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TOXIC ACETYLENE-CONTAINING LIPIDS FROM THE RED MARINE ALGA *LIAGORA FARINOSA* LAMOUROUX[1]

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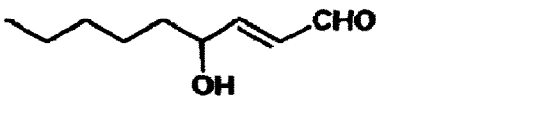
Summary: Four new acetogenins, three of which contain acetylene functionalities, have been isolated from the red seaweed *Liagora farinosa* from both the Pacific Ocean and the Caribbean Sea. Two of these compounds are lethal, at the 5-8 µg/ml range, to the reef-dwelling fish, *Eupomacentrus leucostictus*.

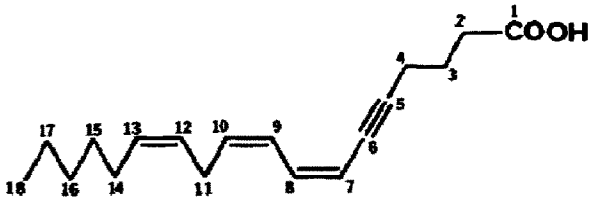
Acetogenins with acetylene functionalities are common components of terrestrial plants, and they are particularly prevalent within the Compositae[2]. In the marine environment, acetylene-containing compounds are more rare, but some have been found in sponges[3], marine pigments[4], and in green algae[5]. In the red algae, the genus *Laurencia* is recognized to produce a group of cyclic ethers with terminal enyne functional groups[6]. We wish to report the isolation and structure elucidation of several acetylene-containing lipids, **1**-**4**, from the tropical red alga *Liagora farinosa* Lamouroux (Nemaliales, Helminthocladiaceae). The aldehyde **1**, and the major metabolite **2**, show unexpected acute toxicity toward the reef-dwelling fish *Eupomacentrus leucostictus*[7], at 8 µg/ml and 5 µg/ml concentrations, respectively. At 10 µg/ml, the hydroxyacid **3** showed chronic toxicity after 24 hours. The monoglyceride **4** was not toxic in this assay.

L. farinosa was collected both from Carrie Bow Cay, Belize (Caribbean Sea), and Los Frailes Bay, Baja California (Pacific Ocean), in early 1979, and transported to La Jolla in ethanol. The samples were subsequently extracted with chloroform and the reduced extract chromatographed over silica gel. Further purifications of column fractions by HPLC (silica gel) led to the isolation of **1**-**4**, in increasing order of polarity. Both the Caribbean and Pacific collections gave identical yields of these metabolites as 18% of the extracts (2% dry wt. yield).

4-Hydroxy-2E-enal (**1**), $C_9H_{16}O_2$ by mass spectral analysis, was isolated as 1% of the extract by HPLC (40% EtOAc/trimethylpentane(TMP)), and showed $[\alpha]_D$ 0° (c 1.1, $CHCl_3$). The presence of unsaturated aldehyde and hydroxyl groups in this molecule were indicated by IR absorptions at 1680 and 3500 cm^{-1}, and the former functional group gave rise to UV absorption at 217 nm (ε 12,000, MeOH). ^1H and ^{13}C NMR data (Tables 1 & 2) were informative in the assignment of structure **1**. Coupling of 5 Hz between the C-3 olefin and the C-4 methine proton securely placed the hydroxyl group at C-4.



1



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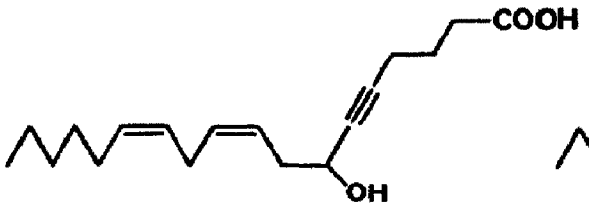

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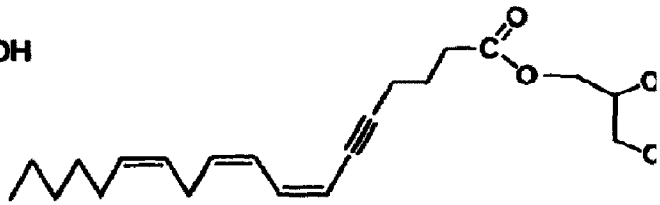

4

Octadec-5-yne-7Z,9Z,12Z-trienoic acid (2), the major metabolite of L. farinosa (10% extrac was isolated by HPLC (40% EtOAc/TMP). Treatment of 2 with CH_2N_2 yielded the corresponding met ester which analysed for $C_{19}H_{28}O_2$ by high resolution mass spectrometry (M^+ m/e 288.2072, 1.9% d from calc.). Hydrogenation (Pt/Et_2O) yielded methyl octadecanoate, which was identical with a commercial sample. The six degrees of unsaturation for 1 were accounted for by one acid carbon group, three olefinic bonds, and one non-terminal acetylene group, as determined by IR $\gamma_{C=O}$ 17 cm^{-1}, and ^{13}C and ^{1}H NMR (Tables 1 & 2). UV absorption at 273 nm (ε=29,000) indicated a triene diene-yne constellation, and the latter case was confirmed by extensive spin decoupling experim Specifically, the protons of the C-9 through C-13 homoconjugated diene system were readily inte related as in Table 1. Hence, the chromophore in question must arise from the conjugation of o these double bonds with a conjugated enyne group. The placement of the acetylene function at C C-6 was deduced by sequential decoupling experiments beginning by relating C-2 through C-4 and through C-13. Olefin coupling constants of 11 Hz or less indicated an all Z olefin geometry.

7-Hydroxy-5-yne-9Z,12Z-dienoic acid (3), was isolated as 5% of the extract by HPLC (75% Et TMP). The acid showed $[\alpha]_D$ 6.8° (c 1.5, $CHCl_3$), and analysed for $C_{18}H_{28}O_3$ by mass spectrometry The 5 degrees of unsaturation in this formula were accounted for by one acid carbonyl, one acet functionality, and two olefinic bonds. The lack of UV absorption illustrated that the diene-yn chromophore was not intact. Acetylation (Ac_2O/py) yielded a monoacetate, the proton NMR charac istics of which showed it to be a 2° acetate (a shift δ4.39, 1H, dt(6,1)⟶δ 5.50). As in 2, plete ^{1}H NMR decoupling studies allowed the structure assignment as in 3 (Table 1). The C-9 - homoconjugated diene was found to be intact, and flanked by a methylene group at C-8. The C-8 methylene was coupled to the alcohol methine, hence the hydroxyl group was securely placed at C As with 2, ^{13}C NMR features were in strong support of the assigned structure.

Table 1. 220 MHz ^{1}H NMR Data for 1-4. Assignments made by decoupling experiments. ($CDCl_3$)

C#	1	2	3	4
1	9.57 d(8)	—	—	—
2	6.35 dd(16,8)	2.50 m	2.50 m	2.52 m
3	6.84 dd(16,5)	1.80 m	1.85 m	1.90 m
4	4.44 dd(10,5)	2.50 m	2.31 dt(6,1)	2.52 m
5	1.62 dd(10,7)	—	—	—
6	1.3 m	—	—	—
7		5.47 dd(11,11)	4.39 dt(6,1)	5.44 d(11)
8		6.64 dd(11,10)	2.50 m	6.40 dd(11,10)
9	0.90 t(6)	6.59 dd(10,10)	5.45 m	6.66 dd(10,10)
10		5.57 dd(10,7)	5.55 m[a]	5.55 dd(10,7)
11		2.95 dd(7,6)	2.82 t(7)	2.95 dd(7,6)
12		5.35 dd(10,6)	5.35 m[a]	5.39 m
13		5.37 dd(10,7)	5.45 m	5.39 m
14		2.10 m(7)	2.02 m	2.08 m
15		1.30 m	1.30 m	1.31 m
16				
17				
18		0.90 t(6)	0.87 t(7)	0.87 t(7)
1'		—	—	4.20 m
2'		—	—	3.90 m
3'		—	—	3.65 m

Table 2. 20 MHz ^{13}C NMR Data for 1-4. Assignments aided by off-resonance decoupling. ($Bz-d_6$)

C#	1	2	3	4				
1	191.9	180.0	178.8	173.0				
2	129.8	33.0	31.9	33.0[a]	12	131.2[b]	128.0[a]	131.2[c]
3	158.0	26.7[a]	26.4	26.7[b]	13	127.2[b]	124.9[a]	127.2[c]
4	69.9	19.2	18.6	19.3	14	27.6[a]	27.7[b]	27.6[b]
5	35.6[a]	79.2	84.1	79.2	15	29.0[a]	29.8[b]	29.6[b]
6	31.0[a]	96.2	82.8	96.2	16	31.8[a]	31.9	31.8[a]
7	24.2[a]	110.0	64.2	110.0	17	22.9	23.0[b]	22.2
8	21.9	131.1[b]	36.6	133.1[c]	18	14.9	14.4	14.2
9	13.3	134.2[b]	131.6[a]	134.2[c]	1'			65.2[d]
10		126.3[b]	130.7[a]	126.3[c]	2'			70.6
11		24.0[a]	24.6[b]	24.3	3'			63.7[d]

a-d assignments may be intermixed

Glyceryl octadec-5-yne-7Z,9Z,12Z-trienoate (4), was isolated as 2% of the extract, and anal ysed for $C_{21}H_{32}O_4$ by mass spectrometry. The six degrees of unsaturation, the highly comparable and ^{13}C NMR features of this metabolite, and its UV absorption at 273 nm (22,000), closely paral ing that of 2, suggested 4 was the glycerol ester of 2. Further, 4 showed $[\alpha]_D$ -1.5° (c 0.5, CH which indicated it was the 1° monoglyceride. To confirm the optical activity, 4 was hydrogenate yield glyceryl octadecanoate, and this glyceride showed $[\alpha]_D$-5.0° (c 0.5, $CHCl_3$). Acetylation ((Ac_2O/py) yielded a diacetate, the ^1H NMR features of which confirmed the C-1' site of initial esterification.

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