Polyacetylenes from a Red Sea Sponge *Callyspongia* Species

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From the methanolic extract of the Red Sea sponge *Callyspongia* sp. six new polyacetylenic compounds, aikupikanynes A−F, together with octahydrosiphonochalyne, were isolated and identified. Their structures, which comprise four hydrocarbons, two alcohols, and one unprecedented α-hydroxy carboxylic acid, were determined by extensive 1D and 2D NMR studies and mass spectral determinations.

Polyacetylenes are a unique class of sponge metabolites of more than 100 compounds reported in the past 20 years. Polyacetylenes are characteristic of sponges in the order Haplosclerida,[2−39] with about 88 compounds reported from five families belonging only to this order. These families include Petrosiidae (50 compounds),[2−21] Callyspongiidae (12 compounds),[22−29] Chalinidae (10 compounds),[30−33] Oceanapiidae (15 compounds),[34,35] and Niphatidae (7 compounds).[36−39] Polyacetylenes vary in chain length and degree of oxygenation and can be roughly divided into four categories: common hydroxylated polyacetylenes (70 compounds), brominated C_{18} acetylenic acid (19 compounds), less common polyacetylenic hydrocarbons (12 compounds), and the rare C_{47} hydroxylated polyacetylenic acid (6 compounds). We now report structures of four hydrocarbons, two alcohols, and one unprecedented α-hydroxy acid.

Petrosiidae sponges (genera *Petrosia* and *Xestospongia*) are characterized by the presence of hydroxylated C_{30} and C_{46} polyactylenes[3,8,10,11,13,18,20,21] as well as brominated C_{18} acetylenic acid,[4] while the highly hydroxylated C_{47} acetylenic acids as well as the hydroxylated C_{30} polyacetylenes are represented in Chalinidae sponges (genera *Haliclona* and *Adocia*).[32,33] Hydroxylated C_{23}−C_{41} and C_{32}−C_{33} Polyacetylenic acids were reported in the family Oceanapiidae (genus *Pellina*).[34,35] Hydroxylated C_{20}−C_{22} polyacetylenes were found in the family Niphatidae (genus *Cribrochalina*).[36−39] Polyacetylenic C_{21}−C_{22} hydrocarbons[23,25,27] are characteristic of the family Callyspongiidae (genera *Siphonochalina* and *Callyspongia*). In addition, C_{23} sulfonated[29] and C_{22}−C_{23} hydroxylated polyacetylenes[23,25,27] are also reported from members of the Callyspongiidae. Recently, a series of C_{32}−C_{35} hydroxylated polyacetylenes were isolated from a Lithistid sponge (*Theonella* sp.) of the order Astrophoridae.[40] Biological activities for the reported polyacetylenes are diverse. These bioactive metabolites have been associated with antifungal,[2,14] antimicrobial,[3] HIV protease inhibitory,[4] HIV reverse transcriptase inhibitory,[5] H^+- and K^+-ATPase inhibitory,[22] antifouling,[23] immunosuppressant,[36] and antitumor activities.[6,7,37,38]

The dichloromethane fraction of a methanolic extract of the sponge *Callyspongia* sp. from the Red Sea was subjected to reversed-phase flash chromatography, Sephadex LH-20 (MeOH−CH_2Cl_2, 1:1), and reversed-phase HPLC to yield six new polyacetylenes, aikupikanynes[41] A−F (**1**, **2**, **4**−**7**), together with the known compound octahydrosiphonochalyne[25] (**3**) (Figure 1). The present paper deals with the structure determination of these compounds. Compound **3**, although known, had been only partially characterized.

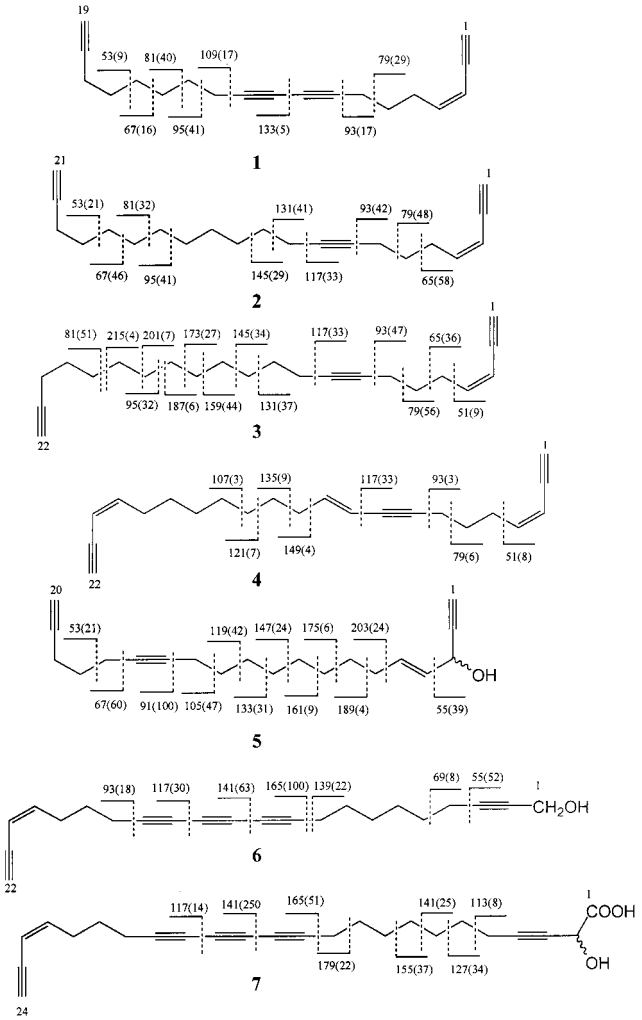

Figure 1. Structures and key MS fragmentation pattern of **1**−**7**.

Results and Discussion

Specimens of the sponge were extracted with MeOH. 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 CH_2Cl_2 extract was fractionated by reversed-phase flash chromatography, and the resulting fractions were

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

	1			**2**			**3**		
C	δ_C	δ_H (M, Hz)	HMBC with H	δ_C	δ_H (M, Hz)	HMBC with H	δ_C	δ_H (M, Hz)	HMBC with H
1	81.2	3.08 (d,1.9)		81.1	3.07 (d, 2.0)		81.1	3.06 (d, 2.3)	
2	80.3		1	80.5		1	80.4		
3	108.6	5.46 (ddd, 10.8,2.2,0.7)		107.9	5.44 (ddt, 11.0, 2.0, 1.1)	1, 5	107.9	5.43 (ddt, 10.6,2.3, 1.5)	
4	145.1	5.96 (dt, 10.8,7.4)	1, 5	146.2	5.99 (dtd, 11.0, 7.5, 0.7)	1,5	146.3	6.03 (dtd, 10.6,7.6, 0.9)	2, 3
5	29.5	2.34 (q, 7.5)	5	30.2	2.32 (dq, 7.5, 1.1)		30.2	2.31 (dq, 7.3, 1.0)	3, 4
6	27.5	1.58−1.51 m	4, 7	28.6	1.45[a] (quin., 7.5)	4	29.9−28.4	1.46 (quin., 7.3)	
7	19.2	2.29 (t, 6.5)		18.6	2.14[b] (dt, 7.5, 1.1)		18.6	2.12	
8	79.0		7	80.4		6, 10	80.7		
9	60.4		7	79.8		7, 11	79.2		
10	65.8		12	18.7	2.12[b] (dt, 7.5, 1.1)		17.9	2.24 (tt, 7.1,2.4)	
11	79.0		12	28.7	1.48[a]		29.9−28.4	1.56 (quin., 7.1)	
12	19.1	2.31 (t, 7.0)		29.3[c]	1.25 m		29.9−28.4	1.67−1.25 m	
13	27.6	1.58−1.51 m		29.1[c]	1.27 m		29.9−28.4	1.67−1.25 m	
14	27.8	1.58−1.51 m		29.1[c]	1.27 m		29.9−28.4	1.67−1.25 m	
15	27.4	1.58−1.51 m	13	29.0[c]	1.27 m		29.9−28.4	1.67−1.25 m	
16	27.8	1.58−1.51 m	17	28.8[c]	1.53 m		29.9−28.4	1.67−1.25 m	
17	18.2	2.19 (dt, 6.6, 2.6)		28.0	1.53 m	19	29.9−28.4	1.67−1.25 m	
18	84.2		17, 19	27.9	1.55 m	19	29.9−28.4	1.67−1.25 m	
19	68.4	1.95 (t, 2.6)		18.3	2.18 (dt, 7.0, 2.6)	18	27.9	1.54 m	20
20				84.5		18, 19, 21	18.3	2.17 (dt, 7.0, 2.6)	
21				68.1	1.93 (t, 2.6)	19	84.5		19,20,21
22							68.0	1.94 (t, 2.5)	

[a,b] Signals are partially overlapped. [c] assignments in the same column may be interchangeable due to proximity of signals.

purified by either SiO_2 column or reversed-phase HPLC to afford compounds **1**−**7**.

Aikupikanyne (**1**) was obtained as a colorless oil, with a molecular formula of $C_{19}H_{22}$, as established by HRDCIMS. The ^{13}C NMR spectrum of **1**, combined with HMQC experiment results (Table 1), showed resonances for eight acetylenic carbons [δ 84.2 (s), 81.2 (d), 80.3 (s), 79.0 (2C, s), 68.4 (d), 65.8 (s), and 60.4 (s)], two sp^2 carbons [δ 145.1 and 108.6], and nine methylene carbons. The ^{1}H NMR spectrum of **1** (Table 1) showed resonances for two terminal acetylenes at 3.08 (d, J = 1.9 Hz, H-1) and 1.95 (t, J = 2.6 Hz, H-19); the chemical shift of H-1 is attributed to an electron withdrawing substituent at C-2. Two olefinic protons at δ 5.96 and 5.46 for the *Z*-double bond at C-3/C-4 ($J_{3,4}$ = 10.8 Hz), together with signals for nine methylenes, accounted for the remaining hydrogens in the molecule. Interpretation of the ^{1}H−^{1}H COSY and HMQC spectra led us to the assembly of the C-1/C-7 unit. The long-range COSY cross-peaks between H-1 and H-3 were indicative of a conjugated enyne moiety. The assignments of the quaternary acetylenic carbons were securely assigned from the HMBC experiment. Correlation between H-1 and C-2, H-19 and C-18, H_2-7 and C-8 and C-9, as well as between H_2-12 and C-11 and C-10 established the location of the acetylenes. A combination of the COSY, HMQC, and HMBC experiments (Table 1) enabled us to establish the structure of **1**. The existence of the fragmentation ion peaks at *m*/*z* 109 and 133 in the MS (Figure 1) confirmed the position of the isolated triple bonds, thus completing the structure of **1**.

Aikupikanyne B (**2**) was obtained as a colorless oil with a molecular formula of $C_{21}H_{30}$ as established by HRDCIMS. Its ^{13}C NMR spectrum, together with an HMQC experiment, revealed the presence of six acetylenic carbons [δ 84.5 (s), 81.1 (d), 80.5 (s), 80.4 (s), 79.8 (s), and 68.1 (d)], two sp^2 carbons [δ 146.2 and 107.9], and 13 methylene carbons (Table 1). The ^{1}H NMR spectrum displayed two terminal acetylenic protons at δ 3.07 (d, J = 2.0 Hz, H-1) and 1.93 (t, J = 2.6 Hz, H-21) due to terminal enyne and terminal alkyne moieties, respectively. ^{1}H−^{1}H COSY and HMQC experiments allowed the assembly of the C-1/C-7 and C-17/C-21 units. The HMBC experiment showed cross-peaks for H-1/C-2, H-1/C-3, H-1/C-4 ($^4J_{CH}$), H-5/C-3, H-5/C-4, H-4/C-6, H-6/C-8, and H-7/C-9. The partial structure of C-1/C-9 was also supported by the appearance of a strong ion peak at *m*/*z* 117 (33%) in the mass spectrum (Figure 1). Additional long-range HMBC correlations for the H-10/C-8 and H-11/C-9 were also observed. On the other hand, correlations between H_2-19 and C-17, C-18, C-20, C-21 and between H_2-18 and C-19 and C-20 were recognized. The *Z*-configuration of C-3/C-4 was deduced from the magnitude of the coupling constant ($J_{3,4}$ = 11.0 Hz). Significant fragmentation ion peaks (*m*/*z* 65, 79, 93, and 131) in the MS (Figure 1) confirmed the position of the isolated triple bond and supported the structure of **2**.

Octahydrosiphonochalyne (**3**) was previously reported from a Red Sea sponge *Siphonochalina* sp.,[25] but only partially characterized. Compound **3** was separated as a mixture with **5**. LREIMS of the mixture showed two molecular ion peaks at *m*/*z* 296 and 284 for **3** and **5**, respectively. Moreover the HRDCIMS displayed two pseudomolecular ion peaks for $[M + NH_4]^+$ at *m*/*z* 314.2847 ($C_{22}H_{36}N$) and 302.3221 ($C_{20}H_{32}ON$), corresponding to the proposed structures of **3** and **5**. The molecular formula of **3** requires seven degrees of unsaturation (three alkyne and one alkene functionalities). The ^{13}C NMR spectrum and HMBC experiment displayed resonances for six acetylenic carbons [δ 84.5 (s), 81.1 (d), 80.7 (s), 80.4 (s), 79.2 (s), and 68.0 (d)], two sp^2 carbons [δ 146.3 and 107.9], three methylene carbons vicinal to triple bonds [δ 18.6, 18.3, and 17.9], and an additional 11 methylene carbons [δ 31.8−27.9]. The ^{1}H NMR spectrum showed resonances for terminal enyne and terminal alkyne protons at δ 3.06 (d, J = 2.3 Hz) and 1.94 (t, J = 2.5 Hz), respectively, as well as two olefinic protons at δ 6.03 and 5.43 for the *Z*-double bond at C-3/C-4 ($J_{3,4}$ = 10.6 Hz). The ^{1}H−^{1}H COSY spectrum, together with the HMBC experiment, enabled us to determine the structure of the C-1/C-7 subunit. Moreover, the position of the isolated triple bond in **3** was confirmed through significant fragmentation ion peaks at *m*/*z* 93 and 117 for the C-1/C-7 and C-1/C-9 fragments, respectively. Further fragment ion peaks in the mass spectrum (Figure 1) confirmed the structure of **3**.

Due to the existence of a terminal enyne attached to three methylenes in the structures of compounds **1**−**3**, similar fragmentation ion peaks (*m*/*z* 79, and 93) in the MS (Figure 1) of these compounds were observed.

Aikupikanyne C (**4**) was isolated as colorless oil, with a

Table 2. ^{1}H and ^{13}C NMR Data of **4**−**6** ($CDCl_3$)

	4			**5**			**6**			**7** (DMSO-d_6)		
C	δ_C	δ_H (M, Hz)	HMBC with H	δ_C	δ_H (M, Hz)	HMBC with H	δ_C	δ_H (M, Hz)	HMBC with H	δ_C	δ_H (M, Hz)	HMBC with H
1	81.6	3.08 (d, 2.2)	3	74.0	2.56 (d, 2.1)		51.3	4.24 (t, 2.3)		170.3		2
2	80.5		1	83.3			78.6		1,4	62.4	4.32, br s.	
3	108.9	5.48 (ddt, 10.8,2.2,1.2)	1, 5	62.8	4.83 m	4	86.0		1,4,5	80.4		2, 5
4	144.8	6.02 (dt, 10.8, 7.5)	5,6	128.3	5.61 (ddt, 15.2,6.0,1.2)	4, 5	18.5	2.20 (tt, 6.8,2.1)		82.4		2, 5, 6
5	29.5	2.45 (dq, 7.5,1.2)	7	134.5	5.91 (dtd, 15.2,6.0,1.1)		27.9	1.58−1.47 m	4	18.0	2.15 (t-like, 6.1)	
6	28.0	1.67 (quin, 7.3)		31.9	2.06 (q, 7.0)	4,5	27.6	1.58−1.47 m	4,8	27.6	1.40 m	
7	19.1	2.37 (dt, 7.2, 1.9)		29.7−28.4	1.67−1.25 m		27.5	1.58−1.47 m	8	27.2	1.48 m	
8	93.7		6,7	29.7−28.4	1.67−1.25 m		27.9	1.58−1.47 m		27.0	1.48 m	
9	77.8		7	29.7−28.4	1.67−1.25 m		19.1	2.30[a]		27.6	1.48 m	
10	109.3	5.42[a]	12	29.7−28.4	1.67−1.25 m		79.0		8	27.6	1.48 m	
11	142.7	5.80 (td, 14.8,7.5)	12	29.7−28.4	1.67−1.25 m		65.9[b]		9	18.2	2.38 (t, 6.6)	
12	30.0	2.27 (dq, 7.5,1.2)		29.7−28.4	1.67−1.25 m		60.4[c]			80.7		10
13	28.9	1.33 m	12	18.7	2.14[a]		60.3[c]			65.0[b]		10
14	28.9	1.33 m	12	79.8			65.8[b]		16	60.14[c]		10
15	27.8	1.54 m		80.7			79.0		17	60.18[c]		17
16	28.8	1.41 m	18	18.7	2.14[a]		19.2	2.29[a] (t, 7.0)		65.1[b]		17
17	28.6	1.41 m	18	28.0	1.59 m	18	27.3	1.58−1.47 m	15, 18	80.9		17, 18
18	30.2	2.31 (dq, 7.0, 1.4)		18.3	2.19 (dt, 6.8,2.8)		29.7	2.34 (dq, 7.4, 1.3)		18.4	2.36 (t, 7.0)	
19	146.2	5.98 (td, 10.8,7.3)	18	84.8		17, 18,20	145.1	5.96 (dtd, 10.8,7.5,0.8)	17,18,20,22	26.9	1.48 m	20
20	108.0	5.44[a]	18, 22	68.1	1.93 (t, 2.8)		108.6	5.46 (ddt, 10.8,2.3,1.3)	18,19,22	29.1	2.24 (q, 6.6)	20, 21
21	80.3		22				80.3		19,20,22	144.9	6.02 (dt, 10.7, 7.5)	
22	81.1	3.07 (d, 2.4)					81.5	3.08 (tdd, 2.3,0.8,0.4)	19,20	108.8	5.51 (br d., 10.7)	23
23										80.5		23
24										84.9	4.08 (br d, 2.0)	21

[a] In each column signals are partially overlapped. [b,c] Assignments with the same superscript in the same column may be interchangeable due to proximity of signals.

molecular formula of $C_{22}H_{28}$ by HRDCIMS. The ^{13}C NMR spectrum, together with the HMQC data (Table 2), indicated the presence of six acetylenic carbons [δ 93.7 (s), 81.6 (d), 81.1 (d), 80.5 (s), 80.3 (s), and 77.8 (s)] and six protonated sp^2 carbons [δ 146.1, 144.8, 142.7, 109.3, 108.9, and 108.0]. In addition, resonances for 10 methylene carbons were also observed. The ^{1}H NMR spectrum of **4** displayed resonances for two terminal enyne protons at δ 3.07 (d, J = 2.4 Hz) and 3.09 (d, J = 2.2 Hz); six olefinic protons at δ 6.02, 5.98, 5.80, 5.48, 5.44, and 5.42 (Table 2), and signals for 10 methylenes. The ^{1}H−^{1}H COSY and HMQC experiments led to the assembly of the C-1/C-7 and C-10/C-22 units of **4**, which were confirmed by the HMBC data (Table 2). The placement of the C-8/C-9 triple bond was supported by HMBC cross-peaks H-6/C-8, H-7/C-8, and H-7/C-9, completing the gross structure of **4**. 3*Z*- and 19*Z*-configurations were determined by ^{1}H coupling constants ($J_{3,4} = J_{19,20}$ = 10.8 Hz), while a coupling constant of 14.8 Hz between H-10 and H-11 supported the suggested *E* configuration on this double bond. The gross structure of **4** was also confirmed from the fragmentation pattern of the $[M]^+$ in the mass spectrum (Figure 1).

Aikupikanyne D (**5**) was isolated with **3** as a mixture. The molecular formula of $C_{22}H_{32}$ for **5** was confirmed from the ion peak at *m*/*z* 314.2847 in the HRDCIMS for $[M + NH_4]^+$. Similar to **3**, the molecular formula of **5** requires seven degrees of unsaturation (one alkene and three alkyne functionalities). The ^{13}C NMR spectrum showed resonances for **6** acetylenic carbons [δ 84.8 (s), 83.3 (s), 80.7 (s), 79.8 (s), 74.0 (d), and 68.1 (d)], two sp^2 carbons [δ 134.5 and 128.3], a carbinol [δ 62.8], signals for three methylene carbons attached to triple bonds [δ 18.7 (2C) and 18.3], and 10 methylene carbons [δ 29.7−28.4]. The ^{1}H NMR spectrum showed resonances for two terminal acetylenes at δ 1.93 (t, J = 2.8 Hz) and 2.56 (d, J = 2.1 Hz) characteristic of a terminal alkyne and a terminal acetylene vicinal to a carbinol, a secondary hydroxyl proton at δ 4.83, two olefinic protons at δ 5.61 and 5.91 ($J_{4,5}$ = 15.2 Hz) for the *E*-double bond at C-4/C-5. A combination of the ^{1}H−^{1}H COSY and HMBC experiments, together with the MS fragmentation pattern, enabled us to confirm the structure of **5**. The position of the isolated triple bond was confirmed from the base peak in the mass spectrum at *m*/*z* 91 for the C-14/C-20 fragment. Further significant fragment ion peaks (Figure 1) confirmed the proposed structure of **5**.

The remaining two compounds, aikupikanynes E (**6**) and F (**7**), are conjugated triynes. This structural feature had previously been encountered in a *Pellina* sp. sponge from Micronesia.[34] The carbon resonances are a poor match for those measured for **6** and **7**, undoubtedly due to the fact that the *Pellina* triynes are hydroxylated at both triyne alpha carbons. However, when our data are compared with those of conjugated triynes flanked by methylenes, which have been reported from a terrestrial Annonaceous tree,[42] the NMR data are comparable.

Aikupikanyne E (**6**) was obtained as colorless oil with a molecular formula of $C_{22}H_{24}O$ as deduced from HRDCIMS. The molecular formula of **6** requires 11 degrees of unsaturation equivalent to one alkene and five alkyne functionalities as shown in the structure (Figure 1). The ^{13}C NMR spectrum together with HMQC data revealed resonances for 10 acetylenic carbons [δ 86.0 (s), 81.5 (d), 80.3 (s), 79.0 (2C, s). 78.6 (s), 65.9 (s), 65.8 (s), 60.4 (s), and 60.3 (s)], two sp^2 carbons [δ 145.1 and 108.6], a primary alcohol [δ 51.3], and signals for nine methylene carbons. The chemical shifts of six quaternary carbons at δ 79.0 (2C), 65.9, 65.8, 60.4, and 60.3, together with two methylenes at δ 19.2 and 19.1, are characteristic of three consecutive triple bonds adjacent to neighboring methylene groups. The ^{1}H NMR spectrum showed resonances for 23 protons, including a terminal acetylene at δ 3.08; two olefinic protons at δ 5.96 and 5.46 ($J_{19,20}$ = 10.8 Hz) for the *Z*-double bond at C-19/

C-20; a terminal primary alcohol at δ 4.24; and nine methylene protons. The $^1H-^1H$ COSY and HMQC experiments led to partial structures of C-1/C-9 and C-16/C-22, which were confirmed by the HMBC data (Table 2). Placement of the C-10/C-15 triyne was deduced by HMBC correlations H-8/C-10, H-9/C-11, H-17/C-15, and H-16/C-14, completing the gross structure of **6**. Significant fragment ion peaks at *m/z* 165 (100), 141 (63), 139 (22), 117 (30), 93 (18), and 55 (52) in the mass spectrum (Figure 1) confirmed the structure of **6**.

Aikupikanyne F (**7**) was obtained as obtained as yellowish oil, with a molecular formula $C_{24}H_{26}O_3$ as established by the HRFABMS. The ^{13}C NMR and DEPT spectra (Table 2) showed signals for 10 acetylenic carbons [δ 84.9 (d), 82.4 (s), 80.9 (s), 80.7 (s), 80.5 (s), 80.4 (s), 65.1 (s), 65.0 (s), 60.2 (s), and 60.1 (s)], two sp^2 carbons [δ 144.9 and 108.8], a carboxylic acid [δ 170.3], a secondary alcohol [δ 62.4], and nine methylene carbons. Similar to **6**, the chemical shifts of the following six quaternary carbons at δ 80.7, 65.0, 60.1, 60.2, 65.1, and 80.9, together with the signals at δ 18.2 and 18.4, were characteristic for a conjugated triyne with neighboring methylene groups.

Compound **7** differs from **6** by the elements $C_2H_2O_2$. Comparison of the ^1H and ^{13}C NMR data of **7** with those of **6** (Table 2) showed the absence of the signals of a primary alcohol (δ 4.24/51.3) and the appearance of new signals for an α-hydroxy carboxylic acid (δ 4.32/62.4 and 170.3). All other signals were comparable to those of **6**. Assignments of proton and carbon chemical shifts were made possible from a combination of $^1H-^1H$ COSY, HMQC, and HMBC data (Table 2). Important fragmentation ion peaks in the MS spectrum (Figure 1) supported the proposed structure of **7**. To the best of our knowledge, this is the first example of a polyacetylenic α-hydroxy carboxylic acid.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on either a General Electric GN Omega 500 spectrometer or Varian Unity INOVA 400 WB instrument (^1H NMR at 500 or 400 MHz; ^{13}C NMR at 125 or 100 MHz, respectively). Mass spectral data were measured on either a VGZAB or a VG 7070 mass spectrometer. HRMS were determined in the DCI (NH_3) and FAB (NBA) modes. HPLC was performed on Ultracarb 5 μ ODS 30 (250 $\times$ 10 mm), Phenomenex.

Animal Materials. The sponge forms a large vase-shaped tube up to 20 cm high and up to 6 cm in diameter. It was collected from a depth of 15−20 m, at Sharm El Sheikh, Egypt, on the Red Sea, on September 11, 1998. The texture is very firm, but the sponge is very elastic and springy. The surface is smooth and characterized by a very regular stellate arrangement of secondary and tertiary fibers. The color in life and in preservative is amber. The sponge is an undescribed species of *Callyspongia* (order Haplosclerida, family Callyspongiidae). A voucher specimen has been deposited at the National History Museum, London (BMNH 1999.12.20.6).

Biological Assays. Assays were performed to determine IC_{50} values (recorded in μg/mL) of selected cancer cell lines, including mouse lymphoma (P-388, ATCC: CCL 46), human lung carcinoma (A-549, ATCC: CL 8), and human colon carcinoma (HT-29, ATCC: HTB 38). Aikupikanynes E and F showed moderate activity in the antitumor testing, with IC_{50} values of 5 and 10 μg/mL, respectively. Aikupikanynes A−D were not tested because of insufficient quantities available for testing.

Extraction and Isolation. A freshly collected specimen (101 g, wet wt) of the sponge was quickly immersed in MeOH on site. The sponge was extracted with MeOH (3 $\times$ 400 mL) at room temperature. The combined extracts were evaporated in vacuo. The concentrated extract was dissolved in 500 mL of MeOH−H_2O (9:1) and extracted with hexane (3 $\times$ 200 mL) to give 230 mg of hexane residue. The methanolic layer was diluted with H_2O to MeOH−H_2O (3:2) and then extracted with CH_2Cl_2 (3 $\times$ 200 mL) to afford 689 mg of CH_2Cl_2 extract, which was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 230/70 mesh), eluting with 40→0% H_2O−MeOH followed by CH_2Cl_2 to obtain six fractions. All fractions were subjected to in vitro antitumor assay against a panel of cancer cells. Guided by the in vitro antitumor testing, fraction 2 (123 mg, IC_{50} 5 μg/mL) was subjected to a Sephadex LH-20 column eluted with MeOH−CH_2Cl_2 (1:1) to give pure **7** (9.2 mg, 0.0092%, wet wt) and impure **6** (45 mg, IC_{50} 2 μg /mL), which was further subjected to a SiO_2 column with hexane−CH_2Cl_2 (3:7) to give **6** (15 mg, 0.015%). Fraction 4 (202 mg) was subjected to chromatography on a Sephadex LH-20 column eluted with MeOH−CH_2Cl_2 (1:1) to afford five fractions. Fraction 4−4 (77 mg, IC_{50} 10 μg /mL) was then purified by reversed-phase HPLC with 65% MeCN→MeCN to give **2** (2.7 mg, 0.0027%) and a mixture of **3** and **5** (2.9 mg, 0.0029%). Attempts to separate this mixture on reversed-phase and normal-phase HPLC were unsuccessful. Fraction 4−5 (20.9 mg, IC_{50} 2 μg/mL) was subjected to purification on reversed-phase HPLC with 80% MeCN→MeCN to yield **1** (4.1 mg, 0.0027%) and **4** (1 mg, 0.001%).

Aikupikanyne A (**1**): colorless oil; NMR data, see Table 1; HRMS (DC) (NH_3) *m/z* 268.2081 (calcd for $C_{19}H_{26}N$, $[M + NH_4]^+$, 268.2065).

Aikupikanyne B (**2**): colorless oil; NMR data, see Table 1; HRMS (DC) (NH_3) *m/z* 300.2691 (calcd for $C_{21}H_{34}N$, $[M + NH_4]^+$, 300.2691).

Octahydrosiphonochalyne (**3**): colorless oil; NMR data, see Table 1; HRMS (DC) (NH_3) *m/z* 314.2847 (calcd for $C_{22}H_{36}N$, $[M + NH_4]^+$, 314.2848).

Aikupikanyne C (**4**): colorless oil; NMR data, see Table 2; HRMS (DC) (NH_3) *m/z* 310.2545 (calcd for $C_{22}H_{32}N$, $[M + NH_4]^+$, 310.2535).

Aikupikanyne D (5): colorless oil; NMR data, see Table 2; HRMS (DC) (NH_3) *m/z* 302.3221 (calcd for $C_{20}H_{32}NO$, $[M + NH_4]^+$, 302.2484).

Aikupikanyne E (**6**): colorless oil; NMR data, see Table 2; HRMS (DC) (NH_3) *m/z* 322.2170 (calcd for $C_{22}H_{28}NO$, $[M + NH_4]^+$, 322.2171).

Aikupikanyne F (**7**): unstable yellowish oil; NMR data, see Table 2; HRMS (FAB) *m/z* 385.1792 (calcd for $C_{24}H_{26}O_3Na$, $[M + Na]^+$, 385.1780).

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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