Cytotoxic Scalarane Sesterterpenes from a Sponge, *Hyrtios erecta*

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Novel scalarane sesterterpenes (**1**−**4**) were isolated from a sponge, *Hyrtios erecta* (order Dictyoceratida). They were characterized by means of spectral analyses, X-ray crystallography, and chemical reactions. Compound **1** showed potent *in vitro* and *in vivo* antitumor activities. In addition, the structure−activity relationship was also discussed using computer-assisted structure matching of **1** and aragusterols.

Marine organisms are well-known as a rich source of physiologically active and structurally diverse substances,[1] especially cytotoxic ones. During the course of programs for discovering pharmacologically active metabolites from marine organisms, we have found a variety of chemically and pharmacologically interesting compounds,[2] including antioxidants, a glucose-uptake enhancer, aldose reductase inhibitors, PAF antagonists, N-type calcium channel modulators,[3] endothelin antagonists,[4] and an endothelin-converting enzyme inhibitor,[5] in the past decade. In addition, since the search for cytotoxic metabolites is one of the most important targets in drug development, we screened the lipophilic extracts of marine organisms for inhibition of cell growth in P388 mouse lymphatic leukemia cell line and found that the lipophilic extract of a sponge, *Hyrtios erecta* Keller 1889 (Thorectidae Bergquist 1978, Dictyoceratida Minchin 1900), showed potent cytotoxicity. Now, we report the structural characterization and *in vitro* and *in vivo* antitumor activities of novel scalarane sesterterpenes (**1**, **2a**,**b**, **3**, and **4**)[6] and known 12-*epi*-scalarin[7] obtained from *H. erecta*,[8] together with altohyrtins[9] previously isolated from a similar species, *Hyrtios altum*. The structure−activity relationship was also discussed using computer-assisted structure matchings of **1** and aragusterols.[10]

Results and Discussion

H. erecta was collected on a scuba diving expedition at Setouchi, Amami-Oshima, Kagoshima Prefecture, Japan, in 1993. It was blended in MeOH and extracted with MeOH. The EtOAc-soluble part of the MeOH extract was partitioned between *n*-hexane and 90% aqueous MeOH. The cytotoxicity against the P388 cell line remained in 90% aqueous MeOH, and the separation was monitored using this assay. The 90% aqueous MeOH extract was separated into five novel scalaranes (**1, 2a**,**b**, **3**, and **4**) together with 12-*epi*-scalarin, many other unidentified scalaranes,[11] and altohyrtins[9] by a combination of Diaion HP-20, silica gel, RP-18 column chromatography, and finally HPLC. Compound **1** was the most active scalarane isolated, but the greatest cytotoxicity was shown by altohyrtins.[9]

Active compound **1** was analyzed for $C_{25}H_{38}O_4$ by HRFABMS ($[M + Na]^+$ *m/z* 425.2627 (Δ +0.4 mmu) and elemental analysis. The IR spectrum indicated the presence of hydroxyl groups (ν_{max} cm^{-1} 3451, 3357) and a carbonyl group (ν_{max} cm^{-1} 1690). Five angular methyl singlets (δ_H 0.86, 0.91, 1.04, 1.09, and 1.14) in the ^1H NMR spectrum (Table 1) were compatible with a scalarane skeleton for **1**. Two signals at δ_H 3.85, 5.88 assignable to protons on the oxygen-bearing carbons coupled to hydroxy protons at δ_H 5.56, 9.41, respectively. The ^{13}C NMR spectrum (Table 1) indicated the presence of a carbonyl (δ_C 216.3), a trisubstituted double bond [δ_C 116.6 (d), 137.2 (s)], an acetal [δ_C 100.0 (d)], and two oxygen-bearing carbons [δ_C 68.3 (t), 81.0 (d)] in addition to five methyl, six methylene, four methine, and four quaternary carbons. Compound **1** was easily dehydrated to a furan derivative **5** (EIMS *m/z* 384 $[M^+]$) during NMR measurement in $CDCl_3$, which may have been triggered by a catalytic amount of H^+ or D^+. Its structure was supported by comparison of NMR data of the furan moiety [δ_H 7.34 (brs), 7.58 (brs); δ_C 120.6 (s), 134.9 (s), 136.8 (d), 138.2 (d)] with those of scalarafuran[12] [δ_H 7.14 (brs), 7.41 (brs); δ_C 120.8 (s), 134.4 (s), 137.2 (d), 138.9 (d)]. The EIMS of **1**, though it did not show a molecular ion, displayed the most abundant and diagnostic fragment ion **I** at *m/z* 205.1618 ($C_{14}H_{21}O$, Δ +2.6 mmu) in Figure 1.

Comparison of fragment ion **I** with **III** (*m/z* 191) characteristic of common scalaranes[13] indicated the presence of a 3-keto structure, which is supported by HMBC connectivities between the carbonyl carbon (C-3) and methylene (2-CH_2) and methyl protons (21, 22-CH_3). The other HMBC correlations, in addition to comparison of corresponding proton and carbon chemical shifts with those of known scalaranes,[14] determined the positions of the hydroxy and acetal functionalities. The structure of **1** was unambiguously determined by X-ray crystallography as shown in Figure 2. On the basis of the octant rule of carbonyl compounds in the CD spectrum,[15] the absolute configuration of **1** was as shown, as indicated by a positive Cotton effect ($[\theta]_{287}$ +2770°) in the CD spectrum.

Compound **2a** was analyzed for $C_{25}H_{40}O_4$ by HR-FABMS $[M+Na]^+$ *m/z* 427.2826 ($C_{25}H_{40}O_4Na$, Δ +0.5 mmu) and elemental analysis. Since the spectral features of **2a** including the most abundant fragment ion (**II**: *m/z* 207.1753, Δ +0.4 mmu) were compatible with a structure with a 3-carbonyl group that was

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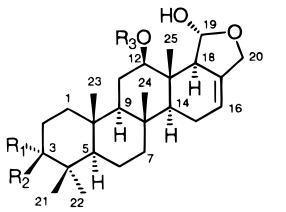
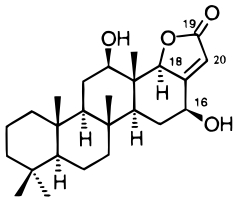
Table 1. ^{1}H and ^{13}C NMR Data of **1** (Py-d_5)

no.	^{13}C δ (mult)	^{1}H δ [mult, J (Hz)]	no.	^{13}C δ (mult)	^{1}H δ [mult, J (Hz)]
1	39.1 (t)	1.27 (ddd, 8.0, 9.6, 13.1)	13	40.6 (s)	
		1.79 (ddd, 4.7, 7.5, 13.1)	14	53.6 (d)	1.18 (dd, 6.1, 10.7)
2	19.3 (t)	2.44 (ddd, 4.7, 8.0, 15.5)	15	27.1 (t)	1.99 (m)
		2.50 (ddd, 7.5, 9.6, 15.5)			2.03 (m)
3	216.3 (s)		16	116.6 (d)	5.51 (m)
4	47.3 (s)		17	137.2 (s)	
5	54.5 (d)	1.31 (dd, 3.4, 12.2)	18	61.8 (d)	2.67 (brs)
6	22.8 (t)	1.35 (dq, 3.4, 12.6)	19	100.0 (d)	5.88 (dd, 5.1, 5.5)
		1.42 (dq, 3.4, 12.6)	20	68.3 (t)	4.38 (dd, 1.8, 11.9)
7	40.8 (t)	0.79 (dt, 3.8, 12.6)			4.74 (dd, 1.8, 11.9)
		1.64 (td, 3.8, 12.6)	21	26.8 (q)	0.86 (s)
8	36.8 (s)[a]		22	21.0 (q)	0.91 (s)
9	57.8 (d)	0.88 (dd, 1.6, 12.6)	23	16.4 (q)	1.04 (s)
10	37.4 (s)[a]		24	16.5 (q)	1.09 (s)
11	34.2 (t)	1.70 (q, 12.0)	25	9.4 (q)	1.14 (s)
		1.86 (ddd, 1.3, 3.7, 12.0)	12-OH		5.56 (d, 3.0)
12	81.0 (d)	3.85 (td, 3.7, 12.0)	19-OH		9.41 (d, 4.9)

[a] Interchangeable.



1 R_1, R_2=O; R_3=H
2a R_1=R_3=H; R_2=OH
2b R_1=H; R_2=OH; R_3=Ac
3 R_1=R_2=R_3=H



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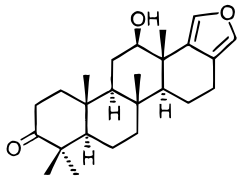

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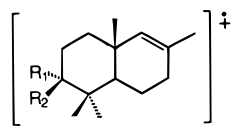

I R_1, R_2=O: *m/z* 205
II R_1=H, R_2=OH: *m/z* 207
III R_1=R_2=H: *m/z* 191

Figure 1. Structures of scalarane sesterterpenes and their EIMS fragment ions.

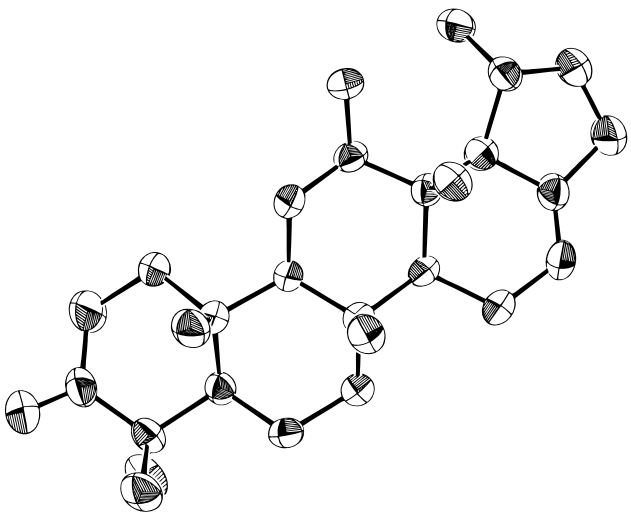

Figure 2. ORTEP drawing of **1**.

reduced to a hydroxyl group, oxidation of **2a** was attempted. On treatment with an excess of PDC in DMF, compound **2a** gave **1** *albeit* in poor yield. Compound **2a**, therefore, could be depicted as shown, together with the NMR data [δ_H 3.16 (H-3α, J = 7.8, 8.7 Hz); δ_C 79.0 (C-3, d)] showing the β-configuration of the 3-hydroxy group (Table 2). The structure of compound **2b** was determined by its alkaline hydrolysis to **2a**, and the structure of compound **3** was also determined by its MS data and comparison of NMR data (Table 3) with those of 12-*epi*-deoxyscalarin.[7] Compound **4** was recently isolated from *H.* cf. *erectus*;[16] its structure was determined unambiguously by X-ray crystallography.

Table 2. ^{1}H and ^{13}C NMR Data of **2a** and **2b** (CD_3OD)

	2a		**2b**	
no.	^{13}C δ (mult)	^{1}H δ [mult, J (Hz)]	^{13}C δ (mult)	^{1}H δ [mult, J (Hz)]
1	39.0 (t)	0.7−1.9 (m)	39.6 (s)	0.7−1.9 (m)
		0.7−1.9 (m)		0.7−1.9 (m)
2	26.0 (t)[a]	0.7−1.9 (m)	28.0 (t)	0.7−1.9 (m)
		0.7−1.9 (m)		0.7−1.9 (m)
3	79.0 (d)	3.16 (dd, 7.8, 8.7)	79.5 (d)	3.15 (dd, 5.7, 10.3)
4	40.4 (s)		40.0 (s)	
5	56.0 (d)	0.7−1.9 (m)	56.7 (d)	0.7−1.9 (m)
6	18.4 (t)	0.7−1.9 (m)	19.0 (t)	0.7−1.9 (m)
		0.7−1.9 (m)		0.7−1.9 (m)
7	42.3 (t)	0.7−1.9 (m)	42.7 (t)	0.84 (brt, 12.8)
		0.7−1.9 (m)		1.76 (td, 2.9, 12.8)
8	37.6 (s)		38.3 (s)[b]	
9	59.2 (d)	0.7−1.9 (m)	59.5 (d)	0.7−1.9 (m)
10	37.8 (s)		38.5 (s)[b]	
11	27.3 (t)[a]	0.7−1.9 (m)	24.6 (t)	1.48 (q, 12.1)
		0.7−1.9 (m)		1.82 (brdd, 3.0, 12.1)
12	81.7 (d)	3.85 (dd, 4.3, 11.5)	84.3 (d)	4.61 (dd, 4.4, 11.5)
13	39.3 (s)		39.2 (s)[b]	
14	53.9 (d)	1.16 (dd, 6.0, 11.2)	55.3 (d)	0.7−1.9 (m)
15	22.7 (t)	2.0−2.1 (m)	23.3 (t)	2.0−2.2 (m)
		2.0−2.1 (m)		2.0−2.2 (m)
16	117.5 (d)	5.54 (dd, 2.3, 8.8)	117.4 (d)	5.52 (brs)
17	135.6 (s)		137.8 (s)	
18	60.9 (d)	2.28 (brs)	62.2 (d)	2.23 (brs)
19	99.6 (d)	5.08 (d, 5.0)	100.7 (d)	
20	68.6 (t)	4.19 (dd, 1.1, 12.6)	69.0 (t)	4.08 (d, 11.1)
		4.37 (dd, 1.8, 12.6)		4.35 (d, 11.1)
21	28.2 (q)	0.78 (s)	28.5 (q)	0.77 (s)
22	17.3 (q)	0.80 (s)	21.5 (q)	0.88 (s)
23	15.6 (q)	0.89 (s)	17.0 (q)	0.90 (s)
24	16.9 (q)	0.94 (s)	17.2 (q)	0.96 (s)
25	8.9 (q)	0.96 (s)	10.3 (q)	0.98 (s)
Ac			15.6 (q)	2.03 (s)
			172.8 (s)	

[a,b] Entries marked with the same symbol are interchangeable.

Scalarane sesterterpenes are characteristic secondary metabolites of some sponges[1] or predators[17] thereof, and some weak pharmacological activities such as cytotoxicity,[18] anti-platelet aggregation,[19] and nerve growth factor (NGF) enhancement[20] have been reported. Generally, they are not functionalized on A- or B-ring carbons, while antitumor compounds obtained here contain unique 3-oxygenated structures[21] for scalaranes. The oxygen constellation on the scalarane was thought

Table 3. ^{1}H and ^{13}C NMR Data of **3** ($CDCl_3$)

no.	^{13}C δ (mult)	^{1}H δ [mult, J (Hz)]	no.	^{13}C δ (mult)	^{1}H δ [mult, J (Hz)]
1	40.0 (t)	0.7−1.9 (m)	13	49.5 (s)	
		0.7−1.9 (m)	14	53.4 (d)	0.7−1.9 (m)
2	18.2 (t)[a]	0.7−1.9 (m)	15	22.1 (t)	2.0−2.2 (m)
		0.7−1.9 (m)			2.0−2.2 (m)
3	41.7 (t)[b]	0.7−1.9 (m)	16	117.3 (d)	5.51 (brs)
4	33.3 (s)		17	134.8 (s)	
5	56.6 (d)	0.7−1.9 (m)	18	59.2 (d)	2.22 (brs)
6	18.6 (t)[a]	0.7−1.9 (m)	19	106.3 (d)	5.29 (dd, 6.6, 4.4)
		0.7−1.9 (m)	20	68.7 (t)	4.22 (td, 1.8, 12.1)
7	42.2 (t)[b]	0.84 (brt, 12.8)			4.52 (dd, 2.1, 12.1)
		1.76 (td, 2.9, 12.8)	21	33.3 (q)	0.81 (s)
8	37.6 (s)		22	21.3 (q)	0.84 (s)
9	58.9 (d)	0.7−1.9 (m)	23	16.6 (q)	0.84 (s)
10	37.6 (s)		24	17.1 (q)	0.86 (s)
11	25.6 (t)	1.48 (q, 12.1)	25	8.8 (q)	0.93 (s)
		1.82 (brdd, 3.0, 12.1)	12-OH		3.56 (d, 3.6)
12	81.2	3.54 (dd, 3.9, 11.4)	19-OH		3.79 (d, 4.4)

[a,b] Entries marked with the same symbol are interchangeable.

Table 4. Tumor Growth Inhibitory Activities of Scalarane (**1**−**4**) and 12-*epi*-Scalarin (48 h Drug Exposure)

compd no.	tumor cell lines	activities IC_{50} (ng/mL)
1	P388	14.5
	MKN-1	57.7
	MKN-7	56.0
	MKN-74	36.8
2a	P388	250.0
2b	P388	505.0
3	P388	>1000
4	P388	>1000
12-*epi*-Scalarin	P388	>1000

Table 5. Life-Prolonging Effect of **1** on P388 Leukemia-Implanted Mice

compd	dose (mg/kg)	wt change (%)	weighed median of survival days	ILS (%)
control		+1.3	8.6	
1	8.0	+0.4	15.0	74.4
	4.0	+0.9	13.0	51.2
	2.0	+2.4	11.3	31.4
	1.0	+2.0	11.4	32.6
	0.5	+3.0	10.7	24.4

to play a significant role for expression of the antitumor activity, which will be discussed later in comparison with those of aragusterols,[10] previously reported as antitumor sterols from a marine sponge, *Xestospongia* sp.

Scalarane sesterterpenes obtained here were tested for *in vitro* cytotoxicity against some tumor cell lines, and *in vivo* mean survival times (MST) and increases of life spans (ILS) in P388 lymphatic leukemia-implanted mice. The activities are summarized in Tables 4 and 5. Compound **1** potently inhibited *in vitro* cell growth with an IC_{50} of 14.5 ng/mL not only against the P388 mouse lymphatic leukemia cell line but also against MNK-1 (IC_{50} 57.7 ng/mL), MNK-7 (IC_{50} 56.0 ng/mL), and MNK-74 (IC_{50} 36.8 ng/mL) human gastric cancer cell lines. The other compounds, **2a**,**b**, **3**, and **4**, showed rather weak or no activity. Intraperitoneal administration of **1** (0.5−8.0 mg/kg) in CDF_1 mice (n = 6) increased MST (10.7−15 days) and ILS (24.4−74.4%), dose-dependently.

Compound **1** could be structurally correlated with aragusterols A (**6**: IC_{50} 22.0 ng/mL in *in vitro* P388 mouse lymphatic leukemia cell line), B (**7**: IC_{50} >3300 ng/mL), C (**8**: IC_{50} 41.0 ng/mL), and their relatives

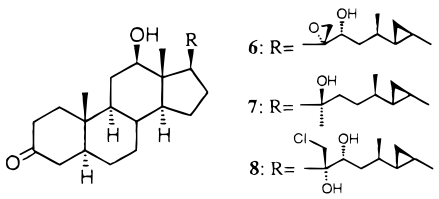

Figure 3. Structures of aragusterols A (**6**), B (**7**), and C (**8**).

(Figure 3).[22] To compare the constellations of **1** and these compounds and to study their structure−activity relationship (SAR), a computer-assisted structure matching[23] of **1** and **8**[10] was attempted using their X-ray crystallographic data. Four respective oxygen atoms of 3-keto, 12(*R*)-hydroxy, 19(*R*)-hydroxy (20(*R*)-hydroxy group in **8**), and hydrofuran groups (22(*R*)-hydroxy group in **8**) were superimposable on each other, with rms (root mean square) of deviation (0.67 Å), where the hydrophobic ring carbons were located near to each other as shown in Figure 4. The structure matching of **7**[24] and **8**, with rms of deviation (0.15 Å) of 17 steroidal ring carbons after fitting, also showed that the hydrogen bonds between respective 12*R*- and 20-hydroxyl groups constrained the conformation of the side chain at C-20 above the steroidal plane in **7** and below the steroidal plane in **8** (Figure 5) because the absolute configurations at C-20 of **7** and **8** were *S* and *R*, respectively.

On the basis of the oxygen constellations and cytotoxic activities of the scalaranes obtained here and aragusterols, the 3-keto group may be significant, because **1** was >20 times as active as 3(*S*)-oxygenated compound **2a**,**b** and also as active as **6** and **8**. 3-Unfunctionalized scalaranes including **3**, **4**, 12-*epi*-scalarin, and other unidentified relatives[11] were all inactive even at a concentration of IC_{50} > 1000 ng/mL. The 12(*R*)- and 19(*R*)-hydroxyl groups may also play important roles because commonly found 3-oxygenated sterols without these functions are usually inactive. The importance of these hydroxy groups and/or the hydrogen bond thereof, though the 22(*R*)-hydroxy group in **8** may additively contribute to its activity, was supported by the SAR between inactive **7** and active **6** and **8**, where the location of the side chains may be important. These results may be accounted for by the assumption that coexistence of the four hydrophilic oxygen functions in preferable constellations is critical for the activity expression.

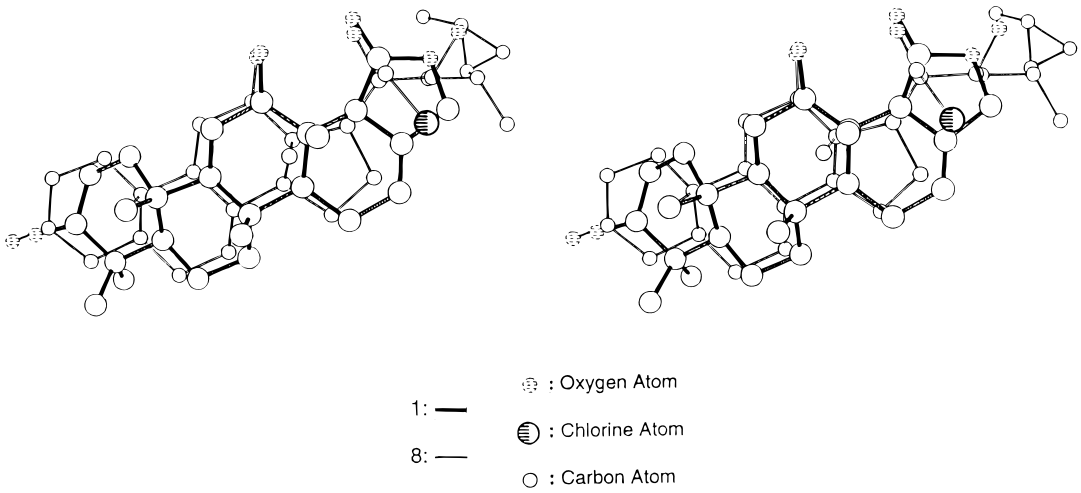

Figure 4. Stereoview of superposed structures of compound **1** and aragusterol C (**8**). Fittings were carried out using four oxygen atoms with rms of deviation (0.67 Å). Compound **1** and aragusterol C (**8**) are shown by thick and thin bonds, respectively.

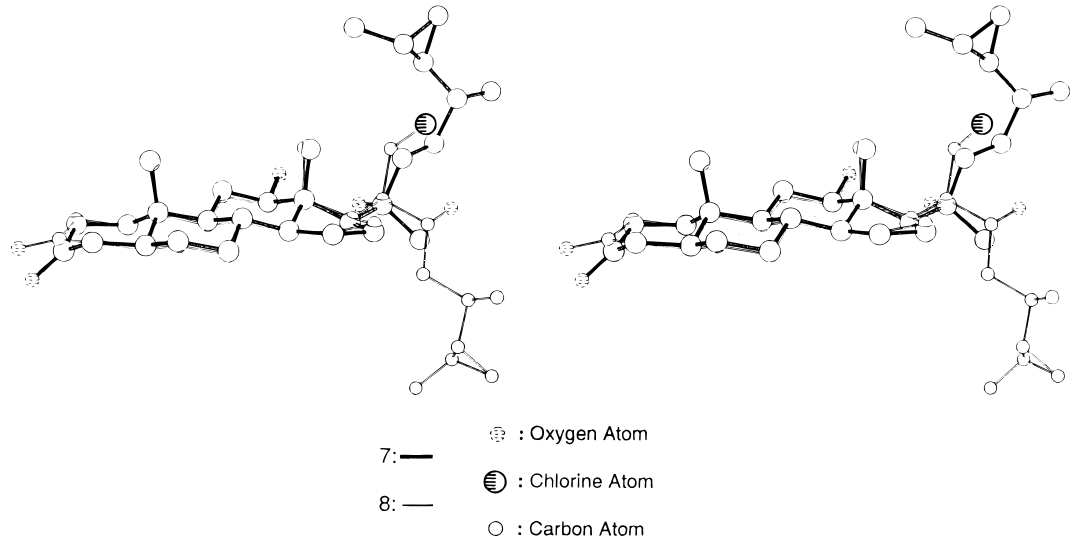

Figure 5. Stereoview of superposed structures of aragusterol B (**7**) and C (**8**). Fittings were carried out using 17 ring carbon atoms with rms of deviation (0.15 Å). Aragusterols B (**7**) and C (**8**) are shown by thick and thin bonds, respectively.

Experimental Section

General Procedures. Melting points were measured on a Yanaco melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-GX400 with TMS as an internal standard. IR spectra were recorded on a Nicole 5SXC and a JASCO Valor-III. UV spectra were recorded on a Shimazu UV-265FW. Mass spectra were recorded on a JEOL JMS-D300. Diaion HP-20 (Nihon Rensui) and silica gel (60−80 mesh; Merck) were used for column chromatography. Packed ODS columns (RP-18: Merck; ODS−H: 6 ∅, 10 ∅, 20 ∅ × 250 mm, Senshu; ODS: 100 ∅ × 300 mm, YMC) were used for HPLC. The fractionation was monitored by testing the cytotoxicity by MTT assay using P388 mouse lymphatic leukemia cell line.

Isolation. *H. erecta* (113.6 kg) was collected on a scuba diving expedition at Setouchi, Amami-Oshima, Kagoshima Prefecture, Japan, in 1993. A voucher specimen (sample no. MC-323) is stored at Biomedical Research Laboratories, Sankyo Co. Ltd. The frozen sample was blended in MeOH and extracted four to five times with MeOH (455 L) at room temperature. The MeOH extract obtained by concentration to dryness under reduced pressure was added to a mixture of EtOAc and water to precipitate a black gum (151.72 g), which was dissolved in $CHCl_3$. After usual treatment, the concentrated EtOAc extract was partitioned between *n*-hexane and 90% aqueous MeOH to give *n*-hexane extract (90.83 g; IC_{50} 5.9 μg/mL) and 90% aqueous MeOH (34.79 g; IC_{50} 2.02 ng/mL).

The 90% aqueous MeOH extract was subjected to Diaion HP-20 (2.5 L) column chromatography. Elution with 60−80% aqueous acetone gave an active fraction (Fr-7, 9.96 g; IC_{50} 2.65 ng/mL), together with inactive fractions (Fr-1−6, -8). Fr-7 was then subjected to silica gel column chromatography (300 g). Elution with $CHCl_3$−30% MeOH/$CHCl_3$ gave six fractions [Fr-7-1 ($CHCl_3$−2% MeOH/$CHCl_3$; 0.55 g; IC_{50} >1000 ng/mL), Fr-7-2 (2−2.5% MeOH/$CHCl_3$; 2.96 g; IC_{50} 0.47 ng/mL), Fr-7-3 (2.5% MeOH/$CHCl_3$; 1.31 g; IC_{50} 2.3 ng/mL), Fr-7-4 (2.5−3% MeOH/$CHCl_3$; 1.01 g; IC_{50} 18.4 ng/mL), Fr-7-5 (4−5% MeOH/$CHCl_3$; 0.45 g; IC_{50} 28.9 ng/mL), Fr-7-6 (5−30% MeOH/$CHCl_3$; 2.21 g; IC_{50} 238 ng/mL)].

Fr-7-2 crystallized to give **4** (411.1 mg) as colorless crystals after recrystallization from MeOH/$CHCl_3$. The

filtrate (2.25 g; IC_{50} 1.93 ng/mL) was separated by preparative HPLC (ODS: 100 ∅ × 300 mm; 50% aqueous ACN) to three active fractions [Fr-7-2-1(190 mg), Fr-7-2-2 (104 mg), Fr-7-2-3 (80 mg)]. Fr-7-2-3 gave additional crystals of **4** (14.8 mg). Fr-7-2-1 was further purified by a combination of silica gel column chromatography (5% MeOH/$CHCl_3$) and preparative HPLC (ODS-H: 20 ∅ × 250 mm; 70% aqueous ACN) to a fraction containing **1**, from which **1** was purified as colorless crystals (30.1 mg) by recrystallization from MeOH. The preparative HPLC (ODS-H: 20 ∅ × 250 mm; 80% aqueous MeOH) of the filtrate gave altohyrtin A (colorless amorphous powder; 11.2 mg) and C (colorless amorphous powder; 9.1 mg), which were identified by HRFABMS and ^1H and ^{13}C NMR spectra. Altohyrtins may be mainly responsible for the cytotoxic activity of this sponge. Fr-7-2-2 was repeatedly purified by silica gel column chromatography (2% MeOH/$CHCl_3$, 66% EtOAc/*n*-hexane) to **2b** (32.9 mg) as a colorless amorphous powder.

The combined fractions [Fr-7-3 and -4 (2.34 g)] were subjected to silica gel column chromatography (200 g). Elution with 70−80% EtOAc/*n*-hexane gave a mixture of manoalides, and then elution with 50% MeOH/$CHCl_3$ gave crude **2a**, which was purified by preparative HPLC (ODS:100 ∅ × 300 mm; 80% aqueous MeOH) followed by recystallization from MeOH/$CHCl_3$ to give **2a** (109.3 mg) as colorless crystals.

The $CHCl_3$ extract of the black gums was subjected to silica gel column chromatography (2.1 kg). Elution with $CHCl_3$−30% MeOH/$CHCl_3$ gave an active fraction (Fr-C-4: 10.5 g; IC_{50} 15.3 ng/mL) with other inactive ones (Fr-C-1−3, 5:83.7 g; IC_{50} >1000 ng/mL). Fr-C-4 was again subjected to silica gel column chromatography (200 g). Elution with 50% EtOAc/*n*-hexane gave Fr-C-4-2 (2.43 g), and subsequent elution with 10% MeOH/$CHCl_3$ gave Fr-C-4-5 (2.248 g). Fr-C-4-5 was purified by silica gel column chromatography and HPLC (ODS-H: 20 ∅ × 250 mm; 75% aqueous MeOH) to altohyrtin A (16.3 mg) and C (3.0 mg). Fr-C-4-2 was purified by silica gel column chromatography and Lobar column chromatography (RP-18; 95% aqueous MeOH) to give 12-*epi*-scalarin (13.6 mg) and **3** (25.3 mg). Compound **3** was recrystallized from MeOH.

Compound **1**: mp 195−196 °C; $[\alpha]^{25}_D$ +40.3° (*c* 0.65, $CHCl_3$); HRFABMS $[M + Na]^+$ *m/z* 425.2627 ($C_{25}H_{38}O_4$Na, Δ +0.4 mmu); EIMS *m/z* 384, 372, 364, 329, 311, 205 (205.1618, $C_{14}H_{21}O$, Δ +2.6 mmu); IR ν_{max} cm^{-1} (KBr) 3451, 3356, 1690, 1463, 1452, 1386, 1322, 1249, 1100, 1052, 978, 956, 815; CD $[\Theta]_{287}$ +2770° (MeOH); *anal.* C 74.63%, H 9.38%, calcd for $C_{25}H_{38}O_4$, C 74.59%, H 9.52%.

Compound **2a**: mp 229−231 °C; $[\alpha]^{25}_D$ +11.4° (*c* 0.56, 50% MeOH/$CHCl_3$); HRFABMS $[M + Na]^+$ *m/z* 427.2826 ($C_{25}H_{40}O_4$Na, Δ +0.5 mmu); EIMS *m/z* 386, 207 (207.1753, $C_{14}H_{23}O$, Δ +0.4 mmu); IR ν_{max} cm^{-1} (KBr) 3425, 1631, 1465, 1451, 1388, 1134, 1098, 1047, 1031, 980; *anal.* C 74.34%, H 9.82, calcd for $C_{25}H_{40}O_4$, C 74.22%, H 9.97%.

Compound **2b**: amorphous powder; $[\alpha]^{25}_D$ −10.5° (*c* 1.51, $CHCl_3$); HRFABMS $[M + K]^+$ *m/z* 485.2661 ($C_{27}H_{40}O_5$K, Δ −0.9 mmu); EIMS *m/z* 387, 386, 368, 358, 340, 325, 207; IR ν_{max} cm^{-1} (KBr) 3442, 1717, 1466, 1452, 1390, 1371, 1254, 1014.

Compound **3**: mp 218−220 °C; $[\alpha]^{25}_D$ +17.1° (*c* 0.31, $CHCl_3$); HRFABMS $[M + Na]^+$ *m/z* 411.2883 ($C_{25}H_{40}O_3$Na, Δ +0.8 mmu); EIMS *m/z* 370, 324, 191 (191.1801, $C_{14}H_{23}$, Δ +0.1 mmu); IR ν_{max} cm^{-1} (KBr) 3479, 3318, 1465, 1387, 1366, 1343, 1312, 1206, 1096, 1060, 1048, 980; *anal.* C 77.17%, 10.20%, calcd for $C_{25}H_{40}O_3$, C 77.27%, H 10.38%.

Compound **4**: mp >260 °C; $[\alpha]^{25}_D$ −2.7° (*c* 0.92, 50% MeOH/$CHCl_3$); HRFABMS $[M + Na]^+$ *m/z* 425.2646 ($C_{25}H_{40}O_4$Na, Δ −2.1 mmu); EIMS *m/z* 384, 207; UV λ_{max} nm (ϵ) 219 (10900); IR ν_{max} cm^{-1} (KBr) 3858, 3533, 3467, 1786, 1751, 1648, 1486, 1459, 1387, 1293, 1156, 1125, 1077, 1052, 873; ^1H NMR (50% $CDCl_3$/CD_3OD) δ 0.71 (3H, s), 0.83 (3H, s), 0.87 (6H, s), 0.90 (3H, s), 0.7−1.9 (15H, m), 2.18 (2H, m), 3.67 (1H, dd, *J* = 4.3, 11.2 Hz), 4.46 (1H, m), 4.62 (1H, brs), 5.94 (1H, d, *J* = 1.8 Hz); ^{13}C NMR (50% $CDCl_3$/CD_3OD) δ 7.1 (q), 16.7 (q), 17.1 (q), 18.8 (t), 19.0 (t), 21.5 (q), 26.3 (t), 31.2 (t), 33.5 (s), 33.7 (q), 38.0 (s), 38.3 (s), 40.5 (t), 42.5 (t), 42.6 (t), 47.5 (s), 48.3 (d), 57.1 (d), 58.8 (d), 68.2 (d), 80.9 (d), 90.9 (d), 111.7 (d), 176.4 (s), 176.6 (s); *anal.* C 74.34, H 9.62, calcd for $C_{25}H_{38}O_4$, C 74.59%, H 9.52%.

Oxidation of 2a with Pyridinium Dichromate. To a solution of pyridinium dichromate (23.6 mg) in DMF (1.0 mL) was added a solution of **2a** (10.6 mg) in DMF (1.0 mL) at room temperature. The mixture was stirred at room temperature overnight. The reaction mixture, after the reaction was complete, was poured into ice−water and extracted with EtOAc. The EtOAc extract (8.9 mg) was purified by preparative TLC (Si-60 F_{254}: 2 mm × 20 cm × 20 cm; Merck) and HPLC (ODS-H: 6 ∅ × 250 mm; 80% aqueous MeOH) to give **1** (1.2 mg), which was identified by means of physicochemical data including optical rotation [$[\alpha]^{25}_D$ +30° (c 0.1, $CHCl_3$)].

Alkaline Hydrolysis of 2b. To a solution of **2b** (10.2 mg) in MeOH (1.0 mL) was added 1 N NaOH (0.4 mL) at room temperature, and the mixture was kept at room temperature for 1 h. After the reaction was complete, water was added to the reaction mixture, and the mixture was extracted with EtOAc. The EtOAc extract obtained by usual workup gave **2a** (9.4 mg), which was identified by means of physicochemical data including optical rotation [$[\alpha]^{25}_D$ +12.4° (*c* 0.33, 50% MeOH/$CHCl_3$)].

Furan derivative **5**: amorphous powder; EIMS *m/z* 384, 366, 205; IR ν_{max} cm^{-1} ($CHCl_3$) 3500−3300, 1700, 1600, 1460, 1395, 1230−1200; ^1H NMR δ 0.87 (3H, s), 0.90 (3H, s), 1.05 (3H, s), 1.15 (3H, s), 1.50 (3H, s), 2.4−2.6 (4H, m), 2.82 (1H, m), 3.88 (1H, dd, *J* = 4.7, 9.7 Hz), 7.34 (brs), 7.58 (brs); ^{13}C NMR δ 16.1 (q), 17.2 (q), 18.2 (t), 19.4 (t), 20.1 (q), 21.0 (q), 21.4 (t), 26.7 (q), 28.8 (t), 34.2 (t), 37.9 (s), 37.5 (s), 39.1 (t), 40.9 (t), 41.2 (s), 47.3 (s), 54.8 (d), 56.0 (d), 57.9 (d), 79.2 (d), 120.6 (s), 134.9 (s), 136.8 (d), 138.2 (d), 216.3 (s).

X-ray Analysis of 1. Crystals recrystallized from MeOH were used for X-ray crystallography. Space group: orthorhombic, $P2_12_12_1$; cell parameters: a = 11.98(5) Å, b = 24.98(5) Å, c = 7.148(1) Å, Z = 4; D_x = 1.25 g/cm^3; R = 0.060 for 1936 reflections [$I > 2\sigma(I)$]. The intensity data were collected on a MacScience MXC18 diffractometer, and the structure was solved using a software package of CRYSTAN. The difference Fourier method revealed the presence of a definite

Table 6. Components of Culture Media

	P388 tumor cell line	MKN tumor cell lines
RPMI-1640	454.5 mL	437 mL
FCS	5% (25 mL)	10% (50 mL)
Streptomycin	2.5 mL (100 U/mL)	2.5 mL (100 U/mL)
Penicillin	100 μg	100 μg
HEDS	0.05 mM	0.05 mM
7.5% $NaHCO_3$	13 mL	13 mL

hydrogen bond between a hydrogen of the 12(*R*)-hydroxy group and an oxygen of the 19(*R*)-hydroxy group (not shown in Figure 2 and 4). Atomic coordinates, bond distances and angles, and torsional angles for **1** and **4** have been deposited at the Cambridge Crystallographic Data Centre.

X-ray Analysis of 4. Crystals recrystallized from $MeOH/CHCl_3$ were used for X-ray crystallography. Space group: orthorhombic, $P2_1$; cell parameters: a = 7.286(2) Å, b = 49.32(1) Å, c = 6.575(1) Å; β = 116.42-(2)°; D_x = 1.26 g/cm^3; Z = 4; R = 0.048 for 3504 reflections [$I > 2\sigma(I)$]. The intensity data collection and structure analysis were carried out as for **1**.

Measurement of Inhibitory Activities against Tumor Cell Growth. Tumor cell lines [1.4×10^3 cells/100 mL medium (mouse leukemia P388 cell line), 5×10^5 cells/100 mL medium (human gastric tumor line MKN-1), and 10^3 cells/100 mL medium (human gastric tumor line MKN-74)] were seeded into a 96-well microtiter plate. The plate was incubated for 24 h at 37 °C in a humidified 5% CO_2−95% air atmosphere. After incubation, the test compounds were dissolved in 100 mL of medium at various concentrations and added to the tumor cell-suspended media, and the plates were incubated further at 37 °C for 48 h in a humidified 5% CO_2−95% air atmosphere. Culture media used were composed of the components listed in Table 6.

After exposure to the test compounds, the tumor cells were washed three times with 200 mL of medium to remove the compounds. The tumor cells were incubated further in drug-free medium for 96 h in a humidified 5% CO_2−95% air atmosphere. Fifty milliliters of MTT (1 mg/l mL medium) solution was added to the culture medium and then incubated for 4 h under the same conditions. The medium was removed from the tumor cells, and 150 μL of DMSO was added to dissolve formazan that formed. The OD of the formazan solution at 540 nm was measured. IC_{50} values that were obtained from the inhibition−concentration curves plotted by measuring OD at various concentrations are shown in Table 4.

Life-Prolonging Effect of 1 on Mice with P388 Leukemia. Six CDF_1 mice per one group (purchased from Charles River Japan Inc., female, 7 weeks old, weighing 20−24 g, were inoculated intraperitoneally with 10^6 viable cells/mouse. One tenth of a milliliter of 10^7 cells/mL of tumor suspension was injected using a syringe with a 27-gauge needle. Test compound was dissolved in a small amount of dimethylacetamide and suspended in 10% polyoxyethylated vegetable oil (Emulphor)-saline. The solution was injected intraperitoneally into mice on days 1, 5, and 9 after tumor inoculation, and the survival time of the mice was observed. Control mice were given neither sample nor vehicle. Antitumor activity was estimated by the increase in lifespan, ILS (%), of mice calculated from the following equation[25]

$$\mathrm{ILS} = (A/B - 1)100$$

where A = weighed median of survival days in treated group and B = weighed median of survival days in untreated control group. The ILS values obtained are summarized in Table 5.

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References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1996**, *14*, 259−302. Many other references are cited here.
(2) (a) Sato, A.; Takahashi, S.; Ogita, T.; Sugano, M.; Kodama, K. *Ann. Rep. Sankyo Res. Lab.* **1995**, *47*, 1−58. (b) Sato, A. *J. Toxicol.-Toxin Rev.* **1996**, *15*, 171−198.
(3) Morishita, T.; Sato, A.; Hisamoto, M.; Oda, T.; Matsuda, K.; Ishii, A.; Kodama, K. *J. Antibiot.* **1997**, *50*, 457−468.
(4) (a) Sato, A.; Watanabe, T.; Iijima, Y.; Haruyama, H. Jpn. Kokai Tokkyo Koho JP0256261. (b) Tsuchiya, N.; Sato, A.; Watanabe, T.; Iijima, Y.; Haruyama, H. *Phytochemistry*, in press.
(5) Sato, A.; Iijima, Y.; Matsushita, Y.; Negishi, A.; Takamatsu, Y.; Haruyama, H.; Kinoshita, T.; Kodama, K.; Ishii, A. Jpn. Kokai Tokkyo Koho JP08208646.
(6) Sato, A.; Sugano, M.; Morishita, T.; Hata, T.; Sasagawa, K.; Kobayashi, T. Jpn. Kokai Tokkyo Koho JP08269085.
(7) Cimino, G.; de Stefano, S.; Minale, L.; Trivellone, E. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1587−1593.
(8) The sponge was identified as *Hyrtios erecta* (order Dictyoceratida) by Prof. P. R. Bergquist, School of Zoological Sciences, University of Auckland, New Zealand.
(9) Kobayashi, M.; Aoki, S.; Kitagawa, I. *Tetrahedron Lett.* **1994**, *35*, 1243−1246.
(10) (a) Iguchi, K.; Fujita, M.; Nagaoka, H.; Mitome, H.; Yamada, Y. *Tetrahedron Lett.* **1993**, *34*, 6277−6280. (b) Iguchi, K.; Shimura, H.; Taira, S.; Yokoo, C.; Matsumoto, K.; Yamada, Y. *J. Org. Chem.* **1994**, *59*, 7499−7502.
(11) These scalaranes were not characterized because they were all inactive against P388.
(12) Walker, R. P.; Thompson, J. E.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 4976−4979.
(13) Cafieri, F.; de Napoli, L.; Fattorusso, E.; Santacroce, C.; Sica, D. *Gazz. Chim. Ital.* **1977**, *107*, 71−74.
(14) Crews, P.; Bescansa, P. *J. Nat. Prod.* **1986**, *49*, 1041−1052.
(15) Velluz, L.; Legrand, M.; Grosjean, M. *Optical Circular Dichroism: Principles, Measurements, and Applications*; Verlag Chemie: Weinheim/Bergstr., Academic Press Inc.: New York, London, 1965; p 79−98.
(16) Ryu, G.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 515−517.
(17) Hochlowski, J. E.; Faulkner, D. J.; Bass, L. S.; Clardy, J. *J. Org. Chem.* **1983**, *48*, 8, 1738−1740.
(18) (a) Rueda, A.; Zubía, E.; Ortega, M. J.; Carballo, J. L.; Salvá, J. *J. Org. Chem.* **1997**, *62*, 1481−1485. (b) Zeng, L.; Fu, X.; Su, J.; Pordesimo, E. O.; Traeger, S. G.; Schmitz, F. J. *J. Nat. Prod.* **1991**, *54*, 421−427.
(19) Nakagawa, M.; Hamamoto, Y.; Ishihama, M.; Hamasaki, S.; Endo, M. *Tetrahedron Lett.* **1987**, *28*, 431−434.
(20) Kobayashi, J.; Mizobe, F. Jpn. Kokai Tokkyo Koho JP06239741.
(21) Jaspars, M.; Jackson, E.; Lobkovsky, E.; Clardy, J.; Diaz, M. C.; Crews, P. *J. Nat. Prod.* **1997**, *60*, 556−561.
(22) Kobayashi, M.; Chen, Y.-J.; Higuchi, K.; Aoki, S.; Kitagawa, I. *Chem. Pharm. Bull.* **1996**, *44*, 1840−1842.
(23) QUANTA software was used for the structure matchings.
(24) The X-ray crystallographical data of aragusterol C were cordially provided by Prof. Y. Yamada, Tokyo College of Pharmacy.
(25) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep. Part 3* **1972**, *3*, 1−103.

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