Luffasterols A−C, 9,11-Secosterols from the Palauan Sponge *Luffariella* sp.

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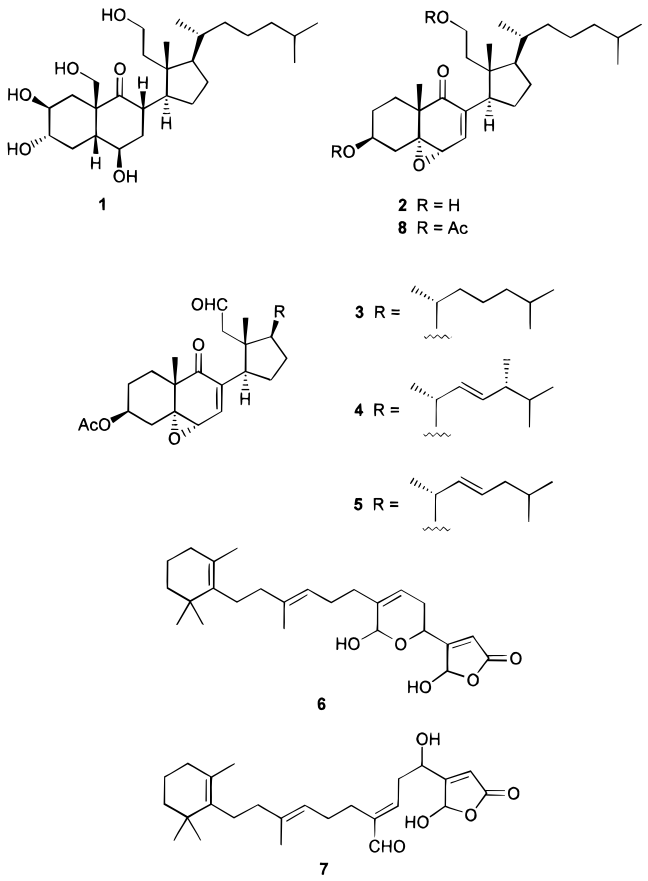
The Palauan sponge *Luffariella* sp. contained manoalide (**6**) and secomanoalide (**7**) as the major metabolites. The minor metabolites, luffasterols A−C (**3**−**5**), are 9,11-secosterols that contain a 3β-acetoxy-5α,6α-epoxy-9-oxo-9,11-secocholest-7-en-11-al ring system joined to three different side chains. The structures of the luffasterols were elucidated by interpretation of spectroscopic data.

Among the bioactive metabolites of sponges are a small group of 9,11-secosterols that have occasionally been found as major metabolites.[1,2] These include herbasterol (**1**), which is an ichthyotoxic and cytotoxic metabolite from *Dysidea herbacea*,[3] and glaciasterols A and B (**2**), the cytotoxic constituents of *Aplysilla glacialis*.[4] 9,11-Secosterols have also been isolated from soft corals.[1,2] In this paper, we report the occurrence of three new 9,11-secosterols, luffasterols A−C (**3**−**5**), as minor metabolites of *Luffariella* sp. from Palau.

Specimens of *Luffariella* sp. were collected by hand from shallow water (−2 m) at Tee marine lake, Palau. The sponge was kept frozen until it was extracted with methanol. Using our standard dereplication techniques, an initial chromatography of the methanolic extract of a small subsample revealed that the major metabolites were the known compounds manoalide (**6**) and secomanoalide (**7**), but a fraction containing the secosterols was also obtained. The chloroform-soluble material was chromatographed on silica gel to obtain manoalide (**6**, 0.43% dry wt) and secomanoalide (**7**, 0.12% dry wt). The hexane-soluble material from the methanol extract was chromatographed on silica to obtain a mixture of secosterols that were separated by HPLC to afford luffasterols A (**3**, 0.023% dry wt), B (**4**, 0.0023% dry wt), and C (**5**, 0.0044% dry wt).

Luffasterol A (**3**), $[\alpha]_D = -33.5°$, was obtained as white needles, mp 139−141 °C. The molecular formula, $C_{29}H_{44}O_5$, was derived from HREIMS and ^{13}C NMR data and indicated eight degrees of unsaturation. The IR spectrum contained bands at 1735 (ester), 1715 (aldehyde), and 1675 cm^{-1} (α,β-unsaturated ketone), and the UV absorption at 255 nm (ϵ 4100) confirmed the presence of the α,β-unsaturated ketone. The ^1H NMR spectrum contained an acetate methyl signal at δ 2.02 (s, 3 H), an aldehyde signal at 9.85 (dd, 1 H, $J = 4$, 1.5 Hz), a signal at 6.84 (br d, 1 H, $J = 4.5$ Hz), which is appropriate for a β-proton on an α,β-unsaturated ketone, and five methyl signals at 0.73 (s, 3 H), 0.83 (d, 3 H, $J = 6.5$ Hz), 0.84 (d, 3 H, $J = 6.5$ Hz), 0.90 (d, 3 H, $J = 6.5$ Hz), and 1.19 (s, 3 H). The ^{13}C NMR spectrum contained the expected 29 signals that included an aldehyde signal at δ 203.5, the ketone carbonyl at 200.3, the acetate carbonyl at 170.1, and olefinic signals at 140.5 and 139.8. At this stage we deduced that the compound was tetracyclic and probably steroidal but since the presence of the aldehyde and ketone moieties suggested a 9,11-secosterol, the A, B, and D rings of the secosterol must be accompanied by one additional ring. The olefinic signal at δ 6.84 was coupled to a signal at δ 3.38 (d, 1 H, $J = 4.5$ Hz) that showed an HMQC correlation to a signal at δ 53.4. In the HMBC experiment, the signal at 6.84 was correlated with signals at 53.4 (C-6), 63.0 (C-5), 200.3 (C-9), and 44.8 (C-14), which implies that C-5 and C-6 are part of an epoxide ring. A signal at δ 4.96 (tt, 1 H, $J = 11$, 4 Hz) was assigned to an axial proton at C-3, which bears the acetoxy group. Further interpretation of the COSY, HMQC, and HMBC data allowed all signals in both the ^1H and ^{13}C NMR spectra to be assigned (Table 1). Comparison of these data with the spectral data[4] for glaciasterol B 3,11-diacetate (**8**) revealed that they were identical except at C-11. The formal name for luffasterol A (**3**) is 3β-acetoxy-5α,6α-epoxy-9-oxo-9,11-secocholest-7-en-11-al.



Luffasterol B (**4**), $[\alpha]_D = -28°$, was obtained as a white solid. The molecular formula, $C_{30}H_{44}O_5$, was derived

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Table 1. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) NMR Data for Luffasterol A (**3**)

C no.	δ_C	δ_H	mult, *J* (Hz)	DQ-COSY	HMBC
1	27.4	2.07	m	H-1′	
		1.73	m	H-1	
2	26.5	2.13	m	H-2′	
		1.65	m	H-2	
3	70.5	4.96	tt, 11, 4	H-4, H-4′	
4	33.9	2.22	dd, 12, 13	H-3, H-4′	C-2, C-3, C-5, C-6
		1.61	m	H-3, H-4	
5	63.0				
6	53.4	3.38	d, 4.5	H-7	C-7, C-8
7	139.8	6.84	br d, 4.5	H-6	C-5, C-6, C-9, C-14
8	140.5				
9	200.3				
10	45.4				
11	203.5	9.85	dd, 4, 1.5	H-12, H-12′	C-12
12	50.5	2.27	dd, 16, 4	H-11, H-12′	C-11, C-13, C-14, C-17
		1.92	dd, 16, 1.5	H-11, H-12	
13	46.4				
14	44.8	3.50	br t, 9.5	H-15	C-7, C-8, C-9, C-12
					C-13, C-16, C-17
15	26.6	1.68	m, 2 H	H-14, H-16	
16	25.9	1.85	m	H-15	
		1.46	m		
17	51.7	1.83	m	H-16	C-13, C-16, C-18, C-20
18	16.6	0.73	s, 3 H		C-12, C-13, C-14, C-17
19	20.9	1.19	s, 3 H		C-1, C-5, C-9, C-10
20	34.8	1.40	m	H-17, H-21	C-17
21	19.3	0.90	d, 3 H, 6.5	H-20	C-17, C-20, C-22
22	35.3	1.33	m	H-23	C-17, C-23
		1.01	m		
23	24.3	1.31	m	H-22	
		1.11	m		
24	39.4	1.09	m, 2 H	H-23	
25	27.9	1.49	m	H-26, H-27	C-23, C-24, C-26, C-27
26	22.5	0.84	d, 3 H, 6.5	H-25	C-24, C-25, C-27
27	22.7	0.83	d, 3 H, 6.5	H-25	C-24, C-25, C-26
OAc	21.2	2.02	s, 3 H		
	170.1				

from HREIMS data. Inspection of the spectral data[5] revealed that luffasterol B (**4**) was identical to luffasterol A (**3**) except that the side chain contained an additional methyl group and olefinic bond, as indicated by a fragmentation ion in the mass spectrum at $m/z = 299$ $(M - AcOH - C_9H_{17})^+$. The ^{1}H NMR spectrum contained new olefinic signals at δ 5.22 (dd, 1 H, $J = 15$, 6 Hz) and 5.19 (dd, 1 H, $J = 15$, 5 Hz) that were assigned to a (22*E*) olefin with the additional methyl group at C-24. The ^{1}H NMR chemical shift data for the methyl groups in the side chain favors the 24*S* configuration,[6] but this method of determination of stereochemistry may not be valid for 9,11-secosterols. Detailed interpretation of the ^{1}H NMR data fully supported the proposed structure for luffasterol B (**4**).

Luffasterol C (**5**), $[\alpha]_D = -22°$, was obtained as a white solid. The molecular formula, $C_{39}H_{42}O_5$, was derived from HREIMS data. Analysis of the spectral data[5] indicated that luffasterol C (**5**) again differed in the side chain. The ^{1}H NMR spectrum contained olefinic signals at δ 5.29 (dt, 1 H, $J = 15$, 6.5 Hz) and 5.24 (dd, 1 H, $J = 15$, 7 Hz) that were assigned to a (22*E*) olefin. Detailed interpretation of the ^{1}H NMR data fully supported the proposed structure for luffasterol C (**5**).

Experimental Section

General Methods. Melting points were obtained on a Mel-Temp apparatus and are uncorrected. Optical rotations were measured on an Autopol III polarimeter using a 1 dm cell, and CD spectra were measured on a Varian Cary 61 spectrometer. Infrared and ultraviolet spectra were recorded on Perkin-Elmer 1600 and Lambda 3B instruments, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 400 spectrometer at 400 and 100 MHz, respectively. DEPT and DQ-COSY experiments were performed using a Varian Unity Inova spectrometer at 300 MHz, and HMQC and HMBC experiments were recorded on a Varian Unity 500 spectrometer. Chemical shifts are reported in ppm based on $\delta_{TMS} = 0$, and coupling constants (*J*) are reported in Hz.

Table 2. Selected ^{1}H NMR Data (400 MHz, $CDCl_3$) for Luffasterols B (**4**) and C (**5**)

	4		**5**	
H no.	δ_H	mult, int, *J* (Hz)	δ_H	mult, int, *J* (Hz)
3	4.96	m, 1 H	4.96	m, 1 H
4	2.22	t, 1 H, 12	2.22	t, 1 H, 12
	1.62	m, 1 H	1.62	m, 1 H
6	3.38	d, 1 H, 4.5	3.38	d, 1 H, 4.5
7	6.82	br d, 1 H, 4.5	6.82	br d, 1 H, 4.5
11	9.86	dd, 1 H, 4, 1.5	9.86	dd, 1 H, 4, 1.5
12	2.23	dd, 1 H, 16, 4	2.23	dd, 1 H, 16, 4
	1.97	dd, 1 H, 16, 1.5	1.97	dd, 1 H, 16, 1.5
14	3.48	br t, 1 H, 9.5	3.48	br t, 1 H, 9.5
18	0.73	s, 3 H	0.73	s, 3 H
19	1.19	s, 3 H	1.19	s, 3 H
20	2.15	m, 1 H		
21	0.98	d, 3 H, 7	0.98	d, 3 H, 6.5
22	5.19	dd, 1 H, 15, 5	5.24	dd, 1 H, 15, 7
23	5.22	dd, 1 H, 15, 6	5.29	dt, 1 H, 15, 6.5
24	1.82	m, 1 H		
26	0.81	d, 3 H, 7	0.83	d, 3 H, 6.5
27	0.79	d, 3 H, 7	0.83	d, 3 H, 6.5
28	0.88	d, 3 H, 7		
OAc	2.02	s, 3 H	2.02	s, 3 H

Animal Material. The sponge *Luffariella* sp. (collection number 95−109) was collected by hand in shallow water (−2 m) at Tee marine lake, Palau, and was frozen within 1 h of collection. The sponge has a fine tertiary fiber network, which is characteristic of the genus *Luffariella*. When compared with our reference specimens of *L. variabilis*, this sponge is more compressible, softer, and has a less harsh fibrous skeleton. A voucher specimen (P-1166) has been deposited in the SIO Benthic Invertebrate Collection.

Extraction and Isolation. The frozen sponge (61 g dry wt) was sliced and extracted with MeOH (2 × 1 L) at room temperature. The crude extract (9.5 g) was partitioned between CH_2Cl_2 and 15% aqueous MeOH, and the organic extract was dried over Na_2SO_4 and then evaporated to obtain a crude organic extract (2.65 g) that was partitioned between hexane and 10% aqueous MeOH to obtain a hexane fraction (0.53 g) that appeared by NMR to contain the majority of the secosterols. The methanolic fraction was diluted with water to 20% aqueous MeOH and extracted with $CHCl_3$. The $CHCl_3$-soluble material (1.08 g) was chromatographed on silica gel to obtain manoalide (**6**, 260 mg, 0.43% dry wt) and secomanoalide (**7**, 70 mg, 0.12% dry wt). The hexane-soluble material was chromatographed on silica gel using a gradient of 5−15% EtOAc in hexane as eluant. The fractions containing the secosterols were pooled, and the secosterols were separated by HPLC on a preparative Microsorb silica column, using 10% EtOAc in hexane as eluant, to obtain, in order of elution, luffasterol B (**4**, 1.4 mg, 0.0023% dry wt), luffasterol A (**3**, 14 mg, 0.023% dry wt), and luffasterol C (**5**, 2.7 mg, 0.0044% dry wt).

Luffasterol A (3): white needles from hexane/EtOAc; mp 139−141 °C; $[\alpha]_D = -33.5°$ (*c* 0.35, $CHCl_3$); IR (film)

1735, 1715, 1675 cm^{-1}; UV (CH_2Cl_2) 255 nm (ϵ 4100); CD (MeOH) 331 nm (+8000), 262 nm (−20 000); ^1H NMR ($CDCl_3$) see Table 1; ^{13}C NMR ($CDCl_3$) see Table 1; EIMS *m/z* (rel int) 472 (15), 428 (38, $[M\text{-}CH_3CHO]^+$), 412 (19, $[M - CH_3COOH]^+$), 368 (28), 352 (16), 315 (39), 299 (25, $[M - CH_3COOH - C_8H_{17}]^+$), 255 (89), 119 (100); HREIMS *m/z* 472.3181, calcd for $C_{29}H_{44}O_5$ 472.3188.

Luffasterol B (4): white solid; $[\alpha]_D = -27.8°$ (*c* 0.047, $CHCl_3$); IR (film) 1730, 1715, 1680, 1240 cm^{-1}; UV (CH_2Cl_2) 256 nm (ϵ 4400); ^1H NMR ($CDCl_3$) see Table 2; EIMS *m/z* (rel int) 484 (9), 440 (19, $[M - CH_3CHO]^+$), 424 (5, $[M - CH_3COOH]^+$), 381 (8), 315 (26), 299 (52, $[M - CH_3COOH - C_9H_{17}]^+$), 255 (90), 119 (100); HREIMS *m/z* 484.3201, calcd for $C_{30}H_{44}O_5$ 484.3189.

Luffasterol C (5): amorphous white solid; $[\alpha]_D = -22°$ (*c* 0.16, $CHCl_3$); IR (film) 1730, 1715, 1680, 1240 cm^{-1}; UV (CH_2Cl_2) 255 nm (ϵ 4200); ^1H NMR ($CDCl_3$) see Table 2; EIMS *m/z* (rel int) 470 (4), 426 (10, $[M - CH_3CHO]^+$), 410 (6, $[M - CH_3COOH]^+$), 315 (16), 299 (41, $[M - CH_3COOH\text{-}C_8H_{15}]^+$), 255 (70), 119 (100); HREIMS *m/z* 488.3372, calcd for $C_{29}H_{46}NO_5$ $[M + NH_4]^+$ 488.3376.

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