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BIOACTIVE MARINE METABOLITES III.[1] A NOVEL POLYACETYLENE ALCOHOL, INHIBITOR OF CELL DIVISION IN FERTILIZED SEA URCHIN EGGS, FROM THE MARINE SPONGE *TETROSIA* SP.

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Abstract: A new C_{30} linear polyacetylene alcohol, triaconta-4,15,26-triene-1,12,18,29-tetrayne-3,14,17,28-tetraol, has been isolated from the sponge *Tetrosia* sp. The compound inhibits mitotic cell division in sea urchin eggs.

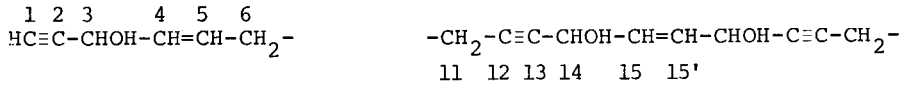
Marine sponges are highly prized by marine natural products chemists since many new compounds with diverse structural features[2,3] and biological activities[4,5] have been isolated during the last dacade. In the course of our continuing studies on bioactive marine metabolites, we found that the ether soluble part of the ethanol extract prepared from a marine sponge, *Tetrosia* sp.[6], collected at Hachijojima island, 300 km south of Tokyo, inhibits the cell division of starfish and sea urchin embryos. The active compound is an unusual polyacetylene alcohol, triaconta-4,15,26-triene-1,12,18,29-tetrayne-3,14,17,28-tetraol(1).

The frozen sponge (1 kg) was extracted with ethanol, and the extract was partitioned between ethyl acetate and water. The organic layer was subjected to TLC-mesh chromatography[7] over Kieselgel H with dichloromethane-ethyl acetate-methanol systems. The active fractions eluted with dichloromethane-ethyl acetate (4:1) and (1:1) were purified on a Lobar column (LiChroprep Si 60 size b, Merck) with dichloromethane-ethyl acetate (9:1). Repeated HPLC on LS-410 ODS (Toyo Soda) with aqueous acetonitrile gave a colorless oil, 230 mg; $[\alpha]_D$ +107° (*c* 0.7, $CHCl_3$).

The compound thus obtained was rather labile at room temperature but stable at -20°C. No ultraviolet absorption was observed above 220 nm. The presence of hydroxyl, disubstituted and terminal acetylene functions was inferred from IR (3300, 2250 and 2100 cm^{-1}) and ^{13}C NMR spectra (δ 62.5d, 58.4d, 86.3s, 79.8s, 83.4s and 73.9d). The ^{13}C NMR spectrum revealed three sp^2 carbons (δ 134.0d, 131.9d and 128.6d), which were also indicated by the IR absorption at 3030 cm^{-1}. Two allylic hydroxyl groups could be seen in the ^{13}C NMR spectrum [δ 62.5d (J_{C-H}=150.9 Hz) and 58.4d (J_{C-H}=146.5)]. In addition, six methylene carbons [δ 31.6t, 28.4t(4C) and 18.7t] were also observed, for a

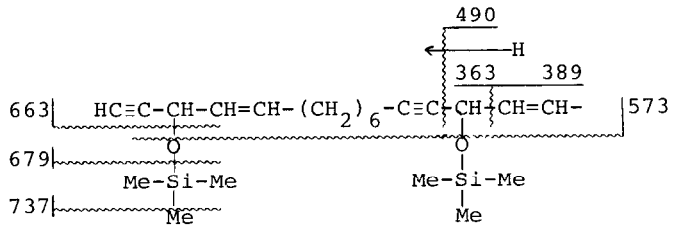
total of fifteen carbon signals. Considering the odd number of carbons, the absence of nitrogen atoms in the elemental analysis[8] and the $(M+H)^+$ ion peak in FDMS at *m/z* 465, it is reasonable to conclude that the molecule is symmetrical and that the molecular formula is $C_{30}H_{40}O_4$, which was also supported by the EIMS of the tetratrimethylsilylether 2 [*m/z* 752 (M^+)] and by the FDMS of the tetraacetate 3 [*m/z* 655 $(M+Na)^+$, 633 $(M+H)^+$]. Moreover, the linear structure of this substance was deduced by the ^1H NMR spectrum, which was augmented by extensive double resonance experiments (Table 1).

Irradiation of the terminal acetylene proton at δ2.60 (d, J=2.5Hz) collapsed a carbinol proton at δ4.85 (ddd, 1.4, 2.5, 6.8 Hz) to a double doublet. This proton at C-3 was in turn coupled to an olefinic proton (H-4) at δ5.62 (ddt, 15.4, 6.8, 1.4 Hz) and allylically to the other olefinic proton (H-5) at δ5.90 (ddt, 15.4, 1.4, 6.7 Hz). The coupling constant of 15.4 Hz between H-4 and H-5 indicated *E* configuration. Proton H-4 was also coupled allylically to the C-6 methylene protons at δ2.08 (m), which were also coupled to the olefinic H-5 proton at δ5.90. These results were consistent with the partial structure A for a terminal portion of the molecule.

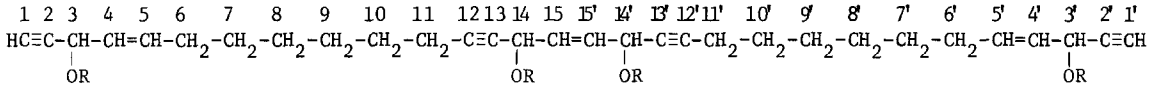


A B

The third olefinic proton (H-15) at δ5.69 (dd, 4.5, 1.4 Hz) was coupled to the carbinol proton H-14 at δ5.27 (ddt, 4.5, 1.4, 2.2 Hz), which was also coupled to methylene protons (C-11) at δ2.21 (dt, 2.2, 7.0 Hz) through an acetylenic bond. This assembly comprised partial structure B for the central part of the molecule. The remaining four methylenes must be accomodated between these partial structures to make up the linear molecule 1. This structure was compatible with EIMS data of the trimethylsilylated derivative as shown below, and with ^1H and ^{13}C NMR data for the tetraacetate (Table 1).



The configuration of the hydroxyls and the geometry of the central C-15,15' olefin are not yet clear.



1: R=H
2: R=TMS
3: R=Ac

Table 1. ^{1}H and ^{13}C NMR Shifts for 1 and 3

	1		3	
C	^{1}H ($CDCl_3$)[a]	^{13}C($CDCl_3$)[b]	^{1}H ($CDCl_3$)[c]	^{13}C($CDCl_3$)[b]
1	2.60 (d, 1H, J=2.5 Hz)	73.9d	2.57 (d, 1H, 2.4)	74.7d
2		83.4s		79.9s
3	4.85 (ddd, 1H, 6.8, 2.5, 1.4)	62.5d	5.80 (ddd, 1H, 6.2, 2.4, 1.0)	64.0d
4	5.62 (ddt, 1H, 15.4, 6.8, 1.4)	128.6d	5.52 (ddt, 1H, 15.2, 6.2, 1.3)	124.6d
5	5.90 (ddt, 1H, 15.4, 1.4, 6.7)	134.0d	6.02 (ddt, 1H, 15.2, 1.0, 6.2)	136.9d
6	2.08 (m, 2H)	31.6t	2.08[f]	31.9t
7	1.41 (m, 2H)			
8	1.32 (m, 2H)[d]	28.4t	1.25-1.50 (m, 8H)	28.5t
9	1.38 (m, 2H)[d]			
10	1.51 (m, 2H)			28.2t
11	2.21 (dt, 2H, 2.2, 7.0)	18.7t	2.21 (dt, 2H, 2.0, 7.0)	18.8t
12		79.8s[e]		76.2s[g]
13		86.3s[e]		87.5s[g]
14	5.27 (ddt, 1H, 4.5, 1.4, 2.2)	58.4d	6.24 (ddt, 1H, 5.5, 2.0, 2.0)	60.3d
15	5.69 (dd, 1H, 4.5, 1.4)	131.9d	5.65 (dd, 1H, 5.0, 2.0)	129.3d
OAc			2.07 (s, 3H)	169.2s
			2.09 (s, 3H)	169.6s
				21.0q(2C)

a: 400 MHz, b: 25 MHz, c: 100 MHz

d, e, g: Assignments may be interchanged

f: The signal overlapped with the acetoxyl methyl signals

All attempts at chemical degradations including Pd-C hydrogenation, and Jones, DDQ, PDC-DMF[9] and MnO_2 oxidation were unsuccessful because of the instability of the molecule.

Compound 1 inhibits the cell division of fertilized sea urchin *Pseudocentrotus depressus* eggs at a concentration of 1 μg/mL.[10] It is also active against *Penicillium chrysogenum*.

Several polyacetylenes have been isolated from sponges,[11-13] molluscs[14] and seaweeds.[15] However, no biological activity has been reported for any of these. It is likely that our compound participates in the defense mechanism of this sponge.

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