Notes

Acetylated Sesterterpenes from the Great Barrier Reef Sponge *Luffariella variabilis*§

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Chemical investigation of the sponge *Luffariella variabilis* collected from the Palm Island group of the Great Barrier Reef, Australia, yielded three new acetylated compounds, 25-acetoxyluffariellin A (**1**), 25-acetoxyluffariellin B (**2**), and 25-acetoxyseco-manoalide (**3**). The structures of the new compounds were elucidated on the basis of interpretation of their spectroscopic data. The known metabolites manoalide (**4**), seco-manoalide (**5**), luffariellin A (**8**), and manoalide monoacetate (**10**) were also isolated. The new acetylated compounds (**1**−**3**) were labile in the sponge tissue when samples were allowed to thaw prior to extraction, but were stable once isolated. Sponge samples that were completely thawed contained only hydroxylated compounds (alcohols). This finding supported the deduction that the acetylated compounds are being enzymatically transformed and/or degraded.

Sponges of the genus *Luffariella* are widespread throughout the Indo-Pacific and have afforded a wealth of bioactive sesterterpenes.[1−5] Manoalide (**4**) was the first of a series of related compounds reported from the Palauan sponge *Luffariella variabilis* by De Silva and Scheuer,[1] who subsequently isolated seco-manoalide (**5**) and (*E*)- and (*Z*)-neomanoalide (**6**, **7**).[2] Kernan and Faulkner[3] reported the presence of two new sesterterpenes, luffariellin A (**8**) and luffariellin B (**9**), in addition to manoalide (**4**) and seco-manoalide (**5**). They also quantified variation of chemistry in 410 Palauan sponges all assigned as *L. variabilis*, with the ratio of these four metabolites being found to vary significantly between different sponge samples.[3] In the current study the isolation of three new acetylated compounds (**1**−**3**) and the previously reported manoalide (**4**),[1] seco-manoalide (**5**),[2] luffariellin A (**8**),[3] and manoalide monoacetate (**10**)[6] is reported from *L. variabilis*, collected from the Palm Island Group of the Great Barrier Reef, Australia. Furthermore, it has been found that sponge storage protocols have a significant effect on the isolated chemistry. This report details the isolation and structure elucidation of three new compounds and the effect of allowing samples to thaw on the presence of these secondary metabolites.The sponge *L. variabilis* collected off Orpheus Island, Australia, was frozen as soon as returned to the surface by immersion in liquid N_2 and kept frozen at −176 °C. The frozen sponge was freeze-dried and extracted with CH_2Cl_2 (3 × 200 mL) at RT. The combined dichloromethane extracts (358 mg) of *L. variabilis* were purified using a series of preparative and semipreparative HPLC chromatography. This process yielded three new sesterterpenes, **1**−**3**, as well as the known compounds manoalide (**4**),[1] seco-manoalide (**5**),[2] luffariellin A (**8**),[3] and manoalide monoacetate (**10**).[6] A number of modifications to previous structural assignments of the known metabolites were recorded during the elucidation of these compounds. The previous isolation of luffariellin A reported doubled proton and carbon signals in the NMR spectra in purified $CDCl_3$ for the protons and carbons around the α-hydroxybutenolide and δ-lactol rings. This was consistent with a mixture of two diastereoisomers in these regions. However, in slightly acidic $CDCl_3$, only a single set of broad signals were observed. This doubling of signals has also been reported for manoalide[7] and related compounds.[8] We also observed a broadening of signals in the NMR spectra for the compounds reported herein.

Compound **1** showed a $[M + Na]^+$ ion in its HRESIMS, consistent with the molecular formula $C_{27}H_{38}O_6$ and, therefore, nine degrees of unsaturation in the form of multiple bonds and rings. The ^1H and ^{13}C NMR data of **1** showed it to contain six double bonds and therefore be tricyclic and, thus, very similar to luffariellin A (**8**).[3] When the ^1H NMR data of **1** and **8** were compared, the differences between the two data sets were the presence of an additional methyl singlet signal at δ_H 2.18 and the downfield shift of the H-25 resonance (δ_H 7.12 in **1** compared to δ_H 6.22 in luffariellin A). The ^{13}C NMR spectral data of **1** compared to that of luffariellin A (**8**) showed additional carbon signals at 168.8 (qC) and 20.1 (CH_3) ppm, and the signals associated with C-3 and C-25 were shifted (δ_C 165.5, 92.4 in **1** compared to δ_C 169.0/168.3, 98.3 in luffariellin A). These differences were consistent with **1** being the 25-acetoxyl derivative of luffariellin A (**8**). The gHMBC NMR data of **1** confirmed this and confirmed the position of the acetoxyl function. A correlation from the methyl signal at δ_H 2.18 to C-25 (δ_C 92.4) was observed as well as correlations from H-25 (δ_H 7.12) to C-2 (δ_C 118.6) and C-3 (δ_C 165.5). These correlations are in agreement with the NMR data for both manoalide monoacetate (**10**) and thorectolide monoacetate (**11**), both of which have an acetoxybutenolide terminus.[8] The Δ^{10} geometry was determined to be *E* on the basis of the ^{13}C NMR chemical shift of C-23 (δ_C 16.2).[9] Compound **1** is therefore 25-acetoxyluffariellin A. Selected ^{13}C NMR assignments and relative configurations were confirmed from the gCOSY, gHSQC, and 1D selective TOCSY spectra of **1**. The chemical shifts of C-13 and C-18 resonated at δ_C 34.2 and 28.8, respectively, in contrast to the values previously reported[3] (C-13 and C-18 at δ_C 25.9 and 34.3, respectively). The stereochemistry at C-14 relative to C-15 was determined by a 1D selective gNOESY experiment. When the H_3-22 signal (δ_H 0.72) was irradiated, NOEs were observed to the H_3-21 signal (δ_H 1.68), the signal for the adjacent methine H-15 (δ_H 1.77), and to both of the signals

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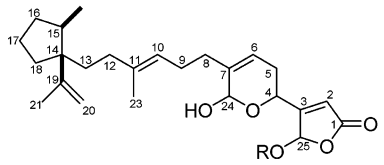
Chart 1



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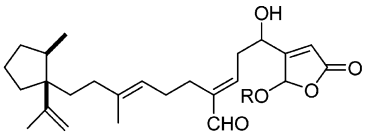

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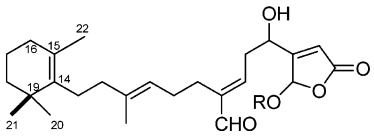

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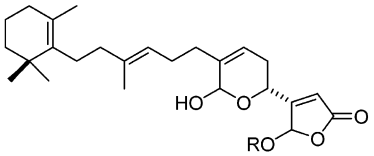

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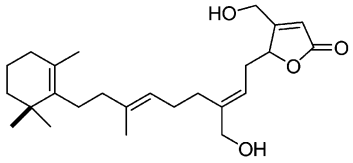

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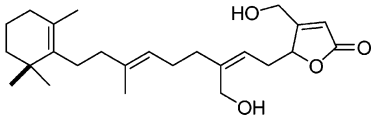

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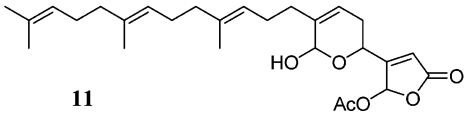

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associated with the exo-methylene H_2-20 (δ_H 4.84, 4.65), indicating these groupings to be on the same side of the ring, which gives **1** the same relative stereochemistry at C-14 and C-15 as that previously reported.[3] We were unable to conclusively determine the relative stereochemistry at C-4 or C-25. The axial nature of H-4 was deduced from its coupling constants (10.9, 4.0 Hz). When the H-4 signal (δ_H 4.77) was irradiated, small NOEs were observed to the signals for H-2, H_2-5, H-6, H-24, H-25, and H_3-25-OAc as well to the signal for the exchangeable proton 4-OH. Consequently the relative stereochemistry at both C-4 and C-25 remains undetermined.

Compound **2** analyzed for $C_{27}H_{38}O_6$ by HRESIMS. Comparison of the NMR data of compound **2** (Tables 1 and 2) with those of luffariellin B (**9**)[3] showed the two data sets to be very similar. Where differences were apparent, they were consistent with **2** being the C-25 acetoxyl derivative of luffariellin B (**9**). These differences included additional signals for an acetate group [δ_C 169.1 (qC), 20.6 (CH_3), δ_H 2.21 (s)] and the shift of the C-3, C-25, and H-25 signals (δ_C 166.1, 93.2 and δ_H 7.21 in **2** compared to δ_C 170.4/169.3, 98.3/97.9 and δ_H 5.40 in luffariellin B). These differences showed **2** to be 25-acetoxyluffariellin B. Similar to luffariellin A, the chemical shifts of C-13 and C-18 for luffariellin B[3] required revision. The relative stereochemistry of C-14 and C-15 was determined by selective gNOESY experiments to be the same as that reported for luffariellin A.[3] The relative stereochemistry at C-4 and C-25 remains unassigned.

Compound **3** had the molecular formula $C_{27}H_{38}O_6$, as determined by HRESIMS, and thus was isomeric with compounds **1** and **2**. Comparison of the 1D NMR data of compound **3** (Tables 1 and 2) with that of seco-manoalide (**5**)[2] showed the two data sets to be similar. In the data set for **3** additional resonances in both the ^1H and ^{13}C NMR spectra [δ_H 2.20 (s); δ_C 169.1 (qC), 20.7 (CH_3)] and a shift in the signals assigned as C-3, C-25, and H-25 (δ_C 165.9, 93.2; δ_H 7.07 respectively in **3**, compared to δ_C 169.0, 99.0; δ_H 6.15 respectively in seco-manoalide) were consistent with the presence of an acetoxyl function at C-25 in **3** rather than the OH of seco-manoalide. As for **1** and **2**, the geometry of the C-10 olefin was determined to be *E* on the basis of the chemical shift of C-23 (δ_C 15.7). These data and all of the other physical data recorded were consistent with **3** being 25-acetoxyseco-manoalide. The relative stereochemistry at C-4 and C-25 remains unassigned.

Optical rotations measured for manoalide (**4**) and manoalide monoacetate (**10**) in this study agree with published values,[1,6] indicating that the absolute stereochemistry at C-4 in both can be assigned as *R*. The optical rotation of seco-manoalide isolated in this study ($[\alpha]_D$ −65.3) differed in magnitude and sign from that reported in the literature ($[\alpha]_D$ +16.2).[2] However, because both compounds are mixtures of diastereoisomers at C-25, it is not possible to comment on the significance of this finding or the absolute stereochemistry of seco-manoalide. The optical rotation measured for luffariellin A ($[\alpha]_D$ −32.0) was of similar magnitude and opposite to that reported in the literature ($[\alpha]_D$ +40.1).[3] The relative stereochemistry at C-14 and C-15 in luffariellin A isolated in the current investigation was shown by selective gNOESY experiments to be the same as reported.[3] Due to both compounds being isolated as mixtures of diastereoisomers, it is not possible to confirm unequivocally the current compound as the antipode of that previously reported.[3]

The presence of the new acetylated compounds (**1**−**3**) in the sponge extracts was significantly affected by the sponge tissue

Table 1. ^{1}H NMR Spectroscopic Data (600 MHz, $CDCl_3$) for Compounds **1**−**3**

	1		**2**		**3**	
position	δ_H (*J* in Hz)	gHMBC	δ_H (*J* in Hz)	gHMBC	δ_H (*J* in Hz)	gHMBC
1						
2	6.10 (s)	1, 3, 4, 25	6.15 (s)	1, 3, 4, 25	6.15 (s)	1, 3, 4, 25
3						
4	4.77 (dd, 10.9, 4.0)	2, 3, 25	4.68 (dd, 7.0, 4.5, 4.0)	2, 3, 5, 6	4.68 (dd, 7.1, 5.2, 4.0)	2, 3, 5, 6,
4-OH			2.42 (d, 4.5)		2.40 (d, 5.2)	
5	2.28 (ddd 17.1, 4.3, 4.0)	3, 4, 6, 7	2.81 (dt, 15.4, 7.1)	4, 6	2.82 (dt, 15.4, 7.1)	4, 6
	2.32 (dd 17.1, 10.9)		2.89 (ddd, 15.4, 7.1, 4.0)		2.91 (ddd, 15.4, 7.1, 4.0)	
6	5.71 (d, 4.3)	4, 5, 8, 24	6.53 (t, 7.1)	4, 5, 7, 8, 24	6.55 (t, 7.1)	4, 5, 7, 8, 24
7						
8	2.14 (m)		2.32 (t, 7.6)	7, 9, 10, 24	2.35 (t, 7.6)	7, 9, 10, 24
9	2.15 (m)		2.08 (m)	8, 10, 11	2.11 (m)	8, 10, 11
10	5.10 (dt, 1.0, 6.7)	8, 9, 12, 23	5.08(br t, 7.2)	8, 9, 12, 13, 23	5.13 (br t, 7.2)	8, 9, 12, 23
11						
12	1.71 (m)		1.68 (m)		1.98 (m)	
	1.76 (m)		1.74 (m)			
13	1.36 (m)		1.32 (m)		2.01 (m)	
	1.40 (m)		1.38 (m)			
14						
15	1.77 (m)		1.76 (m)			
16	1.30 (m)		1.32 (m)		1.91 (t, 6.2)	
	1.94 (m)		1.94 (m)			
17	1.61 (m)		1.61 (m)		1.56 (m)	
	1.72 (m)		1.72 (m)			
18	1.48 (m)		1.46 (m)		1.42 (m)	
	1.75 (m)		1.72 (m)			
19						
20	4.65 (s)	14, 19, 21	4.64 (s)	14, 19, 21	0.99 (s)	18
	4.84 (s)	14, 19, 21	4.83 (s)	14, 19, 21		
21	1.68 (s)		1.68 (s)		0.99 (s)	18
22	0.72 (d, 7.0)	16	0.71 (d, 7.0)	16	1.60 (s)	16
23	1.60 (s)	10, 12	1.56 (s)	10, 12	1.62 (s)	10, 12
24	5.31 (d, 4.5)	6, 8	9.44 (s)	6, 8	9.45 (s)	6, 8
24-OH	2.82 (d, 4.5)					
25	7.12 (s)	2, 3	7.07 (s)	2, 3	7.07 (s)	2
25-OAc	2.18 (s)		2.21 (s)		2.20 (s)	

Table 2. ^{13}C NMR Spectroscopic Data (125 MHz, $CDCl_3$) for Compounds **1**−**3**

	1	**2**	**3**
position	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	169.2, qC	168.7, qC	168.6, qC
2	118.6, CH	119.5, CH	119.8, CH
3	165.5, qC	166.1, qC	165.9, qC
4	61.5, CH	65.9, CH	65.8, CH
5	27.9, CH_2	34.2, CH_2	33.9, CH_2
6	120.6, CH	146.6, CH	146.2, CH
7	136.9, CH	146.2, qC	146.5, qC
8	32.3, CH_2	24.3, CH_2	24.4, CH_2
9	25.6, CH_2	26.4, CH_2	26.7, CH_2
10	122.6, CH	122.1, CH	121.9, CH
11	136.4, qC	137.7, qC	137.7, qC
12	34.7, CH_2	34.8, CH_2	40.1, CH_2
13	34.2, CH_2	34.2, CH_2	27.8, CH_2
14	55.1, qC	55.1, qC	136.9, qC
15	41.8, CH	41.9, CH	127.1, qC
16	30.6, CH_2	31.0, CH_2	32.7, CH
17	20.2, CH_2	20.6, CH_2	19.5, CH_2
18	28.8, CH_2	29.5, CH_2	39.8, CH_2
19	148.0, qC	148.1, qC	35.0, qC
20	111.6, CH_2	111.8, CH_2	28.6, CH_3
21	20.2, CH_3	20.7, CH_3	28.6, CH_3
22	17.7, CH_3	18.2, CH_3	19.8, CH_3
23	16.2, CH_3	16.3, CH_3	15.7, CH_3
24	91.4, CH	194.6, qC	194.2, qC
25	92.4, CH	93.2, CH	93.2, CH
25-OAc	168.8, qC	169.1, qC	169.1, qC
	20 0.1, CH_3	20.6, CH_3	20.7, CH_3

workup protocols. A small number of sponge samples, which were known to contain the acetylated compounds (**1**−**3** and **10**), were subsampled and thawed before being lyophilized and extracted. Only the nonacetylated compounds (**4**, **5**, and **8**) were isolated from the CH_2Cl_2 extracts of these samples. Subsequently, sponge samples (n = 15) were divided into two *in situ* and immediately returned to the surface, where they were immersed in liquid N_2. In the laboratory, one piece of each collected sponge was allowed to thaw for 3 h prior to being freeze-dried, with the remaining piece kept frozen until freeze-dried. In the sponge samples that were kept frozen before being freeze-dried and extracted, the acetylated compounds (**1**−**3** and **10**) together with compounds **4**, **5**, and **8** were isolated. However, in the sponge samples that were allowed to thaw before being freeze-dried and extracted, only the nonacetylated compounds **4**, **5**, and **8** were detected. Once isolated, the acetylated compounds **1**−**3** and **10** are stable, suggesting that their "instability" in the sponges is enzyme mediated. Presumably the enzymes that are active in the freshly collected sponge remain viable in the frozen material. As such, the acetylated compounds may be precursor storage metabolites that can be hydrolyzed enzymatically to the alcohols that have a predetermined function, for example, defense. Activated defenses where biologically inactive acetylated metabolites are enymatically hydrolyzed to biologically active alcohols or aldehydes have been previously reported in the algae *Halimeda* spp.[10] and *Caulerpa taxifolia*, *C. prolifera*, and *C. racemosa*.[11] Recent reports[12] have also postulated enzymatic cleavage of brominated isoxazoline alkaloids into more active monocylic nitrogenous compounds (aeroplysinin-1 and dienones) as an activated defense mechanism after mechanical wounding in the sponge *Aplysina* sp.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco 715 CD polarimeter. UV spectra were measured on a Shimadzu SPD-M10AVP PDA detector. Infrared spectra were taken on a Nicolet Nexus FTIR. ^{1}H and ^{13}C NMR spectra were recorded in neutralized $CDCl_3$ using a Bruker Avance 600 MHz NMR spectrometer

with cryoprobe. Spectra were referenced to residual ^{1}H (δ 7.27) and ^{13}C (δ 77.0) resonances in the deuterated solvents. Both 1D and 2D NMR spectra were recorded using standard Bruker pulse sequences. High-resolution mass spectra were measured with a Bruker BioApex 47e FT-ICR mass spectrometer fitted with an Analytica of Branford electrospray source. Ions were detected in positive mode within a mass range of *m*/*z* 200−1000. Direct infusion of the sample (0.2 mg mL^{-1}) was carried out using a Cole Palmer 74900 syringe pump at a flow rate of 80 μL h^{-1}. HPLC was performed with a Shimadzu LC10-AT pump coupled to either a SPD-M10AVP PDA detector (analytical analyses) or a Shimadzu SPD-10A UV/vis detector (preparative isolations). HPLC columns were purchased from Phenomenex. Compressed gases came from BOC Gases (Townsville, Australia) and were at least 99.99% pure. Purified water was obtained from a MilliQ water purification system (Millipore, Billerica, MA); all other solvents used were HPLC grade (Mallinckrodt, Hazelwood, MO).

Sponge Material. *Luffariella variabilis* (order Dictyoceratida, family Thorectidae) was collected by hand using scuba at depths between 5 and 10 m off Orpheus Island, Australia. A voucher specimen is lodged at the Australian Institute of Marine Science, Queensland, Australia (#27405). Freshly collected sponges were frozen as soon as returned to the surface by immersion in liquid N_2 and kept frozen at −176 °C. The sponges had a dark brown-black exterior and were extensively covered in fouling organisms; interiors were orange-brown.

Extraction and Isolation. The frozen sponge sample for preparative isolation was freeze-dried and extracted with CH_2Cl_2 (3 × 200 mL) at RT. The combined dried extracts (358 mg) were dissolved in MeOH and chromatographed using preparative RP HPLC [Phenomenex, Luna C18 (2), 5 μm, 250 × 21 mm; gradient of CH_3CN-H_2O, 70:30 to 100:0, over 60 min as eluent, flow rate 10 mL/min; UV detection at 254 nm]. A late eluting fraction was found to be rich in manoalide monoacetate (**10**) and was not further purified. The fractions containing compounds **1**−**3** were further purified using semipreparative HPLC [Phenomenex, Luna C18 (2), 5 μm, 250 × 10 mm; gradient of CH_3CN-H_2O, 73:27 to 81:21, over 15 min as eluent, flow rate 4 mL/min; UV detection at 254 nm] to afford 25-acetoxyluffariellin A (**1**), 25-acetoxyluffariellin B (**2**), 25-acetoxyseco-manoalide (**3**), manoalide (**4**), seco-manoalide (**5**), and luffariellin A (**8**). Compounds **4**, **5**, **8**, and **10** were identified by comparison of their NMR data with literature data.[1−3,6]

25-Acetoxyluffariellin A (1): 4.41 mg, 0.08% dry weight; colorless oil; $[\alpha]^{21}_D$ −38.1 (*c* 0.11, $CHCl_3$); UV (PDA, CH_3CN-H_2O, 70:30 to 100:0) λ_{max} (relative absorption) 196 (1), 228 (0.67) nm; IR (film) ν_{max} 3490 (br), 1797, 1766, 1211, 1026, 999 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 481.2577 ($C_{27}H_{38}O_6Na$ [M + Na]$^+$ required 481.2561).

25-Acetoxyluffariellin B (2): 1.14 mg, 0.02% dry weight; colorless oil; $[\alpha]^{21}_D$ −156.1 (*c* 0.06, $CHCl_3$); UV (PDA, CH_3CN-H_2O, 70:30 to 100:0) λ_{max} (relative absorption) 200 (1), 226 (0.84) nm; IR (film) ν_{max} 3518 (br), 2362, 2335, 1761, 1679, 1210 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 481.2547 ($C_{27}H_{38}O_6Na$ [M + Na]$^+$ required 481.2561).

25-Acetoxyseco-manoalide (3): 3.36 mg, 0.06% dry weight; colorless oil; $[\alpha]^{21}_D$ −42.0 (*c* 0.11, $CHCl_3$); UV (PDA, CH_3CN-H_2O, 70:30 to 100:0) λ_{max} (relative absorption) 193 (1), 228 (0.70) nm; IR (film) ν_{max} 3480 (br), 1799, 1681, 1208 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HRESIMS *m*/*z* 481.2556 ($C_{27}H_{38}O_6Na$ [M + Na]$^+$ required 481.2561).

Analyses of Sponge Extracts. Each sponge sample (n = 15) was divided into two portions immediately after collection under water. Both portions of each sponge were placed separately in liquid N_2 on return to the surface. In the laboratory, one portion of each sponge was freeze-dried. The remaining portion of each sponge was allowed to thaw at RT for 3 h before being freeze-dried. All freeze-dried sponge samples were extracted with CH_2Cl_2 (3 × 10 mL). The combined dried extracts were dissolved in DMSO and analyzed using analytical HPLC [Phenomenex, Luna C18 (2), 5 μm, 250 × 4.6 mm; gradient of CH_3CN-H_2O, 73:27 + 0.1% TFA to 81:21 + 0.1% TFA, over 35 min as eluent, flow rate 1 mL/min; UV detection at 254 nm].

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References and Notes

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