Callyspongenols A−C, New Cytotoxic C_{22}-Polyacetylenic Alcohols from a Red Sea Sponge, *Callyspongia* Species

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Investigation of the organic extract of a Red Sea sponge, *Callyspongia* sp., resulted in isolation and identification of three new C_{22}-polyacetylenic alcohols, callyspongenols A−C (**1**−**3**), together with dehydroisophonochalynol (**4**). The structures of **1**−**3** were determined by 1D and 2D NMR studies and mass spectral determinations. Compounds **1**−**4** showed moderate cytotoxicity against P388 and HeLa cells.

Straight-chain polyacetylenes are typical secondary metabolites of sponges of the order Haplosclerida,[2−16] which includes five families, viz., Petrosiidae,[3−6] Callyspongiidae,[7−9] Chalinidae,[7−9] Oceanapiidae,[10,11] and Niphatidae.[12−14] Polyacetylenes vary in chain length and degree of oxygenation pattern. Biological activities reported for polyacetylenes in the literature are diverse and include antifungal,[3] antimicrobial,[4] HIV-protease inhibitory,[5] HIV-reverse transcriptase inhibitory,[6] H^+, K^+-ATPase inhibitory,[7] antifouling,[8] immunosuppressant,[12] α-glucosidase inhibitory,[15] and antitumor effects.[13,14,16]

In our continuing program to search for potential drug leads from marine organisms, we found that a Red Sea sponge, *Callyspongia* sp., showed cytotoxic activity against P388 leukemia cells. Bioassay-guided isolation furnished callyspongenols A−C (**1**−**3**) (Figure 1), together with the known compound dehydroisophonochalynol (**4**).[9] The present paper deals with the isolation and structure determination of **1**−**3** and the biological activities of **1**−**4**.

Frozen specimens of the sponge were extracted with MeOH−CH_2Cl_2 (1:1). The resulting extract was dissolved in 90% MeOH and extracted with hexane; the remaining methanolic solution was diluted with H_2O to 60% MeOH and extracted with CH_2Cl_2. The combined organic extracts were fractionated on ODS and silica gel columns, and the cytotoxic fractions were purified on a C_{18} reversed-phase HPLC to afford **1**−**4**.

Callyspongenol A (**1**) was obtained as a pale yellow oil, with a molecular formula of $C_{22}H_{28}O$, as established by HRFABMS. The ^{13}C NMR spectrum of **1** combined with a HMQC experiment showed resonances for eight acetylenic carbons including seven singlets and one doublet, two monosubstituted olefinic carbons, and an oxygenated methylene attributed to a terminal primary alcohol, together with 11 methylene carbons (Table 1). The ^1H NMR spectrum of **1** showed resonances for 27 protons including a terminal acetylenic unit at δ 3.04, two olefinic protons at δ 5.99 and 5.41 for a *cis*-disubstituted double bond ($J_{19,20}$ = 10.7 Hz), an oxygenated methylene for a terminal primary alcohol at δ 4.21, and signals for 11 methylenes, which accounted for the remaining protons in the molecule

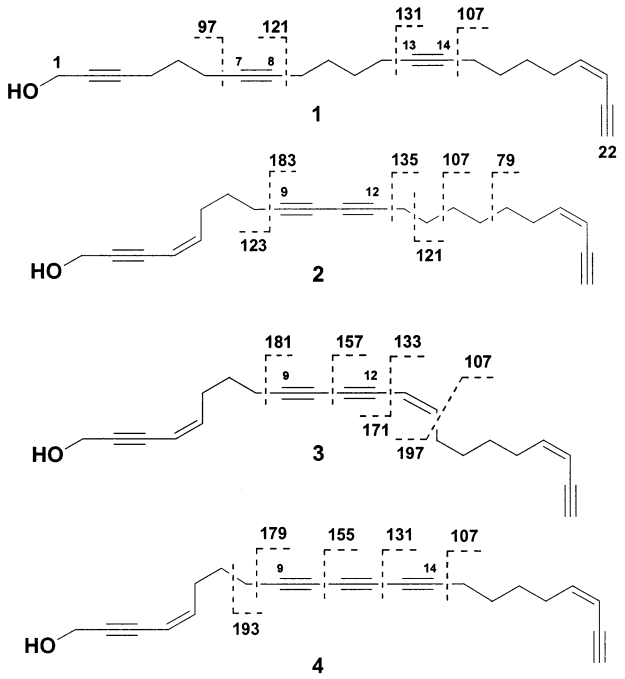

Figure 1. Structures of **1**−**4** and prominent mass spectral fragment peaks (*m*/*z* values)

(Table 1). Interpretation of the COSY, HOHAHA, and HMQC data led to the assembly of the C-1/C-5, C-9/C-12, and C-15/C-22 subunits. HMBC correlations of H_2-1/C-2,3, H_2-4/C-3, H_2-5/C-7, H_2-6/C-7,8, and H_2-9/C-7,8 established the assignment and location of the acetylenic moieties at C-2/C-3 and C-7/C-8, respectively. Similarly, HMBC correlations of H_2-12/C-13 and H_2-15/C-13,14 confirmed the location of the third acetylenic moiety at C-13/C-14. FABMS fragment ion peaks at *m*/*z* 131, 121, 107, and 97 supported the assignment of **1**. Accordingly, compound **1** was assigned as (19*Z*)-1-hydroxydocosa-19-ene-2,7,13,21-tetrayne.

Callyspongenol B (**2**) was obtained as a pale yellow oil with a molecular formula of $C_{22}H_{26}O$ as established by HRFABMS. Its ^{13}C NMR spectrum, together with a HMQC experiment, revealed the presence of eight acetylenic carbons including seven singlets and one doublet, four protonated olefinic carbons, an oxygenated methylene for a terminal primary alcohol, and signals for nine methylene

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Table 1. ^{1}H and ^{13}C Chemical Shift Data of Compounds **1** and **2** ($CDCl_3$)

	1			**2**		
no.	δ_C (mult.)	δ_H [mult., *J*(Hz)]	HMBC (H→C)	δ_C (mult.)	δ_H [mult., *J*(Hz)]	HMBC (H→C)
1	51.2 t	4.21 t (2.0)	C-2, C-3	51.6 t	4.41 br s	C-2, C-3, C-4, C-5
2	78.6 s[a]			91.9 s		
3	86.0 s			82.1 s		
4	18.5 t	2.18 m	C-3	109.6 d	5.51 br d (10.8)	C-2, C-3, C-6
5	28.2 t	1.49 m[c]	C-7	142.8 d	5.87 td (10.8, 7.3)	C-2, C-3, C-6, C-7
6	19.0 t	2.22 t (7.0)	C-7, C-8	29.1 t	2.41 q (7.3)	C-4, C-5, C-7, C-8
7	77.5 s[a]			27.4 t	1.62 quin (7.0)	C-5, C-6, C-8, C-9
8	65.4 s[b]			18.6 t	2.27 t (7.0)	C-9, C-10
9	27.9 t	1.47 m[c]	C-7, C-8	77.1 s[a]		
10	27.2 t	1.37 m[d]		65.6 s[b]		
11	28.1 t	1.37 m[d]	C-13	65.2 s[b]		
12	27.7 t	1.49 m[c]	C-13, C-14	77.8 s[a]		
13	65.1 s[b]			19.1 t	2.23 t (7.0)	C-11, C-12
14	77.1 s[a]			28.1 t	1.50 quin (7.0)	C-12, C-13
15	19.1 t	2.02 q (7.0)	C-13, C-14	28.4 t	1.41m[a]	C-13, C-14
16	28.4 t	1.29 m		28.55 t	1.31 quin (7.0)	C-18
17	28.4 t	1.37 m[d]	C-18	28.49 t	1.36 m[a]	C-17, C-18, C-19
18	30.0 t	2.29 q (7.3)	C-17, C-19, C-20	30.1 t	2.31 q (7.3)	C-17, C-19, C-20
19	145.9 d	5.99 dt (7.3, 10.7)	C-18, C-21	145.9 d	5.97 dt (7.3, 10.8)	C-17, C-18, C-21, C-22
20	108.0 d	5.41 br d (10.7)	C-21, C-22	108.0 d	5.30 br d (10.8)	C-18, C-21, C-22
21	80.4 s			80.5 s		
22	81.2 d	3.04 br d (2.0)	C-21	81.1 d	3.06 br s	C-19, C-20, C-21

[a,b] Assignment may be interchangeable due to proximity of signals. [c,d] In each column signals are partially overlapped.

Table 2. ^{1}H and ^{13}C Chemical Shift Data of Compounds **3** and **4** ($CDCl_3$)

	3			**4**		
no.	δ_C (mult.)	δ_H [mult., *J*(Hz)]	HMBC (H→C)	δ_C (mult.)	δ_H [mult., *J*(Hz)]	HMBC (H→C)
1	51.5 t	4.40 br s	C-2, C-3, C-4, C-5	51.5 t	4.40 br s	C-2, C-3
2	91.9 s			92.0 s		
3	82.7 s[a]			81.9 s		
4	109.7 d	5.51 dd (10.8, 1.5)	C-2, C-3, C-6	109.9 d	5.51 br d (10.4)	C-2, C-3
5	142.7 d	5.86 dt (7.7, 10.8)	C-3, C-6	142.4 d	5.85 dt (7.3, 10.4)	C-3, C-6, C-7
6	29.1 t	2.40 q (7.3)	C-7, C-8	29.1 t	2.38 m	
7	27.3 t	1.65 quin (7.3)	C-9	27.1 t	1.62 quin (7.3)	C-9
8	19.0 t	2.35 m[a]	C-9, C-10, C-11	19.1 t	2.27 m[d]	C-9, C-10, C-11
9	84.4 s[a]			79.8 s[a]		
10	72.2 s			65.7 s[b]		
11	65.5 s			60.3 s[c]		
12	78.7 s			60.5 s[c]		
13	108.1 d	5.45 br d (11.5)	C-11, C-12	66.0 s[b]		
14	147.6 d	5.99 td (11.5, 7.3)	C-12	79.1 s[a]		
15	29.9 t	2.32 m[d]		19.8 t	2.29 m[d]	C-12, C-13, C-14
16	28.1 t	1.42 m[e]	C-14	27.3 t	1.50 m[e]	C-14
17	28.1 t	1.42 m[e]	C-19	27.6 t	1.51 m[e]	
18	29.9 t	2.34 m[d]	C-19	29.5 t	2.31 m[d]	
19	145.7 d	5.96 dt (7.3, 11.5)	C-18	145.1 d	5.99 dt (7.3, 10.4)	C-18, C-21
20	108.2 d	5.41 br d (11.5)	C-18, C-21, C-22	108.6 d	5.44 br d (10.4)	C-21, C-22
21	80.4 s			80.3 s		
22	81.3 d	3.06 br s	C-20	81.5 d	3.06 br s	C-21

[a–c] Assignment may be interchangeable due to proximity of signals. [d,e] In each column signals are partially overlapped.

carbons (Table 1). The ^{1}H NMR spectrum displayed resonances for a terminal acetylenic proton at δ 3.06 (H-22), a terminal primary alcohol (δ 4.41, H_2-1), four olefinic protons (δ 5.97, 5.87, 5.51, and 5.42), and signals for nine methylene protons. The COSY, HOHAHA, and HMQC experiments allowed the assignment of the subunits C-1/C-8 and C-13/C-22 within **2**, which were substantiated by HMBC data (Table 1). HMBC correlations of H_2-7/C-9, H_2-8/C-9, H_2-8/C-10, H_2-14/C-12, H_2-14/C-13, H_2-13/C-11, and H_2-13/C-12 established the unambiguous placement of the conjugated diyne moiety at C-9/C-12. The *Z* geometry of the olefinic moieties within **2** was confirmed by the ^{1}H−^{1}H coupling constants of $J_{4,5} = J_{19,20} = 10.8$ Hz. FABMS fragment ion peaks at *m*/*z* 183, 135, 123, 121, 107, and 79 supported the assignment of **2**. Accordingly, compound **2** was assigned as (4*Z*,19*Z*)-1-hydroxydocosa-4,19-diene-2,9,11,21-tetrayne.

Callyspongenol C (**3**) was isolated as a pale yellow oil, with a molecular formula of $C_{22}H_{24}O$ as determined by HRFABMS. Its ^{13}C NMR spectrum, together with the HMQC data, indicated the presence of eight acetylenic carbons including seven singlets and one doublet, a terminal primary alcohol, three disubstituted olefins, and resonances for seven methylene carbons (Table 2). The ^{1}H NMR spectrum of **3** displayed resonances for a terminal enyne proton at δ 3.06 (H-22), a terminal primary alcohol (δ 4.40, H_2-1), and six olefinic protons at δ 5.99, 5.96, 5.86, 5.51, 5.45, and 5.41, thereby accounting for three disubstituted olefinic bonds (Table 2). In addition, signals for 10 methylenes were observed. Interpretation of the COSY and HMQC data led to the assignment of the C-1/C-8 and C-13/C-22 subunits within **3**, which were confirmed by the HMBC data (Table 2). The placement of the conjugated diyne moiety at C-9/C-12 was inferred from HMBC cross-peaks of H_2-7/C-9, H_2-8/C-9, H_2-8/C-10, H_2-8/C-11, H-13/C-11, H-13/C-12, H-14/C-12, and H_2-15/C-12, completing the gross structure of **3**. The chemical shifts of four

Table 3. Cytotoxicity of Compounds **1**−**4** against P388 and Hela Cells (IC_{50}: μg/mL)

	1	2	3	4	adriamycin[a]
P388	2.2	10.0	2.2	2.2	0.04
HeLa	4.5	10.0	3.9	5.1	0.066

[a] Positive cytotoxicity control.

substituted acetylenic carbons at δ 84.4, 72.2, 65.5, and 78.7 together with resonances at δ 108.1, 147.6, 29.9, and 19.0 were diagnostic of a conjugated endiyne moiety flanked by methylene groups.[8] The coupling constants of $J_{4,5} = 10.8$, $J_{13,14} = 11.5$, and $J_{19,20} = 11.5$ Hz indicated the *Z* geometry of the three olefinic moieties. FABMS fragment ion peaks at *m/z* 197, 181, 171, 157, 133, and 107 supported the assignment of **3**. Thus, compound **3** was assigned as (4*Z*,13*Z*,19*Z*)-1-hydroxydocosa-4,13,19-triene-2,9,11,21-tetrayne.

Dehydroisophonochalynol (**4**) was previously reported from a Red Sea sponge *Siphonochalina* sp.,[9] but only partially characterized using ^{1}H NMR data. Compound **4** was obtained as a pale yellow oil with a molecular formula of $C_{22}H_{22}O$ as deduced from HRFABMS. The complete and unambiguous assignment of **4** was based on interpretation of 1D and 2D NMR (COSY, HMQC, and HMBC) data. The complete NMR data of **4** are shown in Table 2. The cytotoxicity of compounds **1**−**4** against P388 and HeLa cells is summarized in Table 3.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Hitachi 300 spectrometer. NMR spectra were recorded on a JEOL α-600 spectrometer. NMR chemical shifts were referenced to $CDCl_3$ solvent signals (δ_H 7.24; δ_C 77.0). Positive FAB mass spectral data were obtained with a JEOL JMS-700T mass spectrometer.

Animal Material. The sponge was collected in November 2001 by hand using scuba at depths between 9 and 15 m off Hurghada, Egypt, in the Red Sea. The sponge materials were frozen immediately after collection and kept frozen at −20 °C until processed. The sponge is funnel-shaped, pink-colored, and up to 20 cm high, with a terminal aperture of 3−4 cm diameter, and a 1−2 mm thick tube wall. The surface is optically smooth, but irregularly undulated. The sponge shows compressible consistency and was easy to tear. The skeleton of spongin fibers is cored by thin strongyles, 60−85 × 1−2 μm. The ectosomal skeleton is double-meshed, with larger triangular meshes 300−1500 μm in diameter enclosed by primary fibers of 30−55 μm diameter cored by 3−5 spicules, enclosing smaller polygonal meshes of 60−120 μm diameter formed by secondary fibers of 10−15 μm diameter cored by a single spicule. The choanosomal skeleton is a basically rectangular but rather irregular system of primary fibers similar in size and coring to those of the ectosomal skeleton and similar secondary fibers recognizable only by their position and coring with a single spicule, forming large meshes of 250−700 μm diameter. The sponge was classified as an undescribed species of the genus *Callyspongia* (class Demospongiae, order Haplosclerida, family Callyspongiidae). The voucher specimen (No ZMA POR. 17064) was deposited at the Zoological Museum of the University of Amsterdam.

Extraction and Isolation. Frozen specimens (1.2 kg) of the sponge were extracted with MeOH−CH_2Cl_2 (1:1) (3 × 1500 mL) at room temperature. The combined extracts were evaporated in vacuo to obtain a brown residue, which was dissolved in 500 mL of MeOH−H_2O (9:1) and extracted with hexane (3 × 500 mL) to give 1.85 g of a brown residue. The methanol layer was diluted with H_2O to MeOH−H_2O (3:2) and then extracted with CH_2Cl_2 (3 × 500 mL) to afford 2.65 g of a CH_2Cl_2 extract. The combined hexane and CH_2Cl_2 extracts were subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 230/70 mesh), using stepwise elution from 50% MeOH to MeOH, to afford six fractions. Fractions 3 and 4 were combined and evaporated, and the residue (1.48 g) was flash chromatographed on a silica column eluted with hexane−CH_2Cl_2 (1:1) through CH_2Cl_2−MeOH (1:1). Fractions eluted with CH_2Cl_2 (100%) and CH_2Cl_2−MeOH (95:5) were combined, and the resulting residue (210 mg) was finally purified on a C_{18} reversed-phase HPLC column using 80% MeOH to give **1** (3.0 mg, 2.5 × 10^{-4} %), **2** (5.0 mg, 4.1 × 10^{-4} %), **3** (4.2 mg, 3.5 × 10^{-4} %), and **4** (5.1 mg, 4.2 × 10^{-4} %) (based on wet weight).

Callyspongenol A (1): pale yellow oil; UV (MeOH) λ_{max} 216 nm (log ϵ 4.05); ^{1}H and ^{13}C NMR data, see Table 1; positive HRFABMS (3-NBA/NaCl) obsd $[M + Na]^+$ *m/z* 331.2048 (calcd for $C_{22}H_{28}ONa$, 331.2038).

Callyspongenol B (2): pale yellow oil; UV (MeOH) λ_{max} 218 nm (log ϵ 4.04); ^{1}H and ^{13}C NMR data, see Table 1; positive HRFABMS (glycerol) obsd $[M + Na]^+$ *m/z* 329.1884 (calcd for $C_{22}H_{26}ONa$, 329.1881).

Callyspongenol C (3): pale yellow oil; UV (MeOH) λ_{max} 218 nm (log ϵ 4.04); ^{1}H and ^{13}C NMR data, see Table 2; positive HRFABMS (3-NBA/NaCl) obsd $[M + Na]^+$ *m/z* 327.1726 (calcd for $C_{22}H_{24}ONa$, 327.1725).

Dehydroisophonochalynol (4): pale yellow oil; UV (MeOH) λ_{max} 213 nm (log ϵ 4.06); ^{1}H and ^{13}C NMR data, see Table 2; positive HRFABMS (3-NBA/NaCl) obsd $[M + Na]^+$ *m/z* 325.1566 (calcd for $C_{22}H_{22}ONa$, 325.1568).

Bioassays. The cytotoxicity of compounds **1**−**4** against HeLa and P388 cells was evaluated as IC_{50} values and are presented in Table 3. Adriamycin was used as a positive cytotoxicity control. The HeLa (TKG0331) and P388 (TKG0326) cells were a donation from the Institute of Development, Aging and Cancer of Tohoku University, Japan,

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra in $CDCl_3$ for **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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