Haliclonyne, a New Highly Oxygenated Polyacetylene from the Marine Sponge *Haliclona* Species

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Haliclonyne (**1**), a new polyacetylene carboxylic acid, has been isolated from the marine sponge *Haliclona* sp. collected in the Gulf of Eilat. The structure of haliclonyne, a C_{47} oxo-octahydroxy-dienetetrayne carboxylic acid, was elucidated by interpretation of NMR and mass spectra of **1** and two of its derivatives.

As part of our continuing efforts aimed toward the isolation of biologically active compounds from marine invertebrates,[1,2] we isolated a new highly oxygenated polyacetylene compound designated haliclonyne (**1**) from the marine sponge *Haliclona* sp. (family Chalinidae). Marine sponges, particularly, sponges of the genera *Xestospongia*, *Petrosia*, and *Haliclona*, have been shown to be rich sources of high molecular weight polyacetylenic compounds, some of which show significant biological activity.[3−17] Haliclonyne (**1**) possesses a diacetylenic carbinol and a 2-yne-4,5,6-trihydroxy carboxylic acid group as structural features, making it structurally related to osirisynes A−F previously isolated from the sponge *H. osiris*.[18]

Haliclonyne (**1**) was isolated (0.08% dry wt) from the $CHCl_3$−MeOH (1:1) extract of the freeze-dried sponge, *Haliclona* sp., collected at the Gulf of Eilat. The negative FABMS of **1** gave a molecular ion $[M - H]^-$ at *m/z* 811, indicating a molecular weight of 812. Both the ^1H and ^{13}C spectra (Table 1) were complicated, due to overlapping resonances of methylene hydrogens and aliphatic carbons. Nevertheless, the presence of several functional groups was readily recognizable, suggesting five partial structures. The connectivities between these partial structures (**a**−**e**), as well as the size of the linking methylene groups (m, n, p, q), unresolved in the NMR spectra, were established on the basis of MS fragmentations of **1** itself and two of its derivatives (**2** and **3**) (Tables 2−4).

The ^1H and ^{13}C NMR spectra revealed the following moieties: (a) six observed nonprotonated sp carbons (δ_C 77.8−82.9 ppm) and one terminal methine (δ_C 73.4 ppm; δ_H 2.82 ppm); (b) four sp^2 carbons (δ_C 128.9 d to 135.0 d ppm and δ_H 5.30−5.73 ppm) suggesting two disubstituted carbon−carbon double bonds; (c) eight methinoxy groups (δ_C 51.4 d to 76.4 d ppm, δ_H 3.48−4.93 ppm); and (d) a propargyl carboxylic group and a ketone that were recognized by carbon signals at 160 and 213 ppm, respectively. The relatively highfield position of several of the eight methinoxy carbon resonances suggested that they were situated at allylic and propargylic positions.

Four out of five partial structures (**a**−**d**) as depicted in Figure 1 were readily deduced based upon the above data as well as COSY and HMBC correlations (Figures 2 and 3).

COSY correlations between two adjacent olefinic protons (H-43 and H-44) and two vicinal methinols (H-42 and H-45) and correlations from the latter protons to an acetylene proton (H-47), on one side, and to two methylenes (H_2-40 and H_2-41), on the other end, suggested moiety **a** (Figure 2). The structure of **a** was further supported by the highfield chemical shift of C-45 (δ_C 61.4 ppm), which, thus, has to be propargylic.

The elucidation of the structure of moiety **b** began from methinol C-33, which, according to its high resonance (δ_C 51.4 ppm), has to be doubly propargylically situated. Proton-33 exhibited five-bond correlations through the acetylene bonds to methylenes 30 and 36. The latter had additional correlations to methinol H-37 and methylene H_2-38 (Figure 2).

The structure elucidation of moiety **c** started from the allyl alcohol segment H-26 to H-28, which could further be expanded to the vicinal CH_2-29 and methylenes 24 and 25, from the H−H COSY experiment (Figure 2). The structure of segment **d** was mainly based on the IR absorption at 1705 cm^{-1}, the proton chemical shift of the four α-protons H_2-13 and H_2-15 and HMBC correlations (Figure 3).

The assignment of the fifth partial structure (**e**) was less straightforward. H−H COSY correlations between three methinoxy protons indicated the presence of a 1,2,3-trihydroxy group (Figure 1). HMBC correlations established the connectivity between this trihydroxy moiety and a nonprotonated sp carbon (δ_C 80.6) whose counterpart sp carbon (constituting the triple bond) was not seen in the ^{13}C NMR spectrum, most likely because of a long relaxation time or overlapping with another sp carbon. As haliclonyne (**1**) possesses one acetylene terminus and a propargylic carboxylic group (δ_C 160 ppm) on the other end of the molecule, the trihydroxy functionality, next to a triple bond as deduced from the HMBC correlations (Figure 3), has to be connected to this fourth triple bond of the molecule (the other three triple bonds were already accounted for in moieties **a** and **b**). Additional evidence supporting the vicinity of the trihydroxy moiety to the acetylenic bond came from the lowfield shift of carbon 4 in the ^{13}C NMR spectrum (δ_C 63.8).

The structure of moieties **a**−**e** was confirmed by HMBC CH correlations summarized in Figure 3 and Table 1.

The connectivities between the partial structures **a**−**c** were determined based upon long-range correlations in the TOCSY spectrum. A correlation between hydrogens 37 and 42 connected fragments **a** and **b**. In a similar way, a correlation between hydrogens 28 and 30 afforded the connection between partial structures **b** and **c**. The above-discussed moieties count for 504 m.u., still requiring 22

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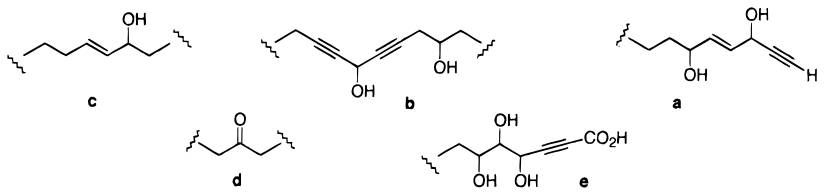


Figure 1. Five partial structures (**a**−**e**) of haliclonyne (**1**).

Table 1. NMR Data of **1**[a,b]

position	δ_C, ppm[c]	δ_H, ppm, mult	*J*, Hz	COSY (^{1}H−^{1}H)	TOCSY (^{1}H−^{1}H)	HMBC (C to H)
1	160 (s)					
2[d]						
3	80.6 (s)					4.47
4	63.8 (d)	4.47 (d)	3.2	3.38	3.38, 3.48	
5	76.4 (d)	3.38 (m)		3.48, 4.47	4.47	
6	72.0 (d)	3.48 (m)		1.25(7)[f], 3.38	1.25(7)[f], 4.47	
7	33 (t)	1.25 (m)		3.48		
8−12[e]	25−30 (t)	1.1−1.4 (m)				
13	42.3 (t)	2.29 (m)				1.40
14	213 (s)					2.29
15	42.3 (t)	2.29 (m)				1.40
16−24[e]	25−30 (t)	1.1−1.4 (m)				
25	31.7 (t)	1.9 (m)		1.25(24),[f] 5.45	3.75, 5.3, 5.45	5.45, 5.3
26	131.3 (d)	5.45 (dt)	15.5, 6.2	1.9, 5.3	1.9, 3.75, 5.3	1.35, 3.75
27	132.8 (d)	5.3 (dd)	15.5, 6.0	5.45, 3.75	3.75, 1.9, 5.45	1.9, 3.75, 1.35
28	72.3 (d)	3.75 (m)		1.35(29),[f] 5.3	5.3, 2.09, 1.9, 5.45	5.45, 5.3
29	36.5 (t)	1.35 (m)		2.09, 3.75	5.45	
30	18.3 (t)	2.09 (m)		1.35(29),[f] 4.93	4.93, 3.75	
31	77.8 (s)					4.93, 2.09
32	82.9 (s)					2.09
33	51.4 (d)	4.93 (m)		2.09, 2.24	2.09, 2.24	
34	79.9 (s)					4.93
35	80.6 (s)					3.59, 2.24
36	27.1 (t)	2.24 (m)		3.59, 4.93	3.59, 4.93	
37	69.3 (d)	3.59 (m)		1.40(38),[f] 2.24	1.40(38),[f] 2.24, 4.07	2.24
38	36.5 (t)	1.4 (m)		2.24		
39[e]	25−30 (t)	1.1−1.4 (m)				
41	36.5 (t)	1.4 (m)		4.07	4.07	
42	70.9 (d)	4.07 (m)		1.10(40),[f] 5.73, 1.40(41)[f]	1.10(40),[f] 5.73, 1.40(41),[f] 5.63, 4.7, 3.59	5.63, 5.73, 1.4
43	135.0 (d)	5.73 (dd)	15.4, 5.5	4.7, 5.63, 4.07	5.63, 2.47, 4.07, 1.40(41)[f]	4.7, 5.63
44	128.9 (d)	5.63 (dd)	15.4, 5.5	5.73, 4.7	5.73, 4.07	4.7, 5.73, 4.07
45	61.4 (d)	4.7 (d)	5.5	2.47, 5.63	2.47, 4.07	5.63, 2.47, 5.73
46	82 (s)					4.7, 5.63
47	73.4 (d)	2.47 (d)	2.1	4.7	4.07, 5.73	4.7

[a] $CDCl_3$, Bruker ARX-500 instrument, chemical shifts refer to TMS ($\delta_H = 0$). [b] $CDCl_3$, Bruker AMX-360 instrument, chemical shifts refer to $CDCl_3$ ($\delta_C = 77.0$). [c] Multiplicities were determined by DEPT and HMQC experiments. [d] Carbon not observed. [e] Overlapping methylenes. [f]Numbers in parentheses are of the corresponding methylene.

Table 2. MS Interpretation of the Fragmentations of Compound **1**

m/z	intensity (%)	fragment
812[a]	3	M
795[a]	2	M−OH
605[b]	15	M−[CH(45)OH−C≡C(47)H]−6×H_2O−CO_2
500[a]	7	M−[C_{1-13} + C_{45-47}]
330[a]	10	M−[C_{1-27}]−OH
223[a]	9	M−[C_{15-47}]−CO_2−H_2O
184[a]	100	H^+O=C_{14-25}
177[c]	100	[C_{2-13}]−2×H_2O

[a] Negative FAB (matrix: TEA). [b] Positive FAB (matrix: DTT/DTE). [c] Positive FAB (matrix: NBA).

Table 3. MS Interpretation of the Fragments of Compound **2**[a]

m/z	intensity (%)	fragment
452[b]	20	[C_{7-36}]
327[b]	58	[C_{15-36}]
191[b]	100	[C_{37-47}]
153[c]	30	[C_{1-6}]
109[c]	28	[C_{2-6}]

[a] Molecular peak was not seen. [b] DEI (100% = 6.6 × 10^6). [c] DEI (100% = 1.6 × 10^6).

Table 4. MS Interpretation of the Fragments of Compound **3**[a]

m/z	intensity	fragment
607[b]	17	[C_{6-45}]−3OH + H
577[b]	16	[C_{6-44}]−3OH
535[b]	15	[C_{8-44}][c]−3OH + H
472[b]	28	[C_{8-37}][c]
430[b]	100	[C_{6-33}]

[a] Molecular peak was not seen. [b]Positive FAB (matrix: DTT/DTE). [c] C6−C7 fragmentation due to McLafferty rearrangement.

methylenes to complete the molecular structure weight of 812. Haliclonyne is, therefore, of the general structure: [**a**−$(CH_2)_m$−**b**−$(CH_2)_n$−**c**−$(CH_2)_p$−**d**−$(CH_2)_q$−**e**] where m+n+p+q = 22. Two simple derivatives of **1** were prepared, to deduce the value of the indices m, n, p, q, namely, the Jones' oxidation product **2** and the $NaIO_4$ oxidative cleavage product **3**. The indices m, n, p, and q were determined to be 4, 1, 10 and 7, respectively, based on the analysis of the MS fragmentation of **1**, **2**, and **3** presented

in Tables 2, 3, 4, thus suggesting the planar structure of **1**.

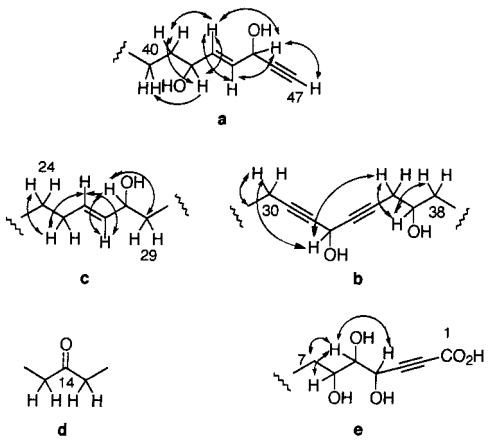


Figure 2. COSY correlations of moieties **a**−**e**.

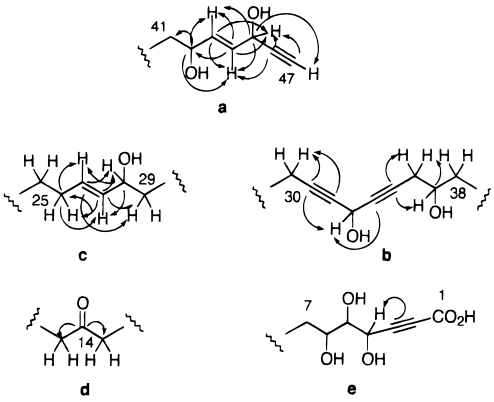
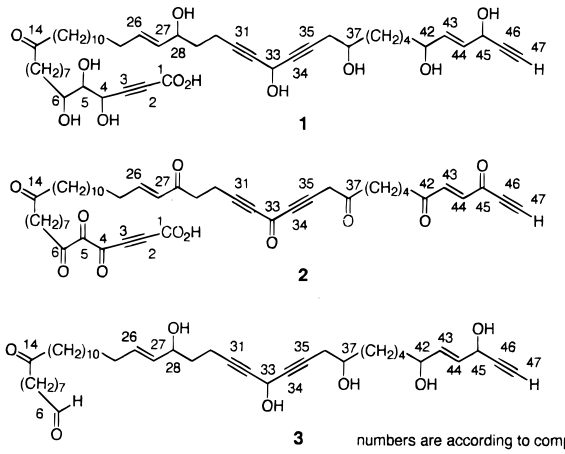


Figure 3. HMBC correlations of moieties **a**−**e**.



Key fragments in the mass spectra of compounds **1**−**3** were the ones at *m*/*z* 177, 191 and 330. Fragment *m*/*z* 177 in the positive FABMS of **1** clearly indicated that q = 7. The peak at *m*/*z* 191 in the DEIMS of **2** pointed to m = 4. Taking into account the q = 7 and m = 4 values, the peak at *m*/*z* 330, in the negative FABMS of **1**, implies that p = 10 and hence that n = 1. The latter values of m, n, p, and q are in full agreement with the other observed major fragments given in Tables 2−4.

As mentioned above, similarities were found between haliclonyne (**1**) and osirisyne F, another C_{47}-polyhydroxylated unsaturated acid.[18] Both compounds possess trihydroxypropargyl carboxylic acid and dihydroxyenyne termini and diyneol and allyl alcohol functionalities. However, the latter two functional groups are differently located, and there are other functionalities in osirisyne F.

Experimental Section

General Experimental Procedures. FABMS and DEIMS were recorded on a Fisons, Autospec Q instrument. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX-500 and AMX-360 spectrometers. All chemical shifts are reported in relation to TMS ($\delta_H = 0$) and $CDCl_3$ ($\delta_C = 77.0$). ^{1}H, ^{13}C, COSY, HMQC, TOCSY, and HMBC spectra were recorded using standard Bruker pulse sequences.

Animal Material. *Haliclona* sp. was collected at the Gulf of Eilat, Israel. The sponge *Haliclona* sp. was collected by hand using scuba (15−25 m depth) in July 1996, in the northern part of the Gulf of Eilat, the Red Sea. A voucher (3752) is deposited at Tel Aviv University. The collected sample was frozen immediately and kept at −20 °C until processed. The sponge fits best the *Haliclona* sp. described by Levi.[19]

Extraction. The freeze-dried sponge (8.5 g) was homogenized and extracted first with EtOAc and then with MeOH−$CHCl_3$ (1:1). The filtered EtOAc extract was evaporated, under reduced pressure, to give a brown gum (540 mg). This gum was subjected to partition by the method of Kupchan et al.[20] The chloroform fraction (130 mg) was chromatographed twice on a Sephadex LH-20 column, eluting with MeOH−$CHCl_3$ (1:1) to afford haliclonyne (0.08%).

Haliclonyne (1): an oil, IR (neat) 1705, 2100, 2250, 3300, 2450 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 360 MHz), see Table 1; FABMS, see Table 2.

Oxidation of 1 to 2. Compound **1** (2 mg) was dissolved in Me_2CO (5 mL) and one drop of Jones' reagent was added, at 0 °C. After 15 min the excess of the oxidant was destroyed by a drop of MeOH. The solvent was removed under vacuum and the residue dissolved in $CHCl_3$ (10 mL). This solution was washed repeatedly with water, saturated bicarbonate solution, and more water and the solvent removed under reduced pressure to afford **2** (R_f value 0.8, EtOAc). For DEIMS, see Table 3.

Reaction of 1 with Sodium Meta-periodate. To a stirred solution of **1** (2 mg) in CH_2Cl_2 (3 mL), saturated bicarbonate solution (0.5 mL) and a granule of $NaIO_4$ were added. After 2 h of stirring, the $NaIO_4$ was filtered out, and the solution was concentrated under vacuum to afford **3**. For FABMS data, see Table 4.

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