Three New Cytotoxic Sesterterpenes from the Marine Sponge *Hyrtios* cf. *erectus*[1]

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Three new cytotoxic sesterterpenes, **1**−**3**, have been isolated from the marine sponge *Hyrtios* cf. *erectus*. Their structures have been determined by interpretation of NMR data. They exhibited cytotoxicity against P-388 murine leukemia cells with IC_{50} values of 0.4−2.1 μg/mL.

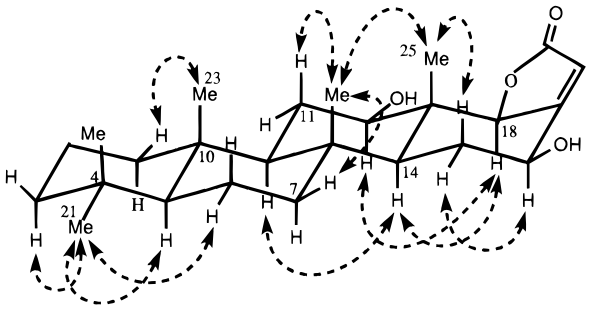
Scalarane sesterterpenes, common metabolites of Dictyoceratida sponges, exhibit a variety of biological activities, for example, antifeedant,[2] ichthyotoxic,[3] antiinflammatory,[4] cytotoxic,[5,6] and platelet-aggregation inhibitory.[7] In our ongoing research program on cytotoxic metabolites from Japanese marine invertebrates, we found significant activity in the MeOH extract of the marine sponge *Hyrtios* cf. *erectus* collected from southern Japan. Bioassay-guided isolation afforded altohyrtins,[8,9] which accounted for most of the cytotoxicity of the sponge. The less active fraction yielded three new pentacyclic sesterterpenes, 16-*O*-deacetyl-16-*epi*-scalarolbutenolide (**1**), 12-*O*-acetyl-16-*O*-deacetyl-16-*epi*-scalarolbutenolide (**2**), and 12-deacetoxy-21-acetoxy-scalarin (**3**), along with the known heteronemin.[10] This paper deals with the isolation and structure elucidation of these new compounds.

The EtOH extract of the frozen sponge (1.2 kg) was partitioned between H_2O and Et_2O, and the ether phase was subjected to Kupchan's partitioning scheme.[11] The active CCl_4 and CH_2Cl_2 fractions were combined and separated by ODS flash chromatography, followed by gel-filtration on Sephadex LH-20. The active fractions were finally purified by reversed-phase HPLC to afford **1**−**3** (**1**, 2.2 mg, 1.8 × 10^{-6}% based on wet wt; **2**, 5.1 mg, 4.2 × 10^{-6}%; **3**, 3.2 mg, 2.6 × 10^{-6}%).

Compound **1** had a molecular formula of $C_{25}H_{38}O_4$ as established by HRFABMS and ^{13}C-NMR data. The ^{1}H-NMR spectrum in $CDCl_3$/CD_3OD (3:1) exhibited signals for five methyl singlets (δ 0.64, 0.75, 0.77, 0.79, and 0.82), three oxygenated methines (δ 3.61, dd, J = 11.5, 4.5 Hz; 4.37, ddd, J = 11.0, 7.5, 1.5; 4.48, d, J = 1.5), and an olefinic proton (δ 5.88, t, J = 1.5) (Table 1). The ^{13}C-NMR spectrum revealed 25 carbons including signals for an α,β-conjugated carbonyl (δ 173.6, 111.6, and 173.6), four quaternary carbons, six methines including three oxygenated methines (δ 67.5, 80.1, 90.2), seven methylenes, and five methyls, which accounted for 36 nonexchangeable hydrogen atoms.

Interpretation of COSY cross peaks led to four partial structures: C1 to C3, C5 to C7, C9 to C12, and C14 to C16. The remaining oxygenated methine (H18, δ 4.48) was long-range-coupled to the H20 olefinic proton (δ 5.88), which in turn was correlated with H16. These structural units were connected through nonprotonated carbons on the basis of HMBC data,[12] which included

Scheme 1. NOESY Correlations for Compound **1**



cross peaks: Me21 (Me22)/C3, C4, C5; Me23/C1, C9, C10, C5; Me24/C9, C8, C14, C7; Me25/C12, C13, C14, C18, and H15/C17, thereby constructing the tetracarbocyclic portion. HMBC cross peaks H20/C17, C18, C19 implied that the α,β-unsaturated γ-lactone was fused to ring D at C17 and C18, which was supported by UV [217 nm (ϵ 7800)] and IR (1750 cm^{-1}) data. Two hydroxyl groups were placed at C12 and C16 on the basis of ^{1}H-NMR data in DMSO-d_6. Therefore, the gross structure of compound **1** could be determined.

^{13}C-NMR data of **1** were similar to those of scalarolbutenolide (**4**)[13] except for the absence of an acetyl group in **1**, thus indicating that **1** was the deacetyl derivative of **4**. This was substantiated by NOESY data (Scheme 1)[14] together with coupling constant analysis. H12 was coupled to H11β with J = 11.5 Hz, which suggested that H12 was axial. H16 was also assigned as axial on the basis of a coupling constant of 11.0 Hz between H16 and H15β. Similarly, H18 was assigned as axial, because intense NOESY cross peaks were observed between H18 and H12 and between H18 and H14. Therefore, **1** was 16-*O*-deacetyl-16-*epi*-scalarolbutenolide.

Compound **2** had a molecular formula of $C_{27}H_{40}O_5$, C_2H_2O larger than **1**. Again, ^{1}H- and ^{13}C-NMR data were quite similar to those of **1**, except for an additional acetyl group in **2**. A significant downfield shift of H12 (1.38 ppm) in **2** implied that **2** was 12-*O*-acetyl-16-*O*-deacetyl-16-epi-scalarolbutenolide. This was confirmed by acetylation of both **1** and **2** with Ac_2O/pyridine, which gave rise to 12-*O*-acetyl-16-*epi*-scalarolbutenolide (**5**).

Compound **3** was an isomer of **2**, but NMR data were significantly different. Interestingly, the ^{1}H-NMR spectrum measured in $CDCl_3$ exhibited well-separated signals in the aliphatic region, while the low-field signals were broad, probably due to tautomerism in the hemiacetal group. In contrast, the ^{1}H-NMR spectrum

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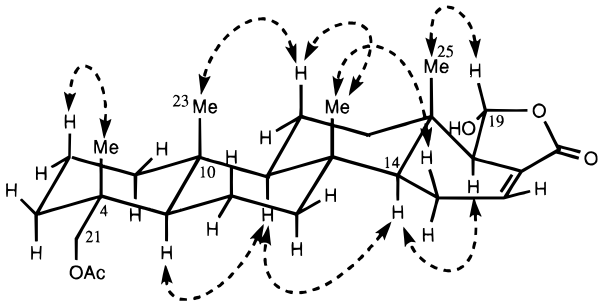
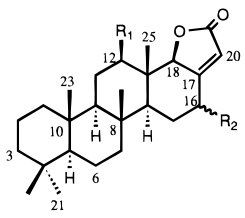
Table 1. NMR Data of Compounds **1** and **3** at 300 K

	1 in $CDCl_3/CD_3OD$ (3:1)		**1** in DMSO-d_6		**3** in $CDCl_3$	
	$\delta^{13}C$	$\delta^{1}H$ (mult, *J* in Hz)	$\delta^{13}C$	$\delta^{1}H$ (mult, *J* in Hz)	$\delta^{13}C$	$\delta^{1}H$ (mult, *J* in Hz)
1	39.8	α 0.77 (m)	39.8	0.78 (m)	39.4	0.78 (m)
		β 1.66 (m)		1.63 (m)		1.69 (m) 39
2	18.0	*β* 1.32 (m)	18.3	1.38 (m)	17.8	1.46 (m)
		α 1.52 (m)		1.57 (m)		1.60 (m)
3	42.4	α 1.07 (dt, 13.0, 13.0, 4.0)	41.7	1.09 (m)	35.8	1.32 (2H, m)
		β 1.33 (m)		1.29 (m)		
4	32.9		33.6		36.5	
5	56.4	0.77 (m)	56.5	0.77 (m)	50.4	1.07 (m)
6	18.2	*β* 1.36 (m)	18.3	1.32 (m)	18.0	1.38 (2H, m)
		1.56 (m)		1.49 (m)		
7	42.4	α 0.93 (m)	41.9	0.93 (dt, 13.0, 5.5)	41.7	0.92 (m)
		β 1.76 (m)		1.73 (br d, 13.0)		1.67 (m)
8	37.5		37.8		37.6	
9	58.2	0.84 (m)	57.9	0.85 (br d, 12.0)	61.2	0.87 (m)
10	37.3		37.6		37.4	
11	25.4	*β* 1.34 (m)	27.1	1.27 (m)	17.2	1.35 (m)
		α 1.74 (m)		1.54 (m)		1.56 (m)
12	80.1	α 3.61 (dd, 11.5, 4.5)	79.2	3.47 (m)	40.6	1.38 (m)
		β				1.88 (m)
12-OH				4.22 (d, 4.5)		
13	46.5		47.6		35.9	
14	50.0	0.96 (dd, 12.0, 2.5)	49.2	1.10 (br d, 13.5)	54.4	1.28 (dd, 12.5, 9.5)
15	30.6	*β* 1.48 (ddd, 13.0, 12.0, 11.0)	30.9	1.34 (m)	24.0	2.05 (dddd, 20.5, 11.0, 3.5, 3.5)
		α 2.11 (ddd, 13.0, 7.5, 2.5)		1.94 (m)		2.29 (dddd, 20.5, 6.0, 3.5, 3.5)
16	67.5	4.37 (ddd, 11.0, 7.5, 1.5)	67.7	4.35 (m)	136.7	6.87 (brs)
16-OH				5.59 (d, 5.5)		
17	173.6		175.4		127.2	
18	90.2	4.48 (d, 1.5)	89.2	4.66 (s)	59.7	2.50 (brs)
19	173.6		173.8		99.2	5.61 (brs)
20	111.6	5.88 (t, 1.5)	111.2	5.76 (s)	166.8	
21	21.0	0.75 (3H, s)	21.3	0.77 (s)	72.9	3.61 (d, 11.5), 3.83 (d, 11.5)
21-OAc					170.9	
21-OAc					21.0	2.05 (3H, s)
22	32.9	0.77 (3H, s)	33.2	0.82 (s)	17.2	0.88 (3H, s)
23	16.2	0.79 (3H, s)	16.6	0.77 (s)	16.8	0.86 (3H, s)
24	17.1	0.82 (3H, s)	17.2	0.77 (s)	16.4	0.90 (3H, s)
25	6.7	0.64 (3H, s)	7.2	0.52 (s)	15.3	0.79 (3H, s)

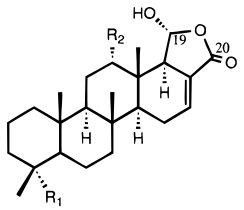
Scheme 2. NOESY correlations for compound **3**



measured in DMSO-d_6 revealed sharp signals in the low-field region, whereas the aliphatic signals overlapped. Interpretation of the NMR data in both solvents revealed the presence of four methyls (δ 0.79, 0.86, 0.88, 0.90) on quaternary carbons; nine methylenes, one of which was oxygenated (δ 72.9); six methines, including a hemiacetal (99.2) and an olefinic (136.7); a disubstituted olefinic carbon (127.2); an ester (166.8); and an acetyl group (21.0 and 170.9). These spectral features indicated that **3** shared a common structure of rings D and E of scalarin (**6**),[15] while the remaining portion was slightly different. The major difference resided in the nature of the acetoxyl group, secondary in **6** and primary in **3**. A C21 instead of a C12α-acetoxyl followed unambiguously from NMR data and was further supported by the absence of the characteristic low-field methyl carbon signal. Stereochemistry of **3** was deduced to be identical with that of scalarin by NOESY experiments (Scheme 2). Thus, compound **3** was 12-deacetoxy-21-acetoxyscalarin, which is the first example of a scalarane sesterterpene functionalized at C21.



1 R_1=OH, R_2=β-OH
2 R_1=OAc, R_2=β-OH
4 R_1=OH, R_2=α-OAc
5 R_1=OAc, R_2=β-OAc



3 R_1=CH_2OAc, R_2=H
6 R_1=CH_3, R_2=OAc

Compounds **1**−**3** were cytotoxic against P-388 leukemia cells with IC_{50} values of 0.4, 2.1, and 0.9 μg/mL, respectively.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Hitachi 330 spectrophotometer. IR spectra were measured with a JASCO FT/IR-5300 infrared spectrometer. ^1H- and ^{13}C-NMR spectra were recorded on a JEOL A500 NMR spectrometer. ^1H and ^{13}C chemical shifts were referenced to solvent peaks: δ_H 3.3 and δ_C 49.9 for $CDCl_3/CD_3OD$ (3:1); δ_H 7.24, δ_C 77.0 for $CDCl_3$; δ_H 2.49, δ_C 39.5 for DMSO-d_6. MS were obtained with a JEOL SX 102 mass spectrometer. HRFABMS were measured using a dual target inlet

probe. Optical rotations were determined with a JASCO DIP-371 digital polarimeter.

Animal Material. The sponge samples were collected by SCUBA at depths of 15−20 m off Yakushima Island, near Kagoshima, 1000 km south of Tokyo, frozen immediately after collection, and kept frozen until used. The sponge was identified by Dr. Rob van Soest, University of Amsterdam, as *Hyrtios* cf. *erectus* Keller, 1889 (class Desmospongiae, order Dictyoceratida, family Irciniidae). A voucher specimen (ZMA POR 11026) was deposited at the Zoological Museum of the University of Amsterdam, The Netherlands.

Extraction and Isolation. The frozen sponge (1.2 kg, wet wt) was extracted by blending with EtOH (2 L × 3). The combined extracts were concentrated and partitioned between H_2O (900 mL) and Et_2O (900 mL × 3). The ether phase was partitioned between *n*-hexane and 90% MeOH. The aqueous MeOH layer was then partitioned between 80% MeOH and CCl_4. This last layer was finally partitioned between 60% MeOH and CH_2Cl_2. The cytotoxic CCl_4 and CH_2Cl_2 fractions (5.7 g) were combined and subjected to flash chromatography on an ODS column (70−230 mesh) with 60%, 80%, 90%, and 100% MeOH. The active 80% MeOH fraction (0.4 g) was gel-filtered on Sephadex LH-20 with *n*-hexane/CH_2Cl_2/MeOH (4:2:1) to afford an active fraction (130 mg), which was finally purified by reversed-phase HPLC (ODS, 10 × 250 mm; 82% MeOH; flow rate 2.0 mL/min; UV detection at 210 nm) to yield compounds **1** (2.2 mg, 1.8×10^{-4}%), **2** (5.1 mg, 4.2×10^{-4}%), and **3** (3.2 mg, 2.6×10^{-4}%).

16-*O*-Deacetyl-16-*epi*-scalarolbutenolide (1): colorless amorphous solid; $[\alpha]^{23}_D$ +19.2° (*c* 0.24, $CHCl_3$); IR (film) 3400 (br), 2930, 2840, 1750, 1650, 1240 cm^{-1}; UV (MeOH) 217 nm (ϵ 7800); FABMS *m/z* 403 [(M + H)$^+$]; HRFABMS *m/z* 403.2820 calcd for $C_{25}H_{39}O_4$ (Δ −2.9 mmu); ^{1}H NMR and ^{13}C NMR (see Table 1).

12-*O*-Acetyl-16-*O*-deacetyl-16-*epi*-scalarolbutenolide (2): colorless amorphous solid; $[\alpha]^{23}_D$ +62.0° (*c* 0.46, $CHCl_3$); IR (film) 3420, 2930, 2850, 1740, 1650, 1240 cm^{-1}; UV (MeOH) 217 nm (ϵ 7500); FABMS *m/z* 445 [(M + H)$^+$]; HRFABMS *m/z* 445.2951 calcd for $C_{27}H_{41}O_5$ (Δ −0.3 mmu); ^{1}H NMR ($CDCl_3$) δ 5.91 (1H, s, H20), 4.99 (1H, dd, *J* = 12.0, 4.5 Hz, H12), 4.50 (1H, s, H18), 4.46 (1H, dd, *J* = 13.2, 7.0 Hz, H16), 2.16 (1H, ddd, *J* = 12.2, 7.0, 3.4 Hz, H15α), 2.10 (3H, s, OAc), 1.80 (1H, br d, *o* = 12.5 Hz, H7*β*), 1.68 (1H, m, H11α), 1.60 (1H, m, H1*β*), 1.58 (1H, m, H2α), 1.55 (1H, m, H6α), 1.52 (1H, m, H11*β*), 1.51 (1H, m, H15*β*), 1.41 (1H, m, H2*β*), 1.38 (1H, m, H6*β*), 1.35 (1H, m, H3*β*), 1.11 (1H, m, H3α), 1.08 (1H, dd, *J* = 10.0, 3.4 Hz, H14), 0.98 (1H, m, H9), 0.97 (1H, m, H7α), 0.88 (3H, s, Me24), 0.84 (3H, s, Me22), 0.83 (1H, m, H1α), 0.82 (3H, s, Me23), 0.80 (1H, m, H5), 0.79 (3H, s, Me21), 0.77 (3H, s, Me25); ^{13}C NMR ($CDCl_3$) δ 170.3 (s), 169.6 (s), 166.4 (s), 111.8 (d), 87.8 (d), 79.2 (d), 68.0 (d), 58.2 (d), 56.0 (d), 50.1 (d), 45.6 (s), 41.6 (t), 41.4 (t), 39.6 (t), 37.3 (s), 37.1 (s), 32.9 (s), 32.7 (q), 30.3 (t), 23.3 (t), 21.2 (q), 20.9 (q), 18.1 (t), 17.6 (t), 17.1 (q), 16.0 (q), 7.1 (q).

12-Deacetoxy-21acetoxyscalarin (3): colorless amorphous solid; $[\alpha]^{23}_D$ −2.3° (*c* 0.66, $CHCl_3$); IR (film) 3440 (br), 2930, 2860, 1755, 1735, 1690, 1240 cm^{-1}; UV (MeOH) 218 nm (ϵ 8300); FABMS *m/z* 445 [(M + H)$^+$]; HRFABMS *m/z* 445.2929 calcd for $C_{27}H_{41}O_5$ (Δ −2.5 mmu); ^{1}H NMR and ^{13}C NMR (see Table 1).

Acetylation of 1 and 2. A solution of **1** (500 μg), Ac_2O (0.25 mL), and pyridine (0.5 mL) was stirred at room temperature overnight. The reagents were evaporated *in vacuo* and purified by SiO_2 column chromatography (CH_2Cl_2/MeOH, 95:5) to yield diacetate **5**, which showed an (M + H)$^+$ ion at *m/z* 487 by FABMS. Acetylation of **2** gave the same product as judged from the ^{1}H-NMR data.

Cytotoxicity Tests. Cytotoxicity was tested against P-388 murine leukemia cells as reported previously.[16]

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