Two New Sesterterpenoids and a New 9,11-Secosterol from *Spongia matamata*

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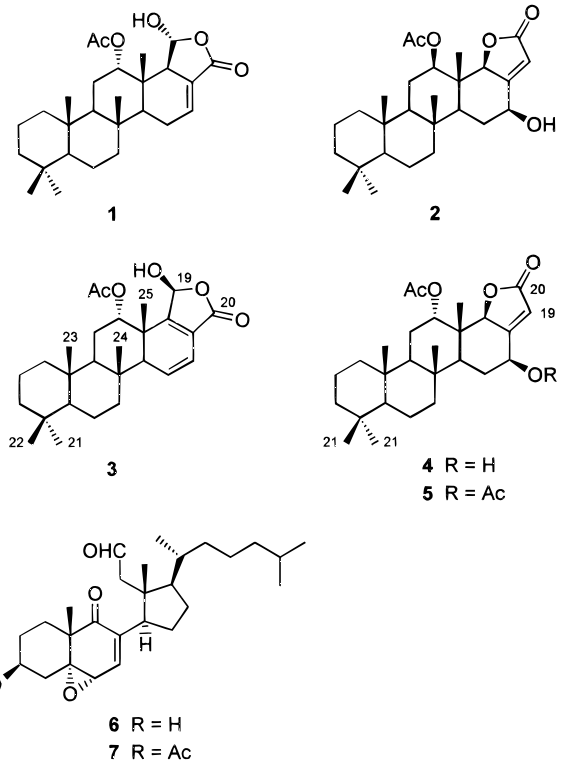
Two novel sesterterpenoids, 12α-acetoxy-19β-hydroxyscalara-15,17-dien-20,19-olide (**3**) and 12α,-16β-diacetoxyscalarolbutenolide (**5**), and a new 9,11-secosterol, 3β-hydroxy-5α,6α-epoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**6**), were isolated from the marine sponge *Spongia matamata* from Palau together with the known compounds scalarin (**1**) and 12α-acetoxy-16β-hydroxyscalarolbutenolide (**4**). The structures were determined by interpretation of spectroscopic data.

Many scalarane sesterterpenoids and 9,11-secosterols have been isolated from sponges of the order Dictyoceratida. Examples include scalarin (**1**) from *Cacospongia scalaris*,[1] 12-deacetoxyscalaradial from *C. mollior*,[2] scalarolbutenolide from *Spongia nitens*,[3] scalarolide from *S. idia*,[4] 16-*O*-deacetyl-16-*epi*-scalarolbutenolide (**2**) from *Hyrtios cf. erectus*,[5] and deoxyscalarin and 3β,6α-dihydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al from *S. officinalis*.[6,7] These compounds exhibit a variety of biological activities. As part of our studies of biologically active metabolites of sponges, we now report the isolation and structural elucidation of two new scalarane sesterterpenoids, 12α-acetoxy-19β-hydroxyscalara-15,-17-dien-20,19-olide (**3**) and 12α,16β-diacetoxyscalarolbutenolide (**5**), along with a novel 9,11-secosterol, 3β-hydroxy-5α,6α-epoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**6**), from *Spongia matamata* de Laubenfels 1954. In addition, we present the spectral data for 12α-acetoxy-16β-hydroxyscalarolbutenolide (**4**), which was reported with limited spectroscopic data as a metabolite of the nudibranch *Chromodoris inornata*.[8]

Specimens of *S. matamata* were collected from a marine lake at Palau, Western Caroline Islands, in June 1995 and were kept frozen until they were extracted with methanol. The dichloromethane-soluble material from the methanol extract was separated by flash chromatography on silica gel, and selected fractions were then purified by HPLC on silica to afford the known sesterterpenes scalarin (**1**, 5.8 mg, 0.005% dry wt) and 12α-acetoxy-16β-hydroxyscalarolbutenolide (**4**, 15.0 mg, 0.013% dry wt),[8] together with three new metabolites, 12α-acetoxy-19β-hydroxyscalara-15,17-dien-20,19-olide (**3**, 11.4 mg, 0.01% dry wt), 12α,16β-diacetoxyscalarolbutenolide (**5**, 10.2 mg, 0.009% dry wt), and 3β-hydroxy-5α,6α-epoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**6**, 8.4 mg, 0.007% dry wt).

12α-Acetoxy-19β-hydroxyscalara-15,17-dien-20,19-olide (**3**), $[\alpha]_D = +84.5°$, was isolated as colorless needles, mp 126−128 °C. The molecular formula, $C_{27}H_{38}O_5$, was established from HRCIMS and ^{13}C NMR data. The IR spectrum contained bands at 3370 (br, hydroxyl), 1770 (butenolide), 1740 (ester), and 1650 cm^{-1} (diene), and the UV spectrum showed an absorbance at 282 nm, which was indicative of a conjugated diene. The ^{13}C NMR spectrum (Table 1) contained two ester carbonyl signals at δ 170.4 and 168.8 and four olefinic signals at 162.2 (C), 132.8 (CH), 127.3 (C), and 117.2 (CH) and,



thus, requires **3** to be pentacyclic. The ^1H NMR spectrum (Table 1) exhibited signals at δ 0.80 (s, 3H), 0.81 (s, 3H), 0.85 (s, 3H), 1.04 (s, 3H), 1.17 (s, 3H), and 2.12 (s, 3H) due to five aliphatic methyl groups and an acetate group, which is appropriate for a pentacyclic scalarane ring system. The olefinic signals at δ 6.10 (dd, 1H, $J = 8$, 2.5 Hz, H-15) and 6.29 (dd, 1H, $J = 8$, 2.5 Hz, H-16) are both coupled to a methine proton signal at 2.60 (t, 1H, $J = 2.5$ Hz, H-14), which allows the conjugated diene to be placed in ring D. The signal for the methine proton on the γ-hydroxybutenolide ring occurs at δ 6.07 (d, 1H, $J = 6$ Hz, H-19) and is coupled to a hydroxyl proton signal at 4.40 (d, 1H, $J = 6$ Hz). The signal at δ 5.30 (dd, 1H, $J = 4$, 2 Hz) was assigned to an equatorial methine proton at C-12. Key HMBC correlations were observed between the H-25 methyl signal at δ 1.17 and the carbon signals at 162.2 (C-18) and 73.7 (C-12). Irradiation of the H-12 signal produced nuclear Overhauser enhancements of H-19 and H-25, which indicated that H-12 is equatorial (12α-acetoxy) and defined the regiochemistry of the butenolide ring. Irradiation of the H-19 signal, which overlaps with the

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Table 1. ^{13}C (100 MHz, $CDCl_3$) and ^1H NMR (400 MHz, $CDCl_3$) NMR Data for 12α-Acetoxy-19β-hydroxyscalara-15,17-dien-20,19-olide (**3**)

C no.	δ_C	δ_H	mult, *J* (Hz)	COSY	HMBC
1	39.8	0.56	m	H-1′	
		1.57	m	H-1	
2	18.4	1.40	m		
		1.60	br s		
3	41.9	1.11	m		
		1.36	m		
4	33.6				
5	56.7	0.83	m	H-6	
6	17.7	1.40	m	H-5	
		1.60	m		
7	40.8	1.00	m	H-7′	
		1.91	m	H-7	
8	37.4				
9	51.9	1.25	dd, 13, 3	H-11	
10	36.8				
11	21.6	1.64	m	H-9, H-11′, H-12	
		1.95	ddd, 15, 4, 2	H-11′, H-12	
12	73.7	5.30	dd, 4, 2	H-11, H-11′	C-9, C-13, C-14
13	41.9				
14	53.1	2.60	t, 2.5	H-15, H-16	C-8, C-13, C-15, C-16 C-18, C-24, C-25
15	132.8	6.10	dd, 8, 2.5	H-14, H-16	C-8, C-13, C-14, C-16 C-17
16	117.2	6.29	dd, 8, 2.5	H-14, H-15	C-14, C-18
17	127.3				
18	162.2				
19	96.9	6.07	br d, 6	OH	C-13, C-17, C-18, C-20
20	168.8				
21	33.2	0.85	s, 3 H		C-3, C-4, C-5, C-22
22	21.3	0.80	s, 3 H		C-3, C-4, C-5, C-21
23	15.8	0.81	s, 3 H		C-1, C-5, C-9, C-10
24	18.9	1.04	s, 3 H		C-7, C-8, C-9, C-14
25	16.4	1.17	s, 3 H		C-12, C-13, C-14, C-18
OAc	21.5	2.12	s, 3 H		
	170.4				
OH		4.40	d, 6	H-19	

H-15 signal, caused a significant enhancement of the acetate methyl signal at δ 2.12 (s, 3 H), which indicated that H-19 must be in the α-orientation. The ^{13}C NMR spectrum revealed the expected 27 carbon signals, most of which were assigned using the HMQC and HMBC experiments. All spectral data, including the DQCOSY and NOEDS experiments, completely support the structure assigned to 12α-acetoxy-19β-hydroxyscalara-15,17-dien-20,19-olide (**3**).

12α-Acetoxy-16β-hydroxyscalarolbutenolide (**4**), $[\alpha]_D$ = +61°, which was eventually found to be a poorly characterized known compound,[8,9] was isolated as pale yellow needles, mp 183−185 °C. A brief summary of the spectral analysis seems appropriate under these circumstances. The molecular formula, $C_{27}H_{40}O_5$, was established from HREIMS and ^{13}C NMR data. The IR spectrum contained bands at 3420 (hydroxyl), 1780 (butenolide), 1745 (ester and butenolide), and 1650 cm^{-1} (unsaturation). The *all-trans* tetracyclic ring system was assigned on the basis of the ^{13}C NMR chemical shifts of the bridgehead methyl groups and the HMBC correlations between the ^1H NMR signals for the corresponding methyl groups and the ^{13}C signals for bridgehead methine groups. The H-25 methyl signal was correlated to the bridgehead methine signal at δ 47.1 (C-14) and to signals at δ 74.3 (C-12) and 82.0 (C-18), both of which must be substituted by ester oxygen groups. This indicated that we were dealing with a member of the scalarolbutenolide group rather than a scalarane.[10] Comparison of the spectral data of **4** with those of 16-*O*-deacetyl-16-*epi*-scalarolbutenolide (**2**)[5] indicated that they had the same substitution pattern and stereochemistry about ring D. The stereochemistry at C-12 was deduced from the two small coupling constants associated with H-12, which must be in the equatorial conformation. 12α-Acetoxy-16β-hydroxyscalarolbutenolide (**4**) was previously reported in an abstract as a metabolite of the nudibranch *Chromodoris inornata* but was not included in the paper that reported other metabolites from *C. inornata*.[8]

12α,16β-Diacetoxyscalarolbutenolide (**5**), $[\alpha]_D$ = +51°, was isolated as a pale yellow amorphous solid, mp 60−62 °C. The molecular formula of $C_{29}H_{42}O_6$ was established from HREIMS and ^{13}C NMR data. ^1H and ^{13}C NMR data (Table 2) were very similar to those of alcohol **4** except for the presence of an additional acetyl group in **5**. The significant downfield shift of H-16 (+1.04 ppm) in **5** indicated that the additional acetate group is at that position, and thus acetate **5** was 12α,16β-diacetoxyscalarolbutenolide. This structural assignment was confirmed by acetylation of alcohol **4** with acetic anhydride in pyridine to obtain acetate **5**.

3β-Hydroxy-5α,6α-epoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**6**), $[\alpha]_D$ = −6.7°, was isolated as colorless needles, mp 171−173 °C. A molecular formula of $C_{27}H_{42}O_4$ was established from HREIMS and ^{13}C NMR data. The structural elucidation of **6** was considerably simplified because Dr. M. V. R. Reddy in our laboratory had recently isolated the corresponding 3-acetate derivative, luffasterol A (**7**), from the marine sponge *Luffariella* sp.[11] Comparison of the ^1H and ^{13}C NMR data (Table 3) showed the two compounds to be identical except for the additional acetate group in **7** and the corresponding downfield shift of the H-3 signal from δ

Table 2. ^{13}C (100 MHz, $CDCl_3$) and Selected ^1H (400 MHz, $CDCl_3$) NMR Data for Butenolides **4** and **5**

	4			**5**		
C no.	δ_C	δ_H	mult, *J* (Hz)	δ_C	δ_H	mult, *J* (Hz)
1	39.8	0.56	m	39.8	0.56	m
		1.59	m			
2	18.4	1.38	m	18.4		
		1.57	m			
3	41.9	1.08	m	41.9		
		1.36	m			
4	33.3			33.3		
5	56.6	0.82	m	56.5		
6	18.2	1.38	m	18.1		
		1.57	m			
7	42.0	1.04	m	42.0		
		1.82	dt, 12.5, 3		1.78	dt, 12.5, 3
8	38.0			38.1		
9	52.2	1.22	dd, 14.5, 1.5	52.1		
10	36.9			36.9		
11	21.6	1.62	m	21.6		
		1.92	ddd, 14.5, 4, 2		1.94	ddd, 14.5, 4, 2
12	74.3	4.86	dd, 4, 3	74.4	4.87	dd, 4, 3
13	43.8			43.9		
14	47.1	1.40	dd, 12, 2	46.9		
15	31.2	1.49	ddd, 12.5, 12, 11	27.6		
		2.18	ddd, 12.5, 7, 2		2.20	ddd, 12.5, 7, 2.5
16	68.3	4.50	ddd, 11, 7, 1.5	69.1	5.54	ddd, 11, 7, 1.5
17	171.9			169.7		
18	82.0	4.89	d, 1.5	82.0	4.94	d, 1.5
19	112.2	5.93	t, 1.5	112.7	5.79	t, 1.5
20	173.0			172.4		
21	33.2	0.84	s, 3 H	33.2	0.84	s, 3 H
22	21.3	0.78	s, 3 H	21.3	0.78	s, 3 H
23	16.1	0.78	s, 3 H	16.1	0.78	s, 3 H
24	17.1	0.87	s, 3 H	17.1	0.87	s, 3 H
25	12.0	0.74	s, 3 H	11.9	0.76	s, 3 H
OAc	21.3	2.09	s, 3 H	21.3	2.10	s, 3 H
	169.3			169.2		
OAc				20.8	2.14	s, 3 H
				167.2		

Table 3. ^{13}C (100 MHz, $CDCl_3$) and Selected ^1H (400 MHz, $CDCl_3$) NMR Data for Secosterols **6** and **7**

	6			**7**		
C no.	δ_C	δ_H	mult, *J* (Hz)	δ_C	δ_H	mult, *J* (Hz)
1	27.8			27.5		
2	30.5			26.5		
3	68.3	3.96	m	70.5	4.96	m
4	37.4			33.9		
5	63.5			63.0		
6	53.5	3.39	d, 4.5	53.4	3.38	d, 4.5
7	139.8	6.84	dd, 4.5, 1	139.8	6.84	dd, 4.5, 1
8	140.5			140.5		
9	200.7			200.3		
10	45.4			45.4		
11	203.5	9.86	dd, 4, 1.5	203.5	9.85	dd, 4, 1.5
12	50.6			50.5		
13	46.4			46.4		
14	44.9	3.51	dd, 9.5, 9	44.8	3.50	dd, 9.5, 9
15	26.6			26.6		
16	25.9			25.9		
17	51.7			51.7		
18	16.7	0.73	s, 3 H	16.6	0.73	s, 3 H
19	21.1	1.19	s, 3 H	20.9	1.19	s, 3 H
20	34.8			34.8		
21	19.3	0.91	d, 6.5	19.3	0.90	d, 6.5
22	35.3			35.3		
23	24.3			24.3		
24	39.4			39.4		
25	27.9			27.9		
26	22.5	0.84	d, 6.5	22.5	0.84	d, 6.5
27	22.6	0.83	d, 6.5	22.7	0.83	d, 6.5
OAc				21.2	2.02	s, 3 H
				170.1		

3.96 in **6** to 4.96 in **7**. The structure of 3β-hydroxy-5α,6α-epoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**6**) was confirmed by acetylation with acetic anhydride in pyridine to obtain luffasterol A (**7**).

Experimental Section

General Experimental Procedures. See ref 11.

Animal Material. The sponge *S. matamata* de Laubenfels 1954 (95-090) was collected by hand using Scuba (at a depth of 2 ft) at Risong marine lake, Republic of Palau, in June 1995, frozen immediately after collection, and kept frozen until used. A specimen of the sponge has been deposited in the SIO Benthic Invertebrate Collection (P-1165).

Extraction and Isolation. The frozen sponge (116 g dry wt) was cut into small pieces and extracted with MeOH (2 × 1 L). The combined MeOH extracts were concentrated and partitioned between CH_2Cl_2 (800 mL) and 15% MeOH in water (800 mL). The CH_2Cl_2 fraction was concentrated and partitioned between hexane (600 mL) and 10% aqueous MeOH (600 mL). The methanolic layer was then partitioned between CH_2Cl_2 (400 mL) and 40% aqueous MeOH (400 mL). The CH_2Cl_2 fraction was concentrated and chromatographed on silica gel using a gradient of 10% EtOAc in CH_2Cl_2 to 100% EtOAc as eluant. The fraction that eluted with 20% EtOAc in CH_2Cl_2 (*ca*. 100 mg) was rechromatographed over silica gel using 50% EtOAc in hexane to obtain four fractions. Purification of fractions 2 and 3 by HPLC (Microsorb, Si 80-199-C5) using 25% EtOAc in hexane afforded 12α-acetoxy-19β-hydroxyscalara-15,17-dien-20,-19-olide (**3**, 11.4 mg, 0.01% dry wt) and 12α,16β-diacetoxyscalarolbutenolide (**5**, 10.2 mg, 0.009% dry wt).

The fraction that eluted with 25% EtOAc in CH_2Cl_2 (*ca.* 50 mg) was rechromatographed on silica gel using 50% EtOAc in hexane to obtain three fractions. Purification of fraction 2 by HPLC using 40% EtOAc in hexane as eluant afforded 12α-acetoxy-16*β*-hydroxyscalarolbutenolide (**4**, 15.0 mg, 0.013%). The fraction that eluted with 30% EtOAc in CH_2Cl_2 (*ca.* 40 mg) was rechromatographed on silica gel using 70% EtOAc in hexane to obtain three fractions. Purification of fraction 2 by HPLC using 50% EtOAc in hexane as eluant afforded 3α-hydroxy-5α,6α-epoxy- 9-oxo-9,11-seco-5α-cholest-7-en-11-al (**6**, 8.4 mg, 0.007% dry wt). The fraction that eluted with 35% EtOAc in CH_2Cl_2 (*ca.* 15 mg) was purified by HPLC using 70% EtOAc in hexane as eluant to obtain scalarin (**1**, 5.8 mg, 0.005% dry wt).

12α-Acetoxy-19*β*-hydroxyscalara-15,17-dien-20,-19-olide (3): colorless needles; mp 126−128 °C; $[\alpha]_D$ = +84.5° (*c* 0.733, $CHCl_3$); IR (film) 3370 (br), 3003, 2930, 2880, 2850, 1770, 1740, 1650, 1460, 1380, 1240 cm^{-1}; UV (CH_2Cl_2) 226 nm (ϵ 5100), 282 nm (ϵ 2400); ^1H NMR ($CDCl_3$, 400 MHz) see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; LREIMS *m/z* (rel int) 399 (M^+ − $COCH_3$, 1), 382 (M^+ − CH_3CO_2H, 24), 367 (100), 364 (21), 349 (37); HRCIMS *m/z* 460.3068, calcd for $C_{27}H_{42}NO_5$ $[M + NH_4]^+$ 460.3068.

12α-Acetoxy-16*β*-hydroxyscalarolbutenolide (4): pale yellow needles; mp 183−185 °C; $[\alpha]_D$ +61.4° (*c* 1.0, $CHCl_3$); IR (film) 3420 (br), 2930, 2850, 1740, 1650, 1460, 1390, 1240 cm^{-1}; UV (CH_2Cl_2) 229 nm (ϵ 7800); ^1H NMR ($CDCl_3$, 400 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; LREIMS *m/z* (rel int) 444 (M^+, 5), 384 (20), 369 (13), 191 (100); HRMS *m/z* 444.2873, calcd for $C_{27}H_{40}O_5$ 444.2876.

12α,16*β*-Diacetoxyscalarolbutenolide (5): pale yellow amorphous solid; mp 60−62 °C; $[\alpha]_D$ +51.0° (*c* 0.33, $CHCl_3$); IR (film) 3430 (br), 2930, 2860, 1760, 1750, 1650, 1460, 1370, 1230 cm^{-1}; UV (CH_2Cl_2) 229 nm (ϵ 8400); ^1H NMR ($CDCl_3$, 400 MHz) see Table 2; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2; LREIMS *m/z* (rel int) 486 (M^+, 7), 426 (10), 411 (9), 366 (9), 191 (100); HRMS *m/z* 486.2971, calcd for $C_{29}H_{42}O_6$ 486.2981.

3*β*-Hydroxy-5α,6α-epoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (6): colorless needles; mp 171−173 °C; $[\alpha]_D$ = −6.7° (*c* 0.24, $CHCl_3$); IR (film) 3420 (br), 2950, 2850, 1716, 1682, 1465 cm^{-1}; UV (CH_2Cl_2) 260 nm (ϵ 3400); ^1H NMR ($CDCl_3$, 400 MHz) see Table 3; ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 3; LREIMS *m/z* (rel int) 430 (M^+, 24), 386 (47), 273 (100), 255 (49); HRMS *m/z* 430.3088, calcd for $C_{27}H_{42}O_4$ 430.3083.

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(9) Comparison of the spectral data of **4** with those provided by Prof. R. Higuchi indicated that the compounds were identical.
(10) Scalarolbutenolide[3] has a different carbon skeleton than that of the scalarins, but unfortunately, the new carbon skelton was not named as such and semi-systematic names are therefore awkward. In order to reduce confusion, we have defined the substituents at C-12 and C-16 as α or *β* rather than use "epi" notation.
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