New Cytotoxic Scalarane Sesterterpenes from the Dictyoceratid Sponge Strepsichordaia lendenfeldi

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NEW CYTOTOXIC SCALARANE SESTERTERPENES FROM THE DICTYOCERATID SPONGE *STREPSICHORDAIA LENDENFELDI*

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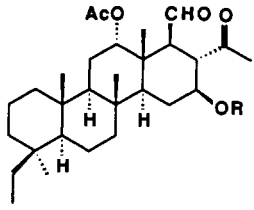
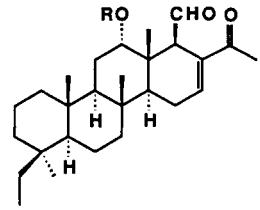
ABSTRACT.—Nine sesterterpenes **1–9** have been isolated from a Dictyoceratid sponge, *Strepsichordaia lendenfeldi*, collected on the Great Barrier Reef, Australia. Seven of these compounds are new, one [**4**] had been reported previously as a semisynthetic product, and one [**5**] had been isolated previously from *Carteriospongia foliascens*. Unambiguous ^{13}C- and ^{1}H-nmr assignments have been made for **1** based on extensive 1D and 2D high field nmr experiments, which enabled ^{13}C-nmr assignments to be made for **2–9**. Significant growth inhibitory effects with tested cancer cell lines are reported for compounds **1–8**.

Dictyoceratid sponges are relatively soft and do not possess calcareous or siliceous spicules for defense. The two families Thorectidae and Spongiidae have been a rich source of sesterterpenes, most of which incorporate the scalarane skeleton (1). A review by Braekman *et al.* (2) in 1985 highlighted the ichthyotoxicity of the scalarane sesterterpenes, and noted the incidence of anti-inflammatory and cytotoxic activity as assed by other authors. The 20,24-bishomoscalaranes are a group of sesterterpenes which appear to be taxonomic markers for the Dictyoceratid sponges, and more specifically for the genera *Phyllospongia* (3–5) and *Carteriospongia* (2, 6–9). [The numbering system which has been used throughout this paper is that proposed by Kazlauskas *et al.* (3) (Figure 1); the numbering for C-21 and C-22 has been (inadvertently?) reversed by Braekman *et al.* (2).] It has been proposed that the 4-ethylscalaranes in the literature all





1 $^{22.4\ 64.1\ 42.7\ 171.5}$ R=$CH_3CHOHCH_2CO$-

2 $^{9.7\ 29.4\ 69.2\ 40.9\ 171.7}$ R=$CH_3CH_2CHOHCH_2CO$-

3 $^{9.0\ 27.6\ 173.0}$ R=CH_3CH_2CO-

4 $^{20.9\ 169.5}$ R=CH_3CO-

5 R=H-

6 $^{9.4\ 26.7\ 71.6\ 170.5\ 21.1\ 38.9\ 169.1}$ R=$CH_3CH_2CH(OCOCH_3)CH_2CO$-

7 $^{9.4\ 26.7\ 71.4\ 173.5\ 27.6\ 9.1\ 38.9\ 169.2}$ R=$CH_3CH_2CH(OCOCH_2CH_3)CH_2CO$-

8 $^{19.8\ 67.4\ 170.3\ 21.1\ 41.1\ 168.9}$ R=$CH_3CH(OCOCH_3)CH_2CO$-

9 $^{10.0\ 29.3\ 69.4\ 42.0\ 171.6}$ R=$CH_3CH_2CHOHCH_2CO$-

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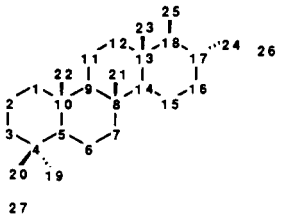

FIGURE 1. Scalarane numbering scheme.

possess a 4β-ethyl group (2,10), despite an earlier report which suggested a 4α-ethyl group (3).

RESULTS AND DISCUSSION

We report here joint work from two laboratories on 20,24-bishomoscalaranes from the Thorectid sponge *Strepsichordaia lendenfeldi* Bergquist (Dictyoceratida) (11) obtained from the Great Barrier Reef, Australia. The petroleum ether extract of a sample collected by the Townsville group was mildly active against the Gram-positive microorganisms *Bacillus subtilis* and *Staphylococcus aureus* and inactive against Gram-negative bacteria and fungi. It was, however, mildly inhibitory against the human bladder carcinoma cell line T24, and not toxic to the human lung cell line MRC5. The CH_2Cl_2 extract was inactive against microorganisms but cytotoxic to both the normal and tumor cell lines.

The petroleum ether extract was fractionated by vlc on Si gel followed by hplc on Si gel to give compounds **1**, **3**, **4**, **6**, and **7**. The major metabolite **1** was readily identified from its spectroscopic properties as a bishomoscalarane sesterterpene with two secondary hydroxyl groups esterified by acetate and 3-hydroxybutanoyl functions. While **1** did not show a molecular ion in the low resolution eims, it gave an ion at *m/z* 578.4057 $[M + NH_4]^+$ in the cims spectrum. The eims showed peaks at *m/z* 205 and 219, corresponding to cleavages through the C ring (2). The ir spectrum showed the presence of hydroxyl (3518 cm^{-1}), saturated aldehyde (2700, 1718 cm^{-1}), and strong ester absorption (1739 cm^{-1}), while the uv spectrum showed no absorption maximum above 210 nm. The ^{1}H-nmr spectrum showed the presence of an aldehyde group (δ 9.47, 1H, s), a methyl ketone residue (δ 2.32, 3H, s), four tertiary methyl groups (δ 0.80, 0.81, 0.83, 0.97, each 3H, s), an ethyl group (δ 0.73, 3H, t, J = 6.5 Hz), and a 3-hydroxybutanoyl group (δ 2.40, 2H, m, H-2′; 4.16, 1H, m, H-3′; 1.21, 3H, d, J = 6.5 Hz, H-4′) in addition to signals at δ 5.11 (1H, m, H-12) and 4.71 (1H, ddd, H-16). Apart from signals for the 3-hydroxybutanyol group, the ^{13}C-nmr spectrum of **1** was almost identical with that of 20,22-dimethyl-19,20-dioxoscalaran-12α,16β-yl diacetate [**4**] (2). By the use of 2D nmr experiments at high field, including COSY, 2D-J resolved, XHCORRD, and COLOC, unambiguous associations of carbons and attached protons were made, and the complete assignment of all carbon and proton signals to the scalarane skeleton in **1** could be made (Table 1 and Experimental). Although several ^{13}C assignments differ from those in some of the earlier literature reports on similar sesterterpenes, agreement with assignments from a recent report (9) where nmr pulse-sequences (and 2D correlations) were used to determine the assignments of the bishomoscalarane nucleus is very good. Our nmr correlations did not permit us to distinguish between the assignments for C-8 and C-10, so for those two atoms we have followed the assignments of Barron *et al.* (9), who had correlation data to support their assignments for those atoms.

The location of the specific esters was assigned by INAPT (1D selective INEPT)

TABLE 1. ^{13}C-nmr Data for **1–9**. Assignments for **1** are Unambiguous.[a] Assignments for **2–9** are Based on Comparison with **1**.[b]

Carbon	Compound								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
C-1	40.2	40.2	40.2	40.1	40.2	40.0	40.0	40.0	40.0
C-2	18.2	18.2	18.2	18.2	18.2	18.1	18.1	18.1	18.1
C-3	36.5	36.5	36.5	36.5	36.6	36.6	36.6	36.6	36.6
C-4	36.1	36.1	36.1	36.0	36.1	36.1	36.1	36.1	36.1
C-5	58.5	58.5	58.5	58.5	58.5	58.5	58.5	58.5	58.5
C-6	17.9	17.9	17.9	17.9	18.0	17.9	17.9	17.9	17.9
C-7	41.6	41.6	41.6	41.6	41.8	41.8	41.8	41.8	41.8
C-8	38.0	38.0	38.0	38.0	37.9	37.8	37.8	37.8	37.8
C-9	52.7	52.7	52.7	52.7	52.8	52.9	52.9	52.9	53.0
C-10	36.9	36.9	36.9	36.8	36.9	36.8	36.9	36.8	36.8
C-11	21.7	21.7	21.7	21.7	21.7	21.9	21.9	21.9	21.9
C-12	74.7	74.7	74.7	74.7	74.8	75.3	75.3	75.3	75.3
C-13	40.5	40.5	40.5	40.4	40.5	40.2	40.2	40.2	40.3
C-14	50.8	50.8	50.7	50.7	51.1[c]	48.9	48.9	48.9	49.0
C-15	25.8	25.8	25.9	25.8	30.4	23.7	23.7	23.7	23.8
C-16	76.2	76.2	75.7	75.8	73.9	142.9	142.9	143.0	143.3
C-17	49.1	49.1	49.1	49.1	52.0[c]	137.1	137.0	137.1	136.9
C-18	58.5	58.5	58.5	58.5	58.2	52.2	52.2	52.2	52.2
C-19	28.4	28.4	28.4	28.3	28.4	28.5	28.4	28.4	28.4
C-20	24.4	24.4	24.4	24.4	24.5	24.5	24.5	24.5	24.5
C-21	16.9	16.9	16.9	16.8	16.9	17.1	17.1	17.1	17.0
C-22	16.8	16.8	16.8	16.7	16.8	16.6	16.6	16.6	16.5
C-23	17.2	17.2	17.2	17.1	17.3	15.2	15.3	15.2	15.3
C-24	210.9	210.9	211.0	210.9	213.2	198.5	198.5	198.5	198.6
C-25	202.1	202.1	202.2	202.1	202.6	200.8	200.9	200.7	201.5
C-26	33.0	32.9	33.0	32.9	33.7	25.1	25.1	25.0	25.0
C-27	8.6	86.	8.6	8.6	8.6	8.6	8.6	8.6	8.6
12-OAc	21.5	21.5	21.5	21.5	21.5				
	170.2	170.2	170.2	170.2	170.2				

[a]Assignments based on XHCORRD, COLOC, INAPT and COSY spectroscopy.
[b]Additional signals see structures.
[c]These assignments may be interchanged.

correlation of the respective protons attached to the secondary oxygenated carbons with the appropriate carbonyl group. Thus, in **1**, irradiation at δ 5.11 (H-12β) gave a ^{13}C signal at 170.2 (acetate C=O) in addition to the signals at 52.7 (C-9), 50.8 (C-14), 40.5 (C-13), and 17.2 (C-23) ppm when delays were optimal to observe $J = 8$ Hz. Similarly, irradiation at δ 5.71 (H-16α) gave a ^{13}C signal at 171.5 (hydroxybutyrate C=O) in addition to the signals at 210.9 (C-24), and 49.1 (C-17) ppm.

The relative stereochemistry of substituents on the C and D rings was assigned on the basis of coupling constants. In compounds **1**, **3**, and **4**, H-15β, H-16α, H-17β, and H-18α are all trans periplanar to one another and linked by vicinal coupling constants of about 11 Hz. The esterified substituent at C-12 in compounds **1**, **3**, **4**, **6**, and **7** was axial; i.e., the attached equatorial proton was coupled to the neighboring H-11α and H-11β with $J<3$ Hz.

The absolute stereochemistry of the secondary alcohol function was determined by application of Mosher's method (12,13). The *R* and *S*-*O*-methylmandelate esters were prepared (14), and the ^{1}H-nmr spectra of the diastereomers showed the expected chemical shift patterns (Figure 2) for a 3*R*-hydroxybutyrate function. This result compares

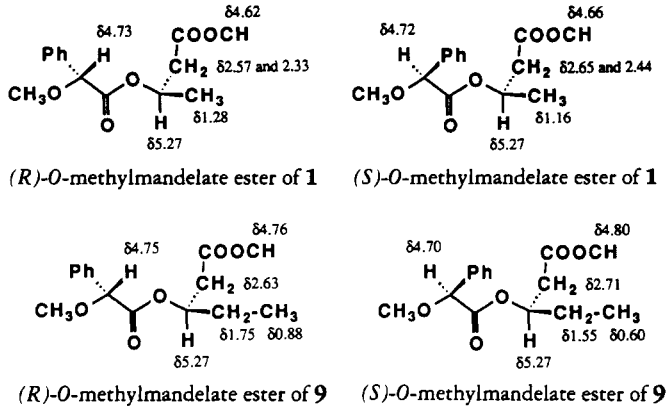

FIGURE 2. Selected ^{1}H-nmr shifts for the *R/S* pairs of *O*-methylmandelate esters of the hydroxybutanoate group in **1** and the hydroxypentanoate group in **9**.

favorably with the *S* absolute stereochemistry determined for the secondary alcohol function in phyllofoliaspongin (5), since the ester in phyllofoliaspongin is a 4-methyl-3-hydroxypentanoate where the priorities for groups around the asymmetric center are reversed relative to **1**.

The crude CH_2Cl_2 extract of a freeze-dried sponge used by the Auckland workers showed similar activity against the Gram-positive bacteria previously described, and in addition was active against the yeast *Candida albicans*. The extract was chromatographed on Sephadex LH20 and Si gel to afford the same compounds, **1**, **3**, **4**, **6**, and **7**, plus three additional sesterterpenes **2**, **5**, and **8**. Again, the INAPT technique was used to verify the relative positions of the different esters: for **2**, irradiation at δ 5.11 (H-12β) gave a ^{13}C signal at 170.2 (acetate C=O) in addition to the same correlations at 52.7 (C-9), 50.8 (C-14), 40.5 (C-13), and 17.2 (C-23) that were observed for **1**. This result clearly places the acetate functionality at C-12; hence the hydroxypentanoate group must be at C-16. These additional examples readily fitted into the pattern of metabolites previously isolated. Compound **5** had previously been reported from *Carteriospongia foliascens* (2), and **4** had been prepared from it by acetylation of the secondary alcohol function.

The Townsville group re-collected a sample of *Str. lendenfeldi* from Sudbury reef. Extraction and fractionation under the previously mentioned conditions yielded the crystalline alcohol **9** in addition to all the previously described metabolites. The absolute stereochemistry of the secondary alcohol function in **9** was also determined by application of Mosher's method, and again the expected chemical shift patterns (Figure 2) for a 3*R*-hydroxybutyrate function were observed.

Agreement among the ^{13}C-nmr spectral values for all comparable carbon positions in **1–9** is extremely good, suggesting that rings A through C are identical in **1–9** and that the substitution patterns in ring D are the same for **1–5** and for **6–9**.

The sesterterpenes reported were responsible for the mild activity against Gram-positive microorganisms observed for the crude extract; compound **1** was active against *B. subtilis* and *Sta. aureus*, inactive against Gram-negative bacteria and fungi. Compounds **3**, **4**, **6**, and **7** were also active against *B. subtilis*, while compounds **2**, **5**, **8**, and **9** were not tested. All of the reported sesterterpenes exhibited moderate cytotoxicity against both P-388 (lymphoid neoplasm from DBA/2 mouse) and A-549 (human lung carcinoma) cells in culture (Table 2). Compounds **2**, **5**, and **8** gave the same approxi-

TABLE 2. Cytotoxicity Results (IC_{50}) for Compounds **1–8** Against P-388 and A549 Cell Lines in Culture.

Compound	Cell Line	
	P388	A-549
1	0.23[a]	0.66
2	~0.5	~0.5
3	0.67	0.67
4	0.91	0.88
5	~0.1	~0.1
6	<0.12	0.25
7	<0.12	0.21
8	~0.2	~0.2

[a]μg/ml.

mate IC_{50} values against HT-29 (human colon carcinoma) and CV-1 (monkey kidney fibroblast) cells as for the P-388 and A-549 cell lines.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ms were determined on a Varian VG 70-SE mass spectrometer. Routine ^{1}H- and ^{13}C-nmr spectra at high field were recorded on a Bruker AM300 (Townsville) or AM400 (Auckland) nmr spectrometer in $CDCl_3$. 2D nmr experiments were performed with Bruker '88 software and the Bruker ASPECT 3000 system. For ^{13}C-^{1}H correlations (J = 125 Hz), the Bruker program XHCORRD was run with 2048 data points and with D_1 = 1.5 sec, D_3 = 0.0038 sec (one bond). For ^{13}C-^{1}H correlations (J = 10 Hz), the Bruker program COLOC was run with 2048 data points and with D_1 = 1.5 sec, D_2 = 0.05 sec. Ir spectra were recorded on a Perkin-Elmer 1600 Ft-ir spectrometer and the uv spectra (in EtOH) on a Varian series 634 spectrophotometer. Optical rotation measurements with a Perkin-Elmer 141 polarimeter were carried out on $CHCl_3$ solutions. Si gel (type 60, Merck) was used for vlc, and plastic-backed plates coated with Si gel F_{254} (Merck) were used for tlc. Hplc was carried out with either a Waters 6000A or 4500A solvent delivery system connected to a Waters U6K injector and a Waters R401 differential refractometer. Hplc columns were from Techsil (250×8 mm, filled with Techsil 5 mm silica) and Hewlett-Packard (250 × 8 mm, filled with Si-100 7 mm). These hplc columns were either in series or alone. All solvents were distilled prior to use.

BIOLOGICAL MATERIAL.—Samples of *Str. lendenfeldi* were collected in November 1989 at Elford Reef near Cairns, North Queensland from a depth of 8 m by scuba diving. The sponge was frozen after collection and freeze-dried prior to extraction. A museum specimen, Holotype AM Z5026, is held in the Department of Zoology, University of Auckland by Prof. P.R. Bergquist, who provided taxonomic identification.

STR. LENDENFELDI METABOLITES.—The freeze-dried sponge (185 g) was extracted with petroleum ether at room temperature three times over 48 h. The solvent was concentrated to give a crude extract (2.6 g), which was subjected to vlc on Si gel. Elution commenced with petroleum ether, followed by increasing portions of CH_2Cl_2 in petroleum ether, and finally EtOAc in CH_2Cl_2. The less polar fractions contained lipid which was discarded after nmr evaluation. More polar fractions were combined and rechromatographed with a variety of solvent mixtures (EtOAc/petroleum ether; Me_2CO/CH_2Cl_2; EtOAc/CH_2Cl_2; Me_2CO/CH_2Cl_2/petroleum ether) on several vlc columns. Final purification in some cases was by repeated hplc separation on Si gel with 30% EtOAc in petroleum ether as solvent. In Auckland, the freeze-dried sponge (260 g) was only extracted twice with CH_2Cl_2, and separation of a 5 g portion of the crude extract (15.1 g) was again achieved by vlc. In this manner, the eight sesterterpenes were isolated.

12α-Acetoxy-16β-(3'R-hydroxybutanoyloxy)-20,24-dimethyl-24-oxoscalaran-25-al [**1**].—Oil (0.60 g): $[\alpha]_D$ +61.3° (c = 1.08); ir ν max (film) 3518 (OH), 1739, 1718 (C=O), 1462, 1391, 1313, 1123, 1018, 953, 798, 788 cm^{-1}; ^{1}H nmr δ 0.62 (m, H-1α), 1.52 (m, H-1β), 1.35 (m) and 1.50 (m, H_2-2), 1.02 (m) and 1.68 (m, H_2-3), 0.93 (m, H-5), 1.55 (m) and 1.40 (m, H_2-6), 1.65 (m) and 0.85 (m, H_2-7), 1.30 (m, H-9), 1.85 (m) and 1.65 (m, H_2-11), 5.11 (br s, H-12β), 1.53 (m, H-14), 2.05 (m) and 1.25 (m, H_2-15),

4.71 (ddd, 10.8, 10.8, 4.8, H-16), 3.08 (dd, 11.0, 10.8, H-17), 3.17 (d, 11.0, H-18), 0.81 (s, H_3-19), 1.45 (m) and 1.1 (m, H_2-20), 0.83 (s, H_3-21), 0.80 (s, H_3-22), 0.97 (s, H_3-23), 9.47 (s, H-25), 2.32 (s, H_3-26), 0.73 (t, 6.5 H_3-27), 2.20 (s, 12-OAc), 1.21 (d, 6.5, H_3-4′), 4.16 (m, H-3′), 2.40 (m, H_2-2′); ^{13}C nmr see Table 1; cims (NH_3) *m/z* $[M+NH_4]^+$ 578 (31%), 548 (15), 457 (40), 439 (37), 415 (20), 397 (100), 369 (66), 315 (38), 205 (26); hrcims (NH_3) *m/z* 578.4059 ($C_{33}H_{52}O_7+NH_4^+$ requires 578.4057).

12α-Acetoxy-16β-(3′-hydroxypentanoyloxy)-20,24-dimethyl-24-oxoscalaran-25-al [**2**].—Oil (16 mg): $[\alpha]_D$ +60.3 (*c* = 0.46); ir ν max (film) 3520 (OH), 1740, 1720 (C=O), ^{1}H nmr δ 9.47 (s, CHO), 5.11 (brs, H-12β), 4.72 (ddd, 11, 11, 4.7 Hz, H-16α), 3.91 (m, H-3′), 3.18 (d, 11.5 Hz, H-18α), 3.09 (dd, 11, 11 Hz, H-17β), 2.33 (s, Me-26), 2.41 (m, H_2-2′), 2.20 (s, 12-OAc), 2.06 (m, H-15α), 1.86 (m, H-2α), 0.97 (s, Me-23), 0.96 (t, 7.4 Hz, Me-5′), 0.83, 0.81, 0.80 (s,s,s, Me-19, Me-21, Me-22), 0.74 (t, 7.4 Hz, Me-27), 0.62 (m, H-1α); ^{13}C nmr see Table 1; cims (NH_3) *m/z* $[M+NH_4]^+$ 592 (9%), $[M]^+$ 574 (9), 534 (13), 499 (10), 474, (20), 457 (45), 439 (83), 397 (100), 385 (33), 369 (39), 205 (27); hrcims (NH_3) *m/z* 592.4191 ($C_{34}H_{54}O_7+NH_4^+$ requires 592.4213).

12α-Acetoxy-16β-propanoyloxy-20,24-dimethyl-24-oxoscalaran-25-al [**3**].—Oil (15 mg): $[\alpha]_D$ +96.0 (*c* = 0.33); ir ν max (CCl_4) 1740, 1715, 1230 cm^{-1}; ^{1}H nmr δ 9.47 (s, CHO), 5.11 (brs, H-12β), 4.66 (ddd, 11, 11, 4.8 Hz, H-16α), 3.19 (d, 11.5 Hz, H-18α), 3.09 (dd, 11.5, 11 Hz, H-17β), 2.33 (s, Me-26), 2.29 (m, MeCH_2COO), 2.21 (s, 12-OAc), 2.07 (m, H-15a), 1.85 (m, H-2α), 1.12 (t, 7 Hz, 16β-CH_3CH_2COO), 0.97 (s, Me-23), 0.83, 0.81, 0.80 (s,s,s, Me-19, Me-21, Me-22), 0.74 (t, 7.4 Hz, Me-27), 0.62 (m, H-1α); ^{13}C nmr see Table 1; cims (NH_3) *m/z* $[M+NH_4]^+$ 548 (50%), $[M+H]^+$ 531 (9), 457 (29), 439 (26), 397 (100), 379 (20), 369 (29), 205 (18); eims *m/z* $[M]^+$ 530 (<1%), $[M-HOAc]^+$ 470 (5), 396 (13), 368 (54), 325 (25), 219 (28), 205 (43); hrcims (NH_3) *m/z* 548.3926 ($C_{32}H_{50}O_6+NH_4^+$ requires 548.3951).

12α,16β-Diacetoxy-20,24-dimethyl-24-oxoscalaran-25-al [**4**].—Oil (11 mg): $[\alpha]_D$ +88.1° (*c* = 0.24); ir ν max (CCl_4) 1740, 1716, 1233 cm^{-1}; ^{1}H nmr δ 9.47 (s, CHO), 5.10 (brs, H-12β), 4.66 (ddd, 11, 11, 4.8 Hz, H-16α), 3.18 (d, 11.5 Hz, H-18α), 3.08 (dd, 11.5, 11 Hz, H-17β), 2.33 (s, Me-26), 2.20 (s, 12-OAc), 2.07 (m, H-15α), 2.05 (s, 16-OAc), 1.85 (m, H-2α), 0.97 (s, Me-23), 0.83, 0.81, 0.80 (s,s,s, Me-19, Me-21, Me-22), 0.73 (t, 7.4 Hz, Me-27), 0.62 (m, H-1α); ^{13}C nmr see Table 1.

12α-Acetoxy-16β-hydroxy-20,24-dimethyl-24-oxoscalaran-25-al [**5**].—Oil (7 mg): $[\alpha]_D$ +95.6° (*c* = 0.27); ^{1}H nmr δ 9.48 (s, CHO), 5.10 (brs, H-12β), 3.59 (ddd, 10.5, 10.5, 4.5 Hz, H-16α), 3.10 (d, 11.5 Hz, H-18α), 2.90 (dd, 11.5, 10.5 Hz, H-17β), 2.40 (s, Me-26), 2.19 (s, 12-OAc), 0.97 (s, Me-23), 0.84, 0.81, 0.81 (s,s,s, Me-19, Me-21, Me-22), 0.74 (t, 7.4 Hz, Me-27), 0.62 (m, H-1α); ^{13}C nmr see Table 1.

12α-(3′-Acetoxypentanoyloxy)-20,24-dimethyl-24-oxoscalar-16-en-25-al [**6**].—Glass (27 mg): $[\alpha]_D$ +44.6° (*c* = 0.33); uv λ max (EtOH) 233 (ε 7800); ir ν max (CCl_4) 1740, 1735, 1670 cm^{-1}; ^{1}H nmr δ 9.38 (d, 3.9 Hz, CHO), 7.09 (m, H-16α), 5.24 (m, H-3′), 4.79 (brs, H-12β), 3.46 (brs, H-18α), 2.69 (m, CH_2-2′), 2.31 (s, Me-26), 2.00 (s, 3′-OAc), 1.85 (m, H-2α), 0.95 (s, Me-23), 0.93 (t, Me-5′), 0.93 0.82, 0.80 (s,s,s, Me-19, Me-21, Me-22), 0.73 (t, 7.4 Hz, Me-27), 0.62 (m, H-1α); ^{13}C nmr see Table 1; cims (NH_3) *m/z* $[M+NH_4]^+$ 574 (5%), $[M+H]^+$ 557 (10), 543 (77), 527 (77), 483 (49), 467 (38), 401 (100), 383 (36), 369 (60), 205 (26); hr desorption eims *m/z* 556.3737 ($C_{34}H_{52}O_6$ requires 556.3764).

12α-(3′-Propanoyloxypentanoyloxy)-20,24-dimethyl-24-oxoscalar-16-en-25-al [**7**].—Oil (18 mg): $[\alpha]_D$ +37.4° (*c* = 0.46); uv λ max (EtOH) 233 nm (ε 8150); ir ν max (CCl_4) 1739, 1734, 1669, 1460, 1387, 1253, 1177, 1083 cm^{-1}; ^{1}H nmr δ 9.38 (d, 4.0 Hz, CHO), 7.09 (m, H-16α), 5.25 (m, H-3′), 4.78 (brs, H-12β), 3.46 (brs, H-18α), 2.70 (m, CH_2-2′), 2.32 (s, Me-26), 2.28 (q, 7.4 Hz, CH_2-2″), 1.85 (m, H-2α), 1.10 (t, 7.6 Hz, Me-3″), 0.95 (s, Me-23), 0.93 (t, Me-5′), 0.93, 0.82, 0.80 (s,s,s, Me-19, Me-21, Me-22), 0.73 (t, 7.4 Hz, Me-27), 0.62 (m, H-1α); ^{13}C nmr see Table 1; desorption eims *m/z* $[M+H]^+$ 571 (1%), $[M]^+$ 570 (1%), 556 (5), 384 (55), 369 (55), 219 (50), 205 (75), 165 (52), 149 (40), 95 (40), 83 (88), 57 (70), 55 (60), 43 (100); hrcims (NH_3) *m/z* 571.3999 ($C_{35}H_{54}O_6+H^+$ requires 571.3999).

12α-(3′-Acetoxybutanoyloxy)-20,24-dimethyl-24-oxoscalar-16-en-25-al [**8**].—Glass (14 mg): $[\alpha]_D$ +47.7° (*c* = 0.27); uv λ max (EtOH) 234 nm (ε 8200); ir ν max (CCl_4) 1740, 1735, 1670 cm^{-1}; ^{1}H nmr δ 9.37 (d, 4.0 Hz, CHO), 7.10 (m, H-16α), 5.33 (m, H-3′), 4.79 (brs, H-12β), 3.43 (brs, H-18α), 2.69 (m, CH_2-2′), 2.33 (s, Me-26), 1.86 (m, H-2α), 1.35 (d, 7, Me-4′), 0.95 (s, Me-23), 0.93, 0.82, 0.80 (s,s,s, Me-19, Me-21, Me-22), 0.73 (t, 7.4 Hz, Me-27), 0.62 (m, H-1α); ^{13}C nmr see Table 1; cims (NH_3) *m/z* $[M+NH_4]^+$ 560 (15%), $[M+H]^+$ 543 (49), 529 (100), 513 (43), 483 (18), 469 (50), 397 (63), 369 (41), 205 (20); hr desorption eims *m/z* 542.3602 ($C_{33}H_{50}O_6$ requires 542.3607).

12α-(3R′-Hydroxybutanoyloxy)-20,24-dimethyl-24-oxoscalar-16-en-25-al [**9**].—A 5-g portion of the extract from a re-collection of the sponge from Sudbury Reef again yielded the same metabolites after vlc

and hplc, but in addition, the crystalline alcohol **9** was obtained (90 mg): needles, mp 155–156° (Me_2CO/hexane); $[\alpha]_D$ +43.0° ($c = 0.57$); uv λ max (EtOH) 234 nm (ε 9140); ir ν max (Nujol) 3570 (OH), 1725, 1709, 1660 cm^{-1}; ^1H nmr δ 9.36 (d, 4.3 Hz, CHO), 7.12 (m, H-16α), 4.76 (t, 2.5 Hz, H-12β), 4.05 (m, H-3′), 3.44 (brs, H-18α), 2.64 (dd, 15.5, 2.8 Hz) and 2.43 (dd, 15.5, 9.4 Hz, CH_2-2′), 2.33 (s, Me-26), 1.87 (brd, 14.7 Hz, H-2α), 1.74 (dt, 9.7, 2.8 Hz, H-11α), 0.98 (t, 7.4, Me-5′), 0.96 (s, Me-23), 0.92, 0.81, 0.79 (s,s,s, Me-19, Me-21, Me-22), 0.72 (t, 7.4 Hz, Me-27), 0.60 (td, 12.5, 4 Hz, H-1α); ^{13}C nmr see Table 1; desorption eims *m/z* $[M]^+$ 514 (1%), $[M - H_2O]^+$ 496 (3), 485 (2), 456 (2), 438 (2), 396 (28), 368 (40), 219 (58), 205 (85), 149 (52), 55 (57), 43 (100); hr desorption eims *m/z* 514.3671 ($C_{32}H_{50}O_5$ requires 514.3658).

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