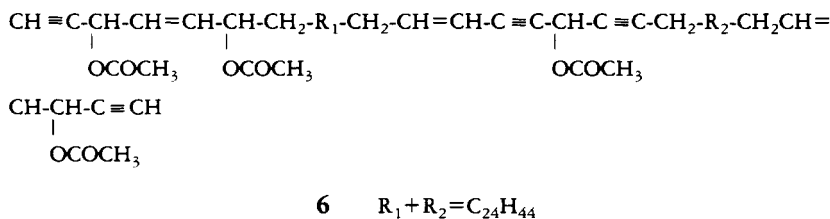
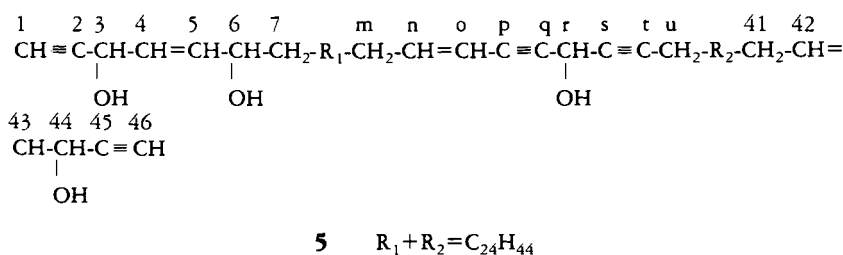
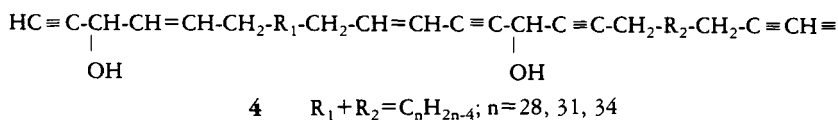
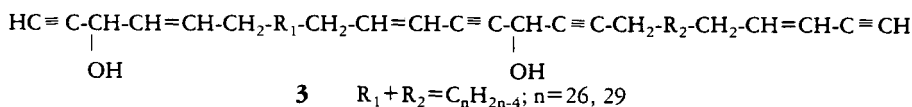
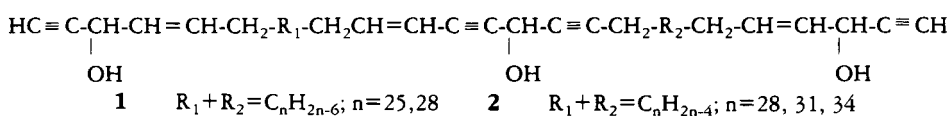


FURTHER HIGH MOLECULAR WEIGHT POLYACETYLENES FROM
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ABSTRACT.—Two further high molecular weight polyacetylenes have been isolated from the sponge *Petrosia ficiformis*. One of these new compounds, (5), shows a C₄₆ straight chain and four hydroxyl groups; the second, isolated as the methyl ester (7), is a C₄₃ straight chain enynoic acid.

Chemical studies (1) on the relationship between the Mediterranean red sponge *Petrosia ficiformis* Poirer and its predator, the nudibranch *Peltodoris atromaculata*, showed the presence in the sponge of two mixtures of unusual polyacetylenic metabolites (1 and 2) characterized by long, unbranched alkyl chains. Studies (2) on a white variety of the sponge, living in dark caves and, because of this, devoid of the red-colored symbiotic alga *Aphanocapsa feldmanni* (3), gave other polyacetylenes (3 and 4) related to, but different from, those of the red variety.

The finding of these unusual compounds stimulated further research on the polyacetylenic metabolites present in *P. ficiformis* in minor amount.

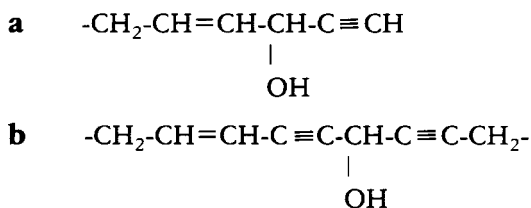


In this paper we report the partial structures of two new polyacetylenes found during a reinvestigation of the more polar metabolites from the ethereal extract of the red variety.

RESULTS AND DISCUSSION

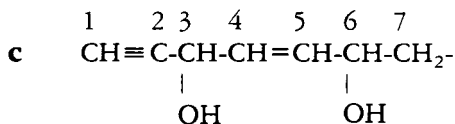
The sponge was extracted as previously (1) reported. Column chromatography on silica gel of the ethereal extract, eluting with light petroleum ether and increasing amounts of Et₂O, yielded, after the fractions containing the known polyacetylenes (**1** and **2**), two other acetylenic fractions, A and B.

The more abundant component (**5**, 0.002% dry weight of the sponge) of A, isolated by preparative hplc (μ Bondapak C-18; MeOH-H₂O, 8:2), showed considerable spectral analogies with the co-occurring polyacetylenes **1** and **2**. Compound **5** exhibited evidence for terminal (ir ν max 3295 and 2110 cm⁻¹) and disubstituted (ir ν max 2205 cm⁻¹) acetylenes, some of which were arranged in an enynic chromophore (uv λ max 232 nm). The nmr spectra were strongly reminiscent of those of **1** and **2**, displaying signals easily assignable to the partial structures **a** and **b**:



The spectra of the new compound showed other structural analogies with the previously reported molecules: the absence of terminal methyls, the presence of a long alkyl chain (strong signal at δ 1.26), and the presence of two disubstituted double bonds [¹H nmr δ 5.25 (bt, 4H), ¹³C nmr δ 130.8 (d), 130.0 (d), 129.5 (d), 128.0 (d)].

In addition, the nmr spectra exhibited signals attributable to the partial structure **c**:



Thus, the ¹H-nmr spectrum showed evidence of a second terminal acetylene proton (H-1, δ 2.56) long-range coupled with the proton (H-3, δ 4.85) of a vinylic carbinol group. The protons of the double bond (H-4 and H-5; complex system centered at δ 5.84) showed couplings both with the proton at δ 4.85 and with a signal at δ 4.16 (H-6, dt) due to the methine proton of the second vinylic alcoholic group, in turn coupled to a methylene group. The comparison of the ¹³C-nmr spectral data of the new compound with those previously reported for **1** and **2** showed that the presence of the additional hydroxy group (C-6, δ 71.6), destroying the terminal symmetry of the molecule, determined characteristic resonances for the carbons of the carbinols in **c** (C-1, δ 74.3; C-3, δ 61.6); the corresponding resonances in **a** are C-46, δ 73.9; C-44, δ 62.6.

In addition, the ¹³C-nmr spectra exhibited, according to the proposed partial structure **c**, signals at δ 135.4, 129.1, and 36.6.

The nmr assignments were confirmed by ¹H [¹H] and ¹³C [¹H] decoupling experiments and also by the nmr data of the acetyl derivative **6** of the new compound which showed the expected downfield shifts for the alcoholic methine protons and the follow-

ing significant ^{13}C -nmr shifts δ 64.0 (C-3), 136.9 and 124.4 (C-4 and C-5), 72.8 (C-6), 34.0 (C-7).

All the reported data are in accordance with a linear structure characterized by units **a** and **c** at the ends of the molecule linked by a long alkyl chain containing unit **b** and two double bonds.

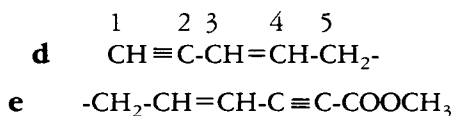
As interpretable mass spectra (ei, fab, ci) were not obtained, the length of the molecule was determined by hydrogenolysis of the acetyl derivative **6** to the parent hydrocarbon. Reduction with W2-Raney Ni yielded, as the main product, a straight chain hydrocarbon $\text{C}_{46}\text{H}_{94}$, identified by glc (8% Dexil 300 on Chromosorb AWDS).

On the basis of this evidence, we can suggest for the new compound the structure **5**, where the positions of the terminal units **a** and **c** can be reversed. It was not possible to localize the positions of the two double bonds in the chain units R_1 and R_2 , but, on the basis of the nmr data, the stereochemistry of all of them was established. The *trans* stereochemistry for the double bonds in the partial structures **a** and **b** was inferred by the ^1H -nmr coupling constants (15 Hz) of the olefinic protons. The same stereochemistry was suggested for the double bonds in **c** on the basis of the comparison of the ^{13}C -nmr data of the two terminal units. In fact, the resonances of carbons 3 and 44 were observed within 0.8 δ . This small shift, due only to the influence of the additional hydroxy group, excluded (4) the presence of differently oriented double bonds in the two units.

The *cis* orientation of the isolated double bonds in the units R_1 and R_2 was inferred from the analysis of the ^{13}C -nmr resonance in the zone of the vinylic methylenes. Only three signals assignable to methylenes were found downfield (δ 36.5, 33.5, 32.0) and were assigned to the vinylic methylenes of units **a** and **b** and to the methylene of unit **c**, while the remaining four resonances assignable to the carbons linked to the other double bonds were observed in the 28.0-26.4 δ zone as expected (4) with *cis*-oriented double bonds.

From the fraction B, after methylation, two components were recovered by silica gel chromatography. The more polar component analyzed by hplc was shown to be a complex mixture of at least seven compounds and is currently under structural investigation.

The less polar fraction yielded, after preparative hplc (μ Bondapak C-18; $\text{MeOH}-\text{CHCl}_3-\text{H}_2\text{O}$, 79:15:6), an ester (**7**; 0.001% dry weight of the sponge). The new compound showed spectral data suggesting the partial structures **d** and **e** at the ends of the molecule:



The monosubstituted acetylene, suggested by the ir spectrum (ν max 3295 cm^{-1}), was arranged in the terminal enyne **d** on the basis of some diagnostic nmr resonances (^1H nmr δ 3.04, H-1; 5.45, H-3; 5.98, H-4; 2.35, $-\text{CH}_2-$; ^{13}C nmr δ 81.1, C-1; 108.2, C-3; 146.1, C-4; 30.3, C-5) and of the uv spectrum (λ max 224 nm). Decoupling experiments confirmed the proposed partial structure. The ir spectrum also exhibited bands at 2200 and 1700 cm^{-1} , due to a disubstituted acetylenic group and to a conjugated carboxylic ester, respectively. The two groups were arranged as shown in the partial unit **e** on the basis of the other maxima in the uv spectrum (λ max 252 and 262 nm), strongly reminiscent of those of methyl hexa-*cis*-4-en-2-ynoate (5). The presence of the partial structure **e** in the new compound was completely confirmed by the nmr spectra showing, besides the expected resonances due to the carbomethoxy group

ANIMAL MATERIAL.—*P. fuciformis* was collected in the bay of Naples, Italy, and identified by Prof. M. Sarà of the University of Genoa.

ANIMAL EXTRACTION.—Fresh material (3 kg dry weight after extraction) was extracted with Me₂CO (3 × 10 liter); after concentration, the aqueous residue was extracted with Et₂O (3 × 200 ml). The combined ethereal extracts were taken to dryness, giving an oily residue (30 g).

FRACTIONATION OF THE EXTRACT.—The oil was chromatographed on a silica gel column using light petroleum ether (bp 40–70°) and increasing amounts of Et₂O. After the fractions containing the previously reported polyacetylenes (**1** and **2**), the tlc monitoring (SiO₂, Et₂O-light petroleum ether, 1:1) allowed combining the more polar material into two main fractions, A and B (Rf 0.4 and 0.25, respectively).

ISOLATION OF 5.—The fraction A (260 mg) yielded by preparative hplc (μ-Bondapak C-18, MeOH-H₂O, 8:2) a colorless oil (**5**, 60 mg, 0.002% dry weight of the sponge). The compound thus obtained showed the following spectral data: ir (liquid film) ν max 3295, 2205 and 2110 cm⁻¹; uv (MeOH) λ max 232 nm (ϵ 15,600) ¹H nmr δ (CDCl₃) 2.56 (H-1, d, J =2 Hz), 4.85 (H-3, dd, J =6 and 2 Hz), 5.84 (complex system, H-4 and H-5), 4.16 (H-6, dt, J =5 and 6 Hz), 6.20 (H-n, dt, J =16 and 6 Hz), 5.50 (H-o, d, J =16 Hz), the AB system due to the protons-n and -o was evidenced by irradiation at δ 2.25, 5.20 (H-r, s), 5.96 (H-42, dt, J =15 and 6 Hz, with irradiation at δ 2.10 the signal collapses in a doublet, J =15 Hz), 5.58 (H-43, dd, J =15 and 6 Hz), 4.85 (H-44, dd, J =6 and 2 Hz), 2.58 (H-46, d, J =2 Hz). Strong signals at δ 5.33 (olefinic protons), 2.10 (allylic protons) and 1.26 (methylene protons of an alkyl chain); ¹³C nmr δ (CDCl₃) 74.3 (C-1); 61.8 (C-3); 135.4 and 129.1 (C-4 and C-5); 71.6 (C-6); 36.6 (C-7); 33.3 (C-m); 145.3 (C-n); 109.2 (C-o); 52.4 (C-r); 18.7 (C-u); 32.0 (C-41); 128.5 (C-42); 134.1 (C-43); 62.6 (C-44); 73.9 (C-46); 80.6, 81.9, 82.6, 83.0, 84.2, 84.7 (acetylenic carbons); 130.8, 130.0, 129.6, 128.2 (carbons of the two double bonds in units R₁ and R₂); 29.7–28.6 (approximately 16 carbons, nonvinylic methylenes in units R₁ and R₂); 28.0, 27.3, 27.2, 26.4 (vinylic methylenes in units R₁ and R₂); ms, the mass spectra did not show interpretable fragments but exhibited only noise signals until ca. 500 mass units.

TREATMENT OF 5 WITH Ac₂O.—Compound **5** (50 mg) was refluxed in Ac₂O (2 ml)-pyridine (2 drops) for 20 min. The obtained product (**6**, 45 mg) was recovered by preparative tlc (SiO₂, light petroleum ether-Et₂O, 9:1, Rf 0.2).

SIGNIFICANT SPECTRAL DATA OF 6.—Ir (liquid film) ν max 1730 cm⁻¹; ¹H nmr δ (CDCl₃) 2.18 (CH₃-CO), all the methine protons of the alcoholic groups were down shifted in the δ 5–6 zone; ¹³C nmr δ (CDCl₃) 75.3 (C-1); 63.0 (C-3); 133.5 and 126.4 (C-4 and C-5); 72.8 (C-6); 34.0 (C-7); 33.2 (C-m); 146.1 (C-n); 108.7 (C-o); 54.0 (C-r); 18.6 (C-u); 32.0 (C-41); 124.4 (C-42); 136.9 (C-43); 64.0 (C-44); 74.6 (C-46); 169.9–169 (3-CO CH₃); 130.8, 130.0, 129.5, 128.0 (carbons of the double bonds in units R₁ and R₂); 86.1, 83.3, 81.9, 79.5, 78.7 (acetylenic carbons); 29.6–28.4 (approximately 16 carbons, nonvinylic methylenes in units R₁ and R₂); 27.9, 27.3, 27.2, 26.2 (vinylic methylenes in units R₁ and R₂).

HYDROGENATION OF 6.—A solution of **6** (40 mg) in MeOH (20 ml) containing W2—Raney Ni (10 mg) was stirred under an atmosphere of hydrogen for 2 h at room temperature. The catalyst was removed by filtration and the solvent evaporated *in vacuo* to yield a white solid (30 mg) which was purified by chromatography on a silica gel column and elution with light petroleum ether. In this manner, the saturated hydrocarbon (C₄₆H₉₄, 5 mg) was recovered and identified by glc by two differential 600 × 3 mm steel columns, 8% Dexil 300 on Chromosorb AWDS; the temperature was programmed from 250 to 360° at 5°/min, retention time 15.4 min. In the ¹H-nmr spectrum (CDCl₃) of the hydrocarbon, only two signals were present at δ 1.26 and 0.9.

ISOLATION OF 7.—The fraction B (400 mg), after methylation with CH₂N₂, gave two spots on tlc (SiO₂, Et₂O-light petroleum ether, 9:1, Rf 0.9 and 0.1, respectively). The mixture was separated by silica gel chromatography, the more polar fraction (200 mg) in hplc was shown to be a very complex mixture of at least seven products, while the less polar fraction (50 mg) after preparative hplc (μ-Bondapak C-18, MeOH-CHCl₃-H₂O, 79:15:6) yielded **7** as the major component (30 mg, 0.001% dry weight of the sponge).

SPECTRAL DATA OF 7.—The ms showed characteristic ions at m/z 630 (M⁺), 599 (M⁺-31), 571 (M⁺-59). The ir spectrum (liquid film) showed major absorption bands at ν 3295, 2200, and 1700 cm⁻¹; uv (CH₃OH) λ max 224, 252, and 265 (sh) nm, (ϵ 20,000, 16,000, and 5,000); ¹H nmr (CDCl₃) δ 3.04 (H-1, d, J =2 Hz), 5.45 (H-3, dd, J =10 and 2 Hz), 5.98 (H-4, dt, J =10 and 7 Hz), 2.35 (C-5 and C-38 hydrogens), 6.24 (H-39, dt, J =7 and 10 Hz), 5.55 (H-40, d, J =10 Hz), 3.81 (-OCH₃, s), 2.85 (2H, t, J =5 Hz) and also strong signals at 5.36 (olefinic protons), 2.05 (allylic protons), 1.28 (methylene protons). ¹³C nmr δ (CDCl₃) 81.1 (C-1), 108.0 (C-3), 146.1 (C-4), 30.3 (C-5), 31.0 (C-38), 151.4 (C-39),

106.5 (C-40), 52.8 (-OCH₃), 25.6 (C-p); 129.9-129.8 (approximately six carbons, olefinic carbons), 29.5-28.9 (approximately 21 carbons), 27.3-27.2 (approximately 4 carbons, vinylic methylenes).

HYDROGENATION OF **7**.—A solution of **7** (15 mg) in MeOH (10 ml) containing W2—Raney Ni (5 mg) was stirred under an atmosphere of hydrogen for 3 h at room temperature. After filtration, evaporation, and chromatography on silica gel column (light petroleum ether-Et₂O, 8:2) the straight chain fatty acid ester, CH₃(CH₂)₄₁COOCH₃ (10 mg), was recovered, *ms m/z* 648 (M⁺). ¹H nmr (CDCl₃) 3.68 (s, 3H), 2.30 (t, 2H), 1.25 (bm), 0.88 (t, 3H).

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