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HIGH MOLECULAR WEIGHT POLYACETYLENES FROM *PETROSIA FICIFORMIS*: FURTHER STRUCTURAL ANALYSIS AND BIOLOGICAL ACTIVITY

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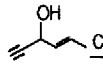
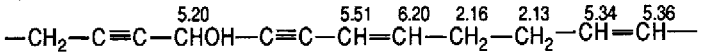
Abstract: The structures of the high molecular weight polyacetylenes from *Petrosia ficiformis* were investigated further. Moreover, their biological activity was examined for the first time and inhibition of sea urchin egg development as well as potent cytotoxic activity were observed.

Several examples of polyacetylenic compounds from marine sponges have been reported[1]. However, because of their extremely long alkyl chain, the polyhydroxy polyacetylenes from *Petrosia ficiformis*[2,3,4] represent an unique class of natural products which, for their structural complexity , have been only partially characterized. The recent suggestion[5] that these compounds could be inhibitors of the development of echinoderm fertilized eggs has prompted our interest in a reinvestigation of the *Petrosia* polyacetylenes, aiming at obtaining both further structural details and a preliminary understanding of their biological properties.

The t.l.c. analysis of the diethyl ether soluble fraction from the acetone extracts of freshly collected red sponges exhibited a chromatographic pattern similar but not identical to those previously described for the red[2] and the white[3] varieties of *P. ficiformis*. SiO_2 column chromatography yielded two main fractions (Rf=0.7 and Rf=0.3, petroleum ether/diethyl ether 1:1). Preparative HPLC (μBondapak C-18, CH_3OH:H_20 9:1) fractionated the most polar fraction into two components, **1** and **2**, with structural features typical of the metabolites found in the red sponge[2]. A similar purification step for the less polar fraction yielded the two C-46 metabolites (**3**,**4**) previously obtained from the white variety of the sponge[3]. The four compounds were named petroformyne-1 (**1**), petroformyne-2 (**2**), petroformyne-3 (**3**) and petroformyne-4 (**4**).

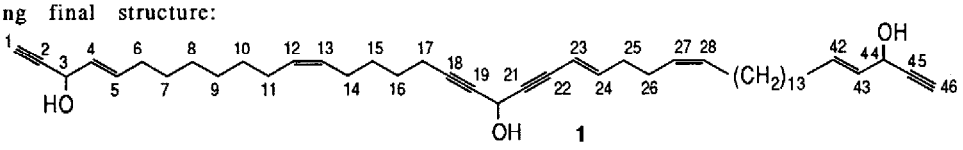
All the petroformynes yielded by reduction[2] the same saturated straight chain hydrocarbon $C_{46}H_{94}$. The spectral data[6] for the four compounds were substantially identical to those reported previously[2,3]. However, a series of ^1H-^1H homonuclear and ^1H-^{13}C heteronuclear 2D NMR experiments led to accurate assignments of almost all the resonances (Table 1). In particular, the analysis of the complex ^1H-NMR set of signals between 2.3 and 2.0 p.p.m. allowed more detailed insights into the structure of petroformyne-1, for which the following signals were observed: 2.22 p.p.m., 2 H, $\underline{CH_2}$-C≡C- (^{13}C at 18.7 p.p.m.); 2.16 p.p.m., 2 H , $\underline{CH_2}$ (^{13}C at 33.2

p.p.m.); 2.13 p.p.m., 2H,  (^{13}C at 26.3 p.p.m.); at 2.08 p.p.m., 4H,  (^{13}C at 31.8 p.p.m.) and, finally, at 2.02 p.p.m., 6H,  (^{13}C at 27.1 p.p.m.). Since the protons at 2.15 p.p.m. did not show, in the ^1H-^1H COSY spectra, any correlation with upfield methylene protons, the above assignments, bearing in mind the previous structural findings[2], led immediately to the partial structure **A**:

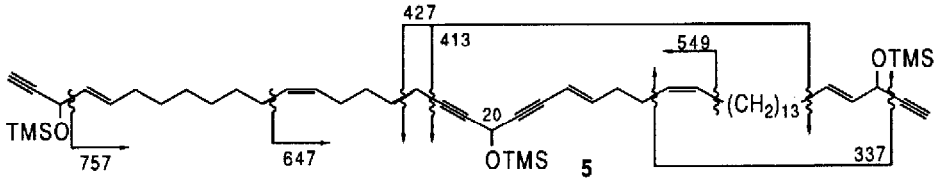


A

Therefore, of the two isolated olefinic double bonds, only one remained to be localized within the molecule. This was achieved by means of ozonolysis (-78°C, CH_2Cl_2), followed by oxydation with H_2O_2, methylation of the bicarboxylic acids with CH_2N_2 and analysis of the esters by GC/MS (Hewlett & Packard 5890, fused silica capillary column, cross-linked methyl silica, ID=0.2 µm, l=25 m) which revealed the presence in the molecule of alkyl chains containing 13, 6 and 4 methylenes. EIMS analysis of the trimethylsilyl (TMS) derivative of petroformyne-1 (**5**), revealing some highly diagnostic peaks (m/z at 337, 30%, 427, 30%, 549, 15%, 647, 15%), led to the following final structure:

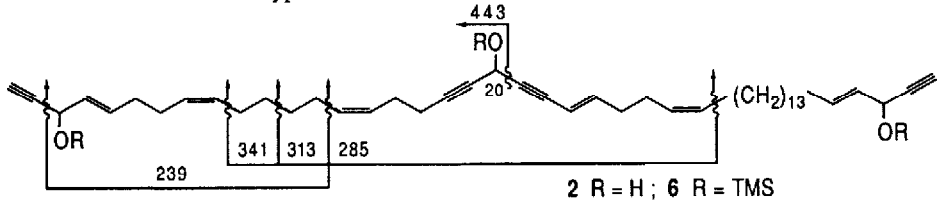


1



5

Petroformyne-2 (**2**) also contains partial structure A, but differs from **1** mainly for the presence of a further double bond. Moreover, in the ^1H-NMR spectrum (Table 1) the protons of the acetylenic methylene (2.30 p.p.m., ^{13}C 19.0 p.p.m.) and of one of the vinylic methylenes (2.28 p.p.m., ^{13}C 26.2 p.p.m.) were downshifted and without any correlation with upfield protons. Analogous shifts and coupling patterns were observed for one of the terminal vinylic methylenes (2.15 p.p.m., ^{13}C 31.7 p.p.m.) and for another of the vinylic methylenes (2.16 p.p.m.,^{13}C 26.5 p.p.m.) Also, the terminal acetylene signal at 2.58 p.p.m. was splitted into two signals, thus indicating that the molecule was no longer symmetrical with regard to the two terminal acetylenic functions. These data, together with the finding of 13 and 4 methylene-containing bicarboxylic acids by ozonolysis, led to suggest the structure **2** by analogy with **1**. The EIMS spectra of the TMS derivative **6** confirmed this hypothesis.



2 R = H ; **6** R = TMS

TABLE 1: Selected ^{1}H and ^{13}C NMR data for compounds 1-4[a]

	1		2		3		4	
n°	δ $^{1}H^{b}$	δ $^{13}C^{c}$	δ $^{1}H^{b}$	δ $^{13}C^{c}$	δ $^{1}H^{b}$	δ $^{13}C^{c}$	δ $^{1}H^{b}$	δ $^{13}C^{c}$
1	2.58	73.9*	2.53	72.9	2.57	74.0	2.58	73.8
3	4.83	62.9	4.82	62.6	4.84	62.8	4.84	62.8
4	5.62	128.6*	5.64	129.2	5.61	128.8	5.62	128.9
5	5.91	134.5	5.92	133.6	5.90	134.4	5.91	134.1
6	2.08	31.6*	2.15	31.7	2.08	31.8	2.08	31.7
7	1.40	28.4*	2.16	26.5	1.40	28.4	1.39	28.5
8	-	-	5.36	130.3*	-	-	-	-
9	-	-	5.42	131.1*	-	-	-	-
10	1.34	28.9	2.02*	27.0*	1.35	29.1	1.36	29.2*
11	2.02	27.2*	1.36	29.1*	2.02*	27.3*	2.02	27.2*
12	5.32	128.1	1.36	29.0*	5.34	129.9	5.35	129.6
13	5.38	131.1	2.02*	26.9*	5.36	130.3	5.40	130.1
14	2.02	27.10*	5.42	130.9*	2.00*	27.0*	2.02	27.1*
15	1.36	29.1*	5.36	130.2*	1.38	28.8*	1.36	28.8
16	1.52	28.0	2.28	26.2	1.55	28.2*	1.52	28.3
17	2.22	18.6	2.30	19.0	2.22	18.7	2.22	18.7
20	5.20	52.9	5.20	52.8	5.20	52.8	5.20	52.9
23	5.51	109.0	5.51	109.0	5.52	109.0	5.52	109.1
24	6.20	146.0	6.20	145.8	6.20	145.8	6.20	145.9
25	2.16	33.2	2.16	33.1	2.16	33.2	2.17	33.2
26	2.13	26.3	2.14	26.2	2.15	26.2	2.14	26.4
27	5.34	129.7	5.32	128.3	5.32	128.2	5.32	128.1
28	5.36	130.1	5.36	129.7	5.36	131.1	5.35	131.0
29	2.02	27.0*	2.03*	27.1*	2.02	27.2*	2.02	27.3*
30	1.36	29.2*	1.37	29.0	1.35	29.1	1.36	29.3*
40	1.40	28.5*	1.38	28.7	-	-	-	-
41	2.08	31.8*	2.08	31.8	-	-	1.42	28.6
42	5.91	134.5	5.88	134.7	1.38	28.7*	2.33	30.2
43	5.62	128.4*	5.62	128.5	1.55	28.1*	6.00	146.1
44	4.83	62.9	4.82	62.9	2.17	18.4	5.43	107.9
46	2.58	74.0*	2.57	73.9	1.92	68.2	3.07	81.1

[a] WM 500 Bruker Spectrometer; $CDCl_3$; TMS=0. Assignments deduced from the analysis of 2D homo- and hetero-nuclear spectra. Similar values belonging to the same structure and marked with an asterisk could be interchanged.

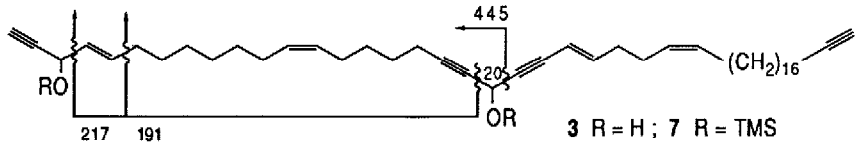
[b] All signals for not reported methylene protons contributed to a large signal at δ 1.26.

[c] Signals assigned to quaternary acetylene carbons were observed between δ 77.8 and 85.6; while the methylene values not reported in the table resonated between δ 29.8 and 29.0.

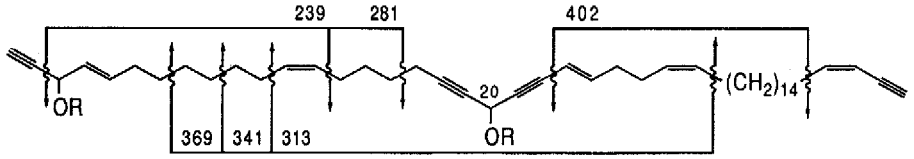
TABLE 2: Pharmacological actions of petroformynes 1-4 (1-4)

	1	2	3	4
A. salina cytotoxicity assay (LD50, p.p.m.)	0.0075	0.003	0.009	0.014
Sea urchin egg assay (50% inhibition, p.p.m.)	50	10	1	10

NMR data (Table 1) for petroformyne-3 (**3**) and petroformyne-4 (**4**) and degradative ozonolysis suggested structures, closely related to **1** also containing the partial structure **A**, which were confirmed by the analysis of the EIMS spectra of their TMS derivatives **7** and **8**. The fragmentation patterns were slightly different from the one shown by TMS petroformyne-1(**5**), probably because of the low stability of these molecules under EI conditions.



3 R = H ; **7** R = TMS



4 R = H ; **8** R = TMS

The four polyacetylenes were tested for cytotoxic activity by means of the *Artemia salina* bioassay and found to be among the most potent substances ever reported for this assay. Furthermore, these compounds inhibited the development of sea urchin fertilized eggs at concentrations ranging from 1 to 50 μg/ml (Table 2).

The findings reported here for the *P. ficiformis* metabolites raise intriguing questions about the biosynthesis and the biological role of these unusual polyacetylenes in both the sponge and its predator nudibranch *Peltodoris atromaculata*.

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5. N. Fusetani , in "*Bioorganic Marine Chemistry*", P.J.Scheuer Ed., **1**, 61, (1987).
6. $[\alpha]_D^{25}$ values for compounds **1**,**2**,**3** and **4** were, respectively, +12.5, +15.0, +10.0 and +6.0 ($CHCl_3$,10 mg/ml).

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