Aurantosides G, H, and I: Three New Tetramic Acid Glycosides from a Papua New Guinea *Theonella swinhoei*

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Received August 24, 2004

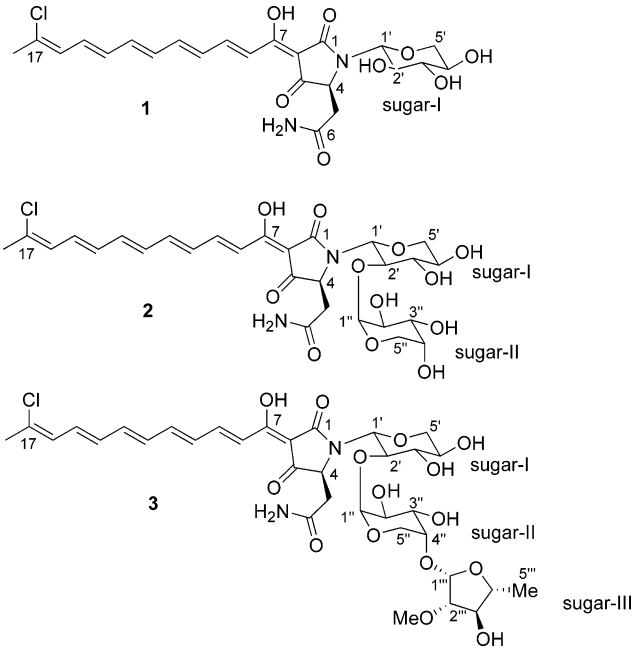
Aurantosides G−I (**1**−**3**) have been isolated from the lithistid sponge *Theonella swinhoei* from Papua New Guinea. Their structures were established by spectroscopic and chemical methods. Compounds **1**−**3** represent new monochloropentaenoyl tetramic acids with mono-, di-, and tri-*N*-saccharide substituents, respectively. Aurantosides G−I (**1**−**3**) failed to show any significant cytotoxicity against the human colon tumor cell line HCT-116.

Lithistid sponges of the family Theonellidae have been recognized as a rich source of structurally novel secondary metabolites with potent and varied biological activities.[1] Among these are the chlorine-containing cytotoxic orange pigments aurantosides A−F[2−5] and rubrosides A−H.[6] Diagnostic structural features of these chlorinated acetogenins include an aglycone portion, consisting of a polyene chain linked to a tetramic acid core, and a *N*-β-glycosidic linkage bearing a saccharide moiety. These naturally occurring tetramic acid glycosides have attracted a great deal of attention due to their wide spectrum of biological activity, e.g., antibiotic,[7] antifungal,[8] and antitumor.[9]

As part of our continuing search for new bioactive metabolites from Papua New Guinea marine invertebrates, the MeOH extract of *Theonella swinhoei* Gray was chemically investigated since it displayed significant cytotoxic and anti-HIV activity. Fractionation of this extract resulted in the isolation of three new tetramic acid glycosides, aurantosides G−I (**1**−**3**). This paper describes the isolation, structure elucidation, and bioactivity of these compounds (**1**−**3**).

The crude aqueous CH_3CN extract of *T. swinhoei* (family Theonellidae) was concentrated under vacuum, then fractionated by flash column chromatography using C18 bonded silica. Further purification by reversed-phase C18 HPLC afforded the new polyenoyltetramic acid glycosides, aurantosides G (**1**, 6.6 mg, 1.35×10^{-2} % yield wet wt), H (**2**, 1.0 mg, 2.04×10^{-3} % yield wet wt), and I (**3**, 1.5 mg, 3.06×10^{-3} % yield wet wt). All three compounds (**1**−**3**) were isolated as bright orange amorphous solids.

The major metabolite aurantoside G (**1**) showed two ions at *m/z* 495 (100) and 497 (33) in the (+)-LRESIMS, indicating the presence of one chlorine atom. A subsequent (+)-HRESIMS measurement established the molecular formula of $C_{23}H_{27}ClN_2O_8$, featuring 11 degrees of unsaturation. The UV spectrum of **1** in MeOH displayed absorptions (λ_{max} 376, 280, and 240 nm) characteristic of a polyene system. These data in conjunction with characteristic features in the NMR spectra (Table 1) of **1** disclosed a polyene tetramic acid glycoside skeleton[10] nearly identical to that of aurantoside A.[2] Correlation spectroscopy data and $^3J_{H/H}$ coupling constant values for **1** were also in excellent agreement with reported values for aurantoside A[2] and were further confirmed by comparison with ^1H and ^{13}C NMR data reported for tetramic acid derivatives in the literature.[8,11]



The structure of **1** differed from that of aurantoside A in that the carbohydrate moiety in **1** was reduced to a monosaccharide unit and the polyene side chain was shortened by one methylene unit. Since the tetramic acid core and the sugar unit in **1** accounted for 6 of the 11 unsaturations required by the formula, **1** must contain a pentenoyl side chain. Placement of the chlorine atom on C-17 was consistent with its ^{13}C chemical shift (δ_C 134.5) and the splitting pattern of the neighboring protons H-16 (d, J = 10.4 Hz) and H_3-18 (s). The pentene unit (C-8−C-18) was connected to the rest of the framework by means of a distinct HMBC correlation between H-9 (δ_H 7.63) and C-7 (δ_C 175.2).

Interpretation of the ^{13}C NMR spectrum of **1** (400 MHz, CD_3OD) was complicated by several closely resonating sp^2-hydridized carbon signals in the region between 120 and 150 ppm. Moreover the ^1H NMR spectrum of **1** recorded in CD_3OD (at 25 °C) revealed two sets of resonances for the

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10.1021/np049721s CCC: $30.25
Published on Web 12/22/2004

Table 1. ^{1}H and ^{13}C NMR (500/125 MHz) Data for Aurantosides G (**1**), H (**2**), and I (**3**) in CD_3OD

	1			**2**			**3**		
position	δ_H (ppm)	multiplicity (*J*, Hz)	δ_C (ppm)	δ_H (ppm)	multiplicity (*J*, Hz)	δ_C (ppm)	δ_H (ppm)	multiplicity (*J*, Hz)	δ_C (ppm)
1			174.9			173.2			176.4
2			*a*			*a*			*a*
3			201.7			*a*			198.1
4	4.30	br s	63.0	4.30	br s	*b*	4.31	br s	65.2
5α	2.80	br m	38.0	2.65	br d (15.9,6.8)	37.0	2.68	dd (15.5,5.6)	38.1
5*β*	2.80	br m		2.78	dd (5.9,4.2)		2.78	dd (15.5,3.8)	
6			174.2			173.2			175.0
7			175.2			173.1			174.3
8	7.23	br d (15.3)	121.5	7.20	br d (14.6)	121.0	7.20	br d (14.4)	*b*
9	7.63	dd (15.3,11.2)	*b*	7.62	dd (14.6,11.7)	145.5	7.62	dd (14.4,11.4)	146.8
10	6.55	dd (14.3,11.2)	132.6	6.56	dd (15.4,11.7)	133.2	6.56	m	134.6
11	6.87	dd (14.3,11.0)	145.5	6.88	dd (15.4,11.0)	144.3	6.90	dd (14.7,11.7)	145.5
12	6.48	m	134.7	6.48	dd (14.9,11.0)	133.4	6.48	dd (14.7,11.4)	134.8
13	6.66	m	140.5	6.62	dd (14.9,10.7)	139.3	6.67	dd (14.9,11.4)	140.5
14	6.49	m	134.3	6.46	m	133.6	6.44	dd (14.9,10.8)	134.3
15	6.68	dd (14.5,10.4)	132.5	6.64	m	131.4	6.67	m	132.6
16	6.31	d (10.4)	126.8	6.30	m	125.7	6.30	d (10.5)	126.9
17			134.5			133.5			135.7
18	2.21	s	26.8	2.21	s	25.6	2.21	s	26.7
1′	4.77	d (8.8)	85.6	4.50	br	85.0	4.50	br	*b*
2′	4.03	dd (8.8,8.8)	71.4	4.50		80.1	4.50		*b*
3′	3.32	br dd (8.8,8.8)	79.2	3.47	dd (9.5,9.5)	78.1	3.46	dd (9.1,9.1)	79.2
4′	3.55	br ddd (10.8,8.8,5.3)	70.9	3.62	ddd (10.7,9.5,5.4)	69.7	3.62	ddd (10.8,9.1,5.6)	70.4
5′α	3.24	dd (11.1,10.8)	69.4	3.21	dd (11.2,10.7)	68.0	3.20	dd (11.1,10.8)	69.2
5′*β*	3.88	dd (11.1,5.3)		3.86	dd (11.2,5.4)		3.86	dd (11.1,5.6)	
1″				5.01	d (2.6)	103.0	5.01	d (2.3)	103.9
2″				3.77	dd (9.3,2.6)	70.0	3.77	dd (9.6,2.3)	71.6
3″				3.78	br d (9.3)	69.2	3.78	br m	70.8
4″				3.40	br m	77.8	3.90	m	76.0
5″α				3.38	dd (12.7,2.7)	64.0	3.56	dd (12.8,3.3)	61.5
5″*β*				3.74	dd (12.7,3.2)		3.69	br d (12.8)	
1‴							5.08	d (4.2)	98.9
2‴							3.65	dd (7.4,4.2)	87.3
3‴							3.87	dd (7.4,6.4)	81.3
4‴							3.75	m	79.7
5‴							1.31	d (6.1)	20.8
OMe							3.35	s	58.3

[a] Not observed (likely due to keto−enol tautomerization). [b] Not assigned.

vinylic protons H-8 and H-9 in a ratio of 3:1. This doubling of the vinylic signals was deemed to be due to keto−enol tautomerization of the adjoining tetramic acid portion of the molecule.[10,12] Similar chemical shift differences ($\Delta\delta$) in the ^{13}C NMR spectrum of **1** for the corresponding carbon resonances corroborated this interpretation. Complications in the NMR spectra due to equilibrium mixtures of tautomers have been reported in the literature for related tetramic acids.[13]

The minor compound aurantoside H (**2**) was assigned the molecular formula $C_{28}H_{35}ClN_2O_{12}$ on the basis of (+)-HRESIMS analysis. Compound **2** was larger than **1** by 132 Da ($C_5H_8O_4$). Furthermore, the ^{1}H NMR spectrum of **2** was very similar to **1**; however, additional resonances in the region δ_H 3.00−4.00 and a doublet at δ_H 5.01 (Table 1) were observed for **2**. These preliminary observations suggested that compounds **1** and **2** were identical with regard to the tetramic acid moiety and the length of the polyene side chain, but varied in their saccharide portion. In comparison with literature values for the known aurantosides,[2−5] the signal at δ_H 5.01 (d, J = 2.6 Hz) was assigned to the equatorial anomeric proton of an arabinopyranose unit (sugar II). The α-orientation of H-1″ (d, J = 2.6 Hz) and H-2″ (dd, J = 9.3, 2.6 Hz) and the *β*-orientation of H-3″ (br d, J = 9.3 Hz) in arabinopyranose were established on the basis of $^3J_{H/H}$ data and DQFCOSY measurements. The presence of two sugars in compound **2** was also confirmed by (+)-LRESIMS analysis, which showed an ion at *m/z* 627 $(M + H)^+$ and an intense fragment ion at *m/z* 495 corresponding to the loss of sugar II. The identities of the sugar moieties in **2**, xylose (sugar I) and arabinose (sugar II), were confirmed by methanolysis of **2** followed by TLC and HPLC comparison with authentic material (see Experimental Section for details). Hence aurantoside H was assigned to structure **2**.

The (+)-HRESIMS of aurantoside I (**3**) indicated a molecular formula of $C_{34}H_{45}ClN_2O_{15}$, featuring 13 degrees of unsaturation. Hence compound **3** was larger than **2** by 130 Da ($C_6H_{11}O_3$). The ^{1}H NMR data of **3** were almost superimposable on those of **2** except aurantoside I had several additional signals in the δ_H 3.00−4.00 region along with three other resonances at δ_H 5.08, 3.35, and 1.31 (Table 1). These data strongly suggested that, besides xylose (sugar I) and arabinose (sugar II), compound **3** also contained a third saccharide moiety. Accordingly, the signal at δ_H 5.08 (d, J = 4.2 Hz) was assigned to the anomeric proton of a 5-deoxy-2-*O*-methylarabinofuranose unit (sugar III), while the signals at δ_H 1.31 (d, J = 6.1 Hz) and 3.35 (s) were assigned to the C-5‴ methyl and the C-2‴ methoxy functions of sugar III, respectively. The trisaccharide assembly in compound **3** was confirmed by prominent fragment ions in the (+)-LRESIMS spectrum at *m/z* 627 $(M + H - \text{sugar III})^+$ and 495 $(M + H - \text{sugar II} + \text{III})^+$. The identities of the sugar units I, II, and III in **3** were further confirmed by comparison to the ^{1}H and ^{13}C NMR data and $^3J_{H/H}$ values of the corresponding sugars in

aurantoside A[2] and hence were assigned the same relative stereochemistry. Hence structure **3** was assigned to aurantoside I.

According to the literature,[2−6] the marine-derived tetramic acid glycosides aurantosides A−F and rubrosides A−H are all derived from L-aspartic acid (identified by GC analysis of the degradation product from periodate/permanganate oxidation with subsequent acid hydrolysis) and carry D-saccharides. This in turn may well be indicative of a common biosynthetic pathway to these polyene tetramic acid metabolites. Thus on the basis of biogenetic reasoning and by comparison of ^{1}H and ^{13}C NMR data with the spectra of related known systems, it can be postulated that the absolute configuration at C-4 of compounds **1**−**3** as well as that of their sugar moieties is identical to those reported for the known metabolites. Furthermore, the sign and magnitude of the optical rotations of **1**−**3** were comparable with those of previously reported aurantosides for which the absolute configuration has been determined. Consequently the absolute configuration of the tetramic acid moiety in aurantosides G−I (**1**−**3**) were assigned as 4*S*, and all the saccharides were assumed to have D-stereochemistry.

Aurantosides G−I (**1**−**3**) failed to show any significant activity against HCT116 cell lines, with wild-type p53 or null mutant p53, when tested in the ranges 0.009−152 , 0.008−124, and 0.006−103 μM for aurantosides G, H, and I, respectively. Aurantosides G, H, and I were found to be inactive in the anti-HIV assay when tested at 1 and 10 μg/mL. Efforts to isolate and characterize the anti-HIV constituents are on going.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV spectra were acquired in spectroscopy grade methanol using a Hewlett-Packard 8452A diode array spectrophotometer. IR spectra were recorded on a JASCO FT/IR-420 spectrometer. NMR spectra were recorded on Varian Unity Inova spectrometers operating at 400/500 and 100/125 MHz, respectively. Chemical shifts are reported in δ (ppm) units and are referenced to residual MeOH (δ_C 49.0; δ_H 3.30) in CD_3OD. Low-resolution mass spectra were obtained using a PE Sciex API III mass spectrometer operating in the ESI mode. High-resolution ESIMS analyses were performed on a Micromass Autospectrometer in the positive electrospray mode. Reversed-phase flash column chromatography was performed on Bakerbond C18 40 μm prep LC packing. Semipreparative HPLC was performed on either a Beckman System Gold or a Agilent 1100 series instrument (diode array detector) using a Phenomenex Luna 5 μm C18 (250 × 10 mm) column. For chiral HPLC, a Shiseido Chiral 5 μm CD-PH (150 × 4.6 mm) column was utilized. All solvents were HPLC grade, purchased from Fisher Scientific. TLC was performed over Kieselgel 60 F_{254} (Merck).

Biological Material. The marine sponge *Theonella swinhoei* Gray (family Theonellidae) was collected by hand using scuba from Milne Bay, Papua New Guinea (S 10°21.55′, E 150°44.70′) in 2001. A voucher specimen of the sample, PNGO1-5-051, is held at the University of Utah.

Extraction and Isolation. The frozen sponge (400 g wet wt) was cut into small pieces and soaked in CH_3CN-H_2O (1:1 v/v, 3 × 1000 mL). The aqueous CH_3CN extract was evaporated to dryness under vacuum, and the resulting crude material was applied onto a RP-silica column pre-equilibrated with aqueous CH_3CN (~90% H_2O). The column was eluted with a step gradient of CH_3CN-H_2O (0−100% CH_3CN) containing 0.05% TFA. The 40% CH_3CN eluate was rechromatographed on C18 silica using an isocratic solvent system of 40% CH_3CN−0.1% aqueous TFA to yield five fractions. The second fraction was further purified on RP-HPLC (Phenomenex 5 μm ODS, 250 × 10 mm) using a gradient of 30%−85% CH_3CN in H_2O (0.05% TFA) over 90 min to yield pure aurantosides G (**1**, 6.6 mg, 1.35 × 10^{-2} % yield wet wt), H (**2**, 1.0 mg, 2.04 × 10^{-3} % yield wet wt), and I (**3**, 1.5 mg, 3.06 × 10^{-3} % yield wet wt).

Aurantoside G (1): orange solid, $[\alpha]^{24}_D$ −116° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 376 (3.57), 280 (3.69), 240 (3.72) nm; IR (film) ν_{max} 3345, 1610, 1560, 1425, 1055 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 495.1523 $(M + H)^+$ (calcd for $C_{23}H_{27}ClN_2O_8$ + H, 495.1534).

Aurantoside H (2): orange solid, $[\alpha]^{24}_D$ −295° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 434 (3.95), 280 (3.79), 240 (3.79) nm; IR (film) ν_{max} 3350, 1610, 1560, 1405, 1075 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 649.1772 $(M + Na)^+$ (calcd for $C_{28}H_{35}ClN_2O_{12}$ + Na, 649.1776).

Aurantoside I (3): orange solid, $[\alpha]^{24}_D$ −210° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 430 (3.72), 282 (3.68), 242 (3.68) nm; IR (film) ν_{max} 3355, 2930, 1675, 1560, 1450, 1205, 1135, 1065, 985, 800, 720 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 779.2417 $(M + Na)^+$ (calcd for $C_{34}H_{45}ClN_2O_{15}$ + Na, 779.2406).

Methanolysis of Sugars. Xylose and arabinose (D- and L-sugars, 5 mg each) were separately refluxed (~90 °C) in 10% HCl−MeOH (15 mL) for 2.5 h. The solvent was removed, and each residue was chromatographed on a silica gel Extract-Clean cartridge (2 g, Alltech) with $CHCl_3-MeOH-H_2O$ (10:3:0.5, v/v). The eluants were concentrated and taken up in CH_3CN-H_2O 1:1 (v/v) for TLC and HPLC analysis of their retention times.

Methanolysis and HPLC Analysis of Compounds 1 and 2. Compounds **1** and **2** (3 mg, each) in 10% HCl−MeOH (1 mL) were separately heated under reflux at 90 °C for 2 h. After evaporation of the solvent, crude residues were passed through a silica gel Extract-Clean cartridge (2 g, Alltech) eluting with $CHCl_3-EtOH-H_2O$ (10:3:0.5, v/v). The resulting sugar fractions were analyzed by co-TLC with authentic sugars (silica gel; developing system, $CH_2Cl_2-EtOH-H_2O$ (8:3:0.5); visualization by 10% H_2SO_4 dip and then heating; methyl-D-xylose (R_f 0.42), methyl-D-arabinose (R_f 0.38)). The identity of each sugar was further confirmed by comparison of its retention time with those of the standards using HPLC under the following conditions: column, Phenomenex Phenosphere 5 μm NH_2 (250 × 4.6 mm); mobile phase, CH_3CN-H_2O (20:80); flow rate, 0.8 mL/min; detection, UV 230 nm; retention times: methyl-D-xylose (t_R, 3.66 min), methyl-D-arabinose (t_R, 3.68 min). An attempt to unambiguously identify the D- and L-sugars following methanolysis of compounds **1** and **2** by HPLC on a chiral stationary phase proved unsatisfactory (column, Shiseido Chiral 5 μm CD-PH (150 × 4.6 mm); mobile phase, CH_3CN-H_2O (30:70); flow rate, 0.5 mL/min; detection, UV 230 nm); co-injection of each hydrolysate with the standards (methyl-D-xylose and methyl-D-arabinose) gave single peaks.

Assay for Cell Viability. HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cell lines were maintained in McCoy's media supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 50 units/mL penicillin and streptomycin, and 10% (v/v) FBS in a humidified incubator at 37 °C and 5% CO_2. Cells were seeded (4 × 10^5 cells/well) to a sterile 96-well plate. The cells were allowed to attach and grow for 24 h at 37 °C and 5% CO_2. Cells were incubated with 0−78 μg/mL aurantoside G, H, or I for 72 h. MTT was added to each well (final concentration, 0.25 mg/mL) and incubated for an additional 3 h. The media was aspirated and 100 mL of DMSO added to the cells. The absorbance was read at 570 nm using a plate reader (Labsystem). IC_{50} values were determined using GraphPad Prism 4.

Acknowledgment. The authors wish to acknowledge Papua New Guinea government for permits to collect the sponge. We thank J. Olsen, Department of Medicinal Chemistry of the University of Utah, for his assistance in performing NMR experiments at 500 MHz. P. Krishna at the University

of Utah Mass Spectrometry and Proteomics Core Facility is acknowledged for performing the LRESIMS experiments. The HRESIMS data were acquired at the University of Iowa High Resolution Mass Spectrometry Facility. This project was funded by NIH grant CA 36622.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra and ESI mass spectral data for aurantosides G, H, and I. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP049721S