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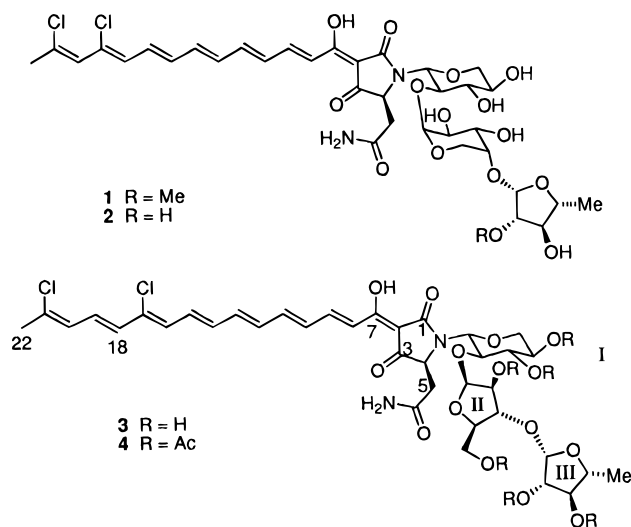
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A new tetramic acid glycoside, aurantioside C (**3**), was isolated from the sponge *Homophymia conferta* and the structure was determined by detailed 1D and 2D NMR analysis of the natural product and a hexaacetate derivative.

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 cytotoxic⁴ and anti-tumor⁵ macrolides, cytotoxic cyclic peptides,⁶ and antifungal glycopeptides.⁷ Among the cytotoxic metabolites reported are aurantiosides 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, aurantioside C (**3**), which differs from aurantioside 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/CH₂Cl₂ extracts of the sponge were combined and subjected to solvent partitioning¹¹ to afford hexane, CH₂Cl₂ and *n*-butanol soluble materials. The

n-butanol soluble fraction was toxic to brine shrimp, LC₅₀ ~90 µg/mL. Flash chromatography on silica gel followed by C₁₈ RP-HPLC yielded **3** as a red-colored amorphous powder. Aurantiosides 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 ¹³C NMR data pointed to a molecular formula of C₃₇H₄₆Cl₂N₂O₁₅. 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 (H₂O) showed absorptions characteristic of a polyene system (422, 252 cm⁻¹) like that of aurantiosides 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 ¹³C NMR spectra) and sample decomposition. After experimentation with polar and nonpolar solvents it was found that the optimum solvent system was CD₂Cl₂: CD₃OD (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 aurantiosides 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 ¹³C 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–CH₂), HMQC, HMBC data (H-5 to C-6; H-9 to C-7)

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

no.	δ _H m	<i>J</i> in Hz	HMBC ¹³ C correl	no.	δ _H m	<i>J</i> 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 CD₂Cl₂CD₃OD (10:1), 45 °C.

Table 2. ¹³C NMR Data **3** and **4**

no.	3 ^{a,b}	4 ^{c,d}	no.	3 ^{a,b}	4 ^{c,d}
1	<i>e</i>	174.8	20	125.2 d	125.2
2	<i>e</i>	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		<i>g</i>

^a Assignments and multiplicity based on HMQC and HMBC. ^b CD₂Cl₂CD₃OD (10:1). ^c CD₂Cl₂. ^d Assignment aided by HMQC and analogy. ^e Not observed. ^f Not observed in ¹³C 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 ¹³C 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 CH₂N₂ 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 ¹³C 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 pseudomolecular 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 argued⁸ 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, LC₅₀ ~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 C₁₈ 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 ¹H/¹³C 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 stronglyloxea 700–900 μ m 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 μ m 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–CH₂Cl₂ (1:1, 2×1000 mL). All extracts were combined, the solvents removed in vacuo, and the residue subjected to solvent partitioning as described previously¹¹ to afford hexane (2.0 g), CH₂Cl₂ (1.7 g), and *n*-BuOH (8.6 g) soluble fractions. The *n*-BuOH solubles were fractionated by vacuum flash chromatography over silica gel (SiO₂) using increasing amounts of MeOH in CHCl₃ 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% H₂O–MeCN containing 0.1% TFA as eluent. The HPLC fraction containing pure auranoside C (**3**) was partitioned between MeCN/CH₂Cl₂/H₂O to remove TFA. Evaporation of the organic phase yielded pure auranoside C (**3**).

Auranoside C (3): $[\alpha]_D^{20} = -480$ [*c* 0.2, CH₂Cl₂:MeOH (10:1)]; IR (film) ν_{\max} 3360 (br, OH, –CO–NH₂), 2925, 1684 (br), 1140 (C–O); UV–vis (H₂O) 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); ¹H and ¹³C 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 auranoside C (3). To 78 mg of a fraction containing auranoside C (¹H NMR analysis) and substantial amounts of salt was added 5 mL Ac₂O, 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 CH₂Cl₂. After evaporation of the solvent, the residue was subjected to C₁₈ RP-HPLC separation using 13% H₂O in MeOH with 0.1% TFA as eluent. The orange-colored fraction containing the acetylated product was partitioned vs CH₂Cl₂. Evaporation of the CH₂Cl₂ layer left 7.3 mg of auranoside C hexacetate (**4**). ¹H NMR (CD₂Cl₂) 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 C₄₉H₅₈Cl₂N₂O₂₁, 1081.2987).

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