
(4) Kitagawa, I.; Kobayashi, M.; Katori, T.; Yamashita, M. *J. Am. Chem. Soc.* **1990**, *112*, 3710−3712.
(5) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Saki, R.; Higa, T.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 6225−6228.
(6) Hamada, T.; Sugawara, T.; Matsunaga, S.; Fusetani, N. *Tetrahedron Lett.* **1994**, *35*, 719−720.
(7) Bewley, C. A.; Faulkner, D. J. *J. Org. Chem.* **1994**, *59*, 4849−4852.
(8) Matsunaga, S.; Fusetani, N.; Kato, Y.; Hirota, H. *J. Am. Chem. Soc.* **1991**, *113*, 9690−9692.
(9) Schmidt, E. W.; Harper, M. K.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60*, 779- 782.
(10) McLaughlin, J. L.; Chang, C. J.; Smith, D. L. In *Studies in Natural Products Chemistry*; Rahman, A., Ed.; Elsevier: Amsterdam, 1991; pp 383−405.
(11) Fu, X.; Schmitz, F. J.; Lee, R. H.; Papkoff, J. S.; Slate, D. L. *J. Nat. Prod.* **1994**, *57*, 1591−1594.
(12) Nolte, M. J.; Steyn, P. S.; Wessels, P. L. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1057−1065.

NP980283X