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Bioactive Polyacetylenes from the Marine Sponge *Petrosia* sp.

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Four new bioactive polyacetylenes have been isolated from the marine sponge *Petrosia* sp. Their structures including absolute stereochemistry have been elucidated by spectral and chemical methods.

Polyacetylenes represent a unique class of sponge metabolites and possess a variety of biological activities ranging from antimicrobial, cytotoxic to enzyme inhibitory activities.[1] During a search for biologically active constituents of marine invertebrates, we found that the methanol extract of a sponge of the genus *Petrosia*[2] collected from Sukumo Bay showed lethality to brine shrimp and inhibitory activity against cell division of fertilized ascidian eggs. Bioassay-directed fractionation of the extract led to isolation of five compounds, all possessing both activities. The dichloromethane soluble fraction of the extract was subjected successively to Sephadex LH-20 ($MeOH/CH_2Cl_2$ 1:1), silica gel (benzene/AcOEt), and reverse phase high-performance liquid ($MeOH/H_2O$) chromatographies to yield four new polyacetylenes, **1**-**4**, together with petrosynol (**5**)[3] in the yields of 0.0025%, 0.0018%, 0.0025%, 0.0024%, and 0.0076%, respectively. The present paper deals with the structural determination of these new compounds.

Compound **1** was isolated as an amorphous solid, $[\alpha]_D^{22}$ +10° (*c* 0.14, $CHCl_3$), and had IR absorptions indicative of the presence of hydroxyl (3600 and 3400 cm^{-1}), terminal acetylene (3300 cm^{-1}), and disubstituted acetylene (2220 cm^{-1}) groups. The molecular formula, $C_{30}H_{46}O_4$, was deduced by high resolution EIMS (*m/z* 470.3412, M^+, Δ +1.6 mmu). The ^{13}C NMR spectrum[4] assisted with INEPT experiments showed fifteen carbon signals attributable to nine methylene, two oxygenated methine, and four acetylene carbons. These data suggested that **1** had a symmetrical molecular structure as petrosynol (**5**).[3] This was also supported by ^1H NMR data[4] which showed signals due to one acetylenic proton [δ 2.47 (d, *J*=2.0 Hz)] and two carbinol protons [δ 4.37 (td, *J*=6.6 and 2.0 Hz) and 4.46 (m)]. Moreover, interpretation of the ^1H-^1H and ^1H-^{13}C COSY spectra and HMBC experiments revealed the presence of structural units **A** and **B** (Fig. 1). In unit **A**, a long-range coupling of 2.0 Hz was observed between the acetylenic proton at δ 2.47 and the carbinol proton at δ 4.37. The symmetrical unit **B** was established by an HMBC correlation between the methylene protons at δ 1.88 and the adjacent equivalent methylene carbon at δ 33.69 (C_{15} and C_{16} positions).

The absolute configuration of **1** was determined by the modified Mosher's method.[5] The validity of the method to the polyacetylenes from the sponge was confirmed by the application to petrosynol (**5**), the absolute configuration of which had been elucidated by the CD allylic benzoate method.[3] The (*S*) and (*R*)-MTPA esters

of **5** were prepared by treatment of **5** with (*S*) and (*R*)-MTPA acids and DCC/DMAP in dichloromethane. Assignment of the proton NMR signals was achieved by using the 2D-COSY spectra. The Δδ values (δ_S-δ_R) thus obtained are summarized in the structure **5a** (Fig. 2), indicating the same (*S*)-configuration for C_3, C_{14}, C_{17}, and C_{28} as that determined by the CD method. In the same manner, the MTPA derivatives of **1** were examined and the results are depicted in the structure **1a** (Fig. 2). On the whole, the signs of Δδ values for characteristic protons of **1a** are opposite to those of **5a**, showing the (*R*)-configuration for C_3, C_{14}, C_{17}, and C_{28}. Therefore, the structure of compound **1** is assigned as (3*R*,14*R*,17*R*,28*R*)-1, 12, 18, 29-triacontatetrayne-3,14,17,28-tetraol.

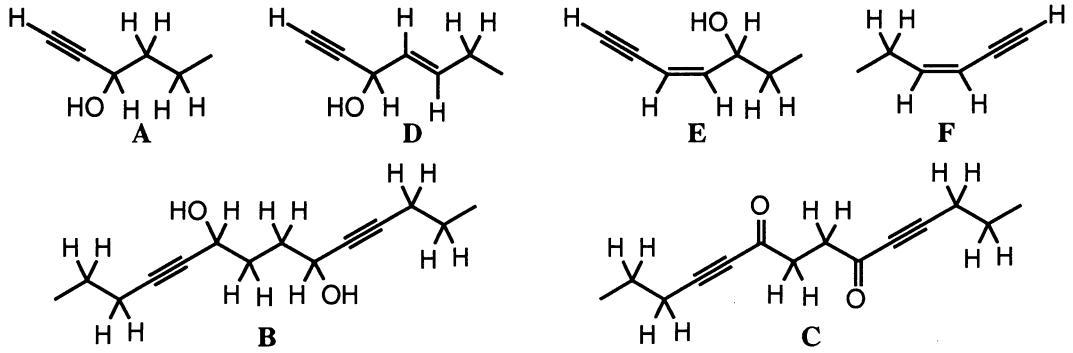


Fig. 1. Structural units established by ^{1}H-^{1}H COSY and HMBC experiments.

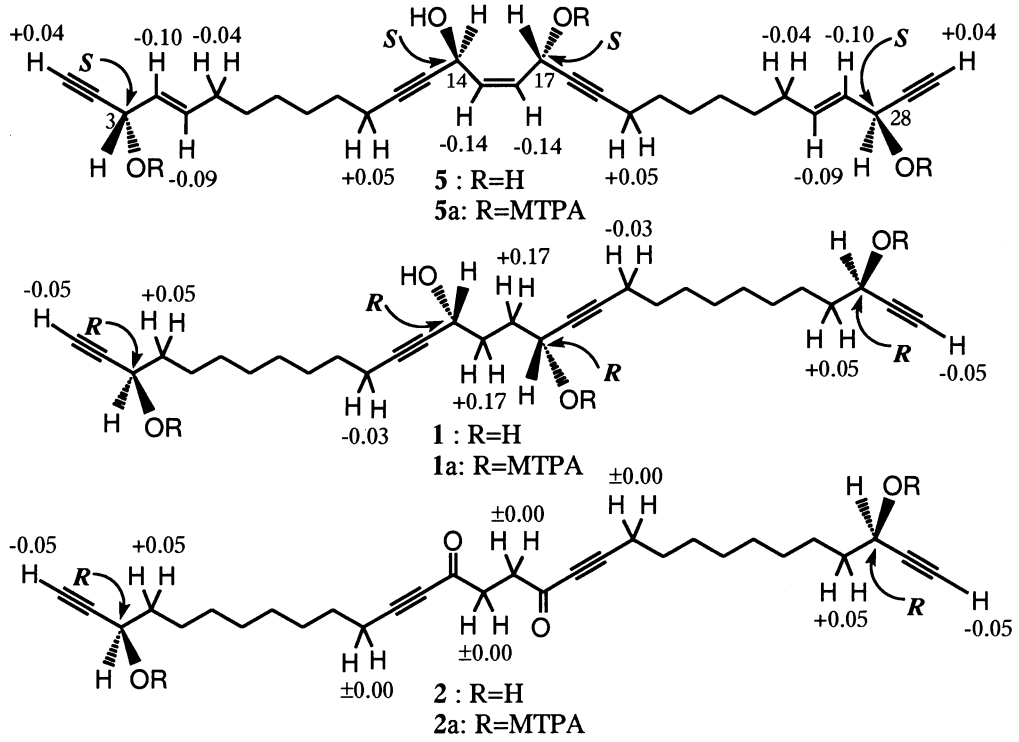
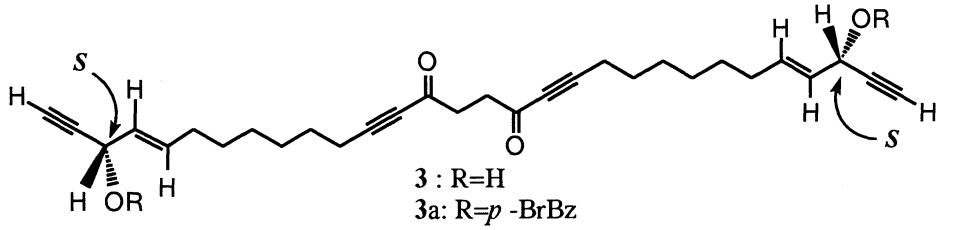


Fig. 2. Δδ values (ppm) obtained for the MTPA esters of petrosynol (**5**), and compounds **1** and **2**.

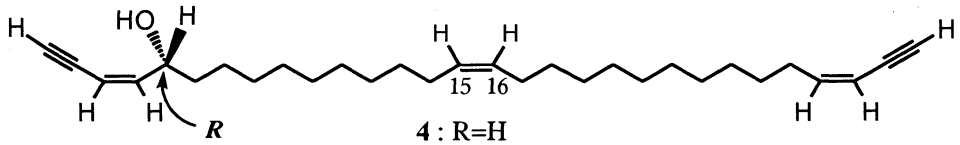
Compound **2** was obtained as a pale yellow oil, $C_{30}H_{42}O_4$ (HRFABMS: *m/z* 489.2971, M^++Na, Δ -1.0 mmu), $[\alpha]_D^{22}$ +22° (*c* 0.18, $CHCl_3$), IR ($CHCl_3$) 3600, 3400, 3300, 2200, and 1675 cm^{-1}, and showed spectral data similar to those of **1** except that the hydroxyl groups at C_{14} and C_{17} in **1** was replaced by the

ketonic groups (δ_C 185.47). The ^1H and ^{13}C NMR spectra[6] in combination with the ^1H-^1H COSY and HMBC experiments revealed the presence of the structural units **A** and **C**, the latter of which was also supported by UV absorptions at 224.8 sh and 220.8 nm (ε 28500 and 29400), suggesting the presence of conjugated ynones. The absolute configuration of **2** was also determined by the modified Mosher's method to be 3*R*,28*R*-configuration as shown in structure **2a** (Fig. 2). These evidence led to the straightforward assignment of the structure of **2** as (3*R*,28*R*)-3,28-dihydroxy-1,12,18,29-triacontatetrayne-14,17-dione.

Compound **3** was isolated as a pale yellow oil, $C_{30}H_{38}O_4$ (HRFABMS: *m/z* 485.2663, M^++Na, Δ -0.5 mmu), $[\alpha]_D^{22}$ +6.0° (*c* 0.12, $CHCl_3$), IR ($CHCl_3$) 3600, 3400, 3300, 2200, and 1680 cm^{-1}, UV (EtOH) 226.8 sh and 221.6 nm (ε 21100 and 21900). Inspection of ^1H and ^{13}C NMR spectra[7] of **3** and **2** established the close structural similarity of these two compounds. The difference between **3** and **2** resided solely in the presence of two trans-disubstituted double bonds [δ_H 5.60 (ddt, *J*=15.1, 6.4, and 1.5 Hz) and 5.89 (dtd, *J*=15.1, 6.8, and 1.5 Hz); δ_C 128.65 and 134.09] in **3**. ^1H-^1H COSY and HMBC experiments implied the presence of the structural unit **D** in addition to **C**, showing the location of the double bonds at C_4 and C_{27}. The absolute configuration of **3** was determined by the CD spectrum of the *p*-bromobenzoate **3a**, which was derived from **3** by treatment with *p*-bromobenzoyl chloride in pyridine, based on the exciton chirality method of allylic benzoate.[8] The UV spectrum of **3a** showed a *p*-bromobenzoate π-π* transition at 248.4 nm (ε 54500), in which region the CD spectrum showed a negative Cotton effect, λ_{ext} 248.6 nm, Δε -2.04 in ethanol, indicating a counterclockwise relationship between the double bond and *p*-bromobenzoate chromophores. Thus, the structure of **3** was assigned as (3*S*,28*S*)-3,28-dihydroxy-4,27-triacontadiene-1,12,18,29-tetrayne-14,17-dione.



3 : R=H
3a: R=*p* -BrBz



4 : R=H
4a: R=*p* -BrBz

Compound **4** was obtained as pale yellow oil, $C_{30}H_{48}O$ (HREIMS: *m/z* 424.3686, M^+, Δ -1.9 mmu), $[\alpha]_D^{20}$ -14° (*c* 0.28, $CHCl_3$), IR ($CHCl_3$) 3600, 3420, 3300, 2100, 1665, and 1615 cm^{-1}, UV (EtOH) 222.8 nm (ε 37300), and had spectroscopic properties somewhat different from those of **1**-**3**. The ^1H and ^{13}C NMR spectra[9] of **4** showed the presence of a secondary hydroxyl group [δ_H 4.67 (1H, brq, *J*=6.6 Hz); δ_C 70.12], together with two terminal acetylenes and three cis-disubstituted double bonds. Furthermore, ^1H-^1H COSY and HMBC experiments established two structural units **E** and **F**. These facts indicated that **4** has an unsymmetrical structure. The location of the isolated double bond at C_{15}-C_{16} was evident from the fragment ions at *m/z* 175 ($C_{13}H_{21}O^+$-H_2O), 177 ($C_{13}H_{21}^+$), 229 ($C_{17}H_{27}O^+$-H_2O), and 231 ($C_{17}H_{27}^+$). The absolute configuration of **4** was also determined by the CD spectrum of the *p*-bromobenzoate **4a**. It displayed a positive Cotton effect [λ_{ext} (EtOH) 243.4 nm (Δε +3.41)], so the absolute configuration of C_5 position of **3** was *R*. From the evidence outlined above, the structure of **4** was proposed as (5*R*)-3,15,27-triacontatriene-1,29-diyn-5-ol.

The cooccurrence of closely related polyacetylene alcohols with opposite stereochemistry, as described above, is of biogenetic interest. Compounds **2**, **3**, and **4** inhibited the cell division of fertilized ascidian (*Styela partita*) eggs with IC_{50} values of 30, 5.0, and 25 μg/ml,[10] respectively, and compounds **1**, **2**, **3**, and **4** displayed toxicity in the brine shrimp lethality bioassay (LC_{50}=30, 0.1, 0.3, and 5.0 μg/ml, respectively).[11]

References

1) N. Fusetani, H. Li, K. Tamura, and S. Matsunaga, *Tetrahedron*, **49**, 1203 (1993).

2) The sponge was identified by the late Dr. T. Hoshino, Mukaishima Marine Biological Laboratory, Hiroshima University, to whom we are grateful.

3) N. Fusetani, T. Shiragaki, S. Matsunaga, and K. Hashimoto, *Tetrahedron Lett.*, **28**, 4313 (1987).

4) **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23-1.42 (16H,m), 1.44 (4H, m, 5-, 26-H_2), 1.49 (4H, m, 10-, 21-H_2), 1.72 (4H, m, 4-, 27-H_2), 1.88 (4H, dt, *J*=15.1, 8.3 Hz, 15-, 16-H_2), 2.20 (4H, td, *J*=6.8, 2.0 Hz, 11-, 20-H_2), 2.27 (4H, brs, OH), 2.47 (2H, d, *J*=2.0 Hz, 1-, 30-H), 4.37 (2H, td, *J*=6.6, 2.0 Hz, 3-, 28-H), 4.46 (2H, m, 14-, 17-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.65 (C_{11}, C_{20}), 24.90 (C_5, C_{26}), 28.56 (C_{10}, C_{21}), 28.70, 28.87, 29.05, 29.26 (C_6-C_9, C_{22}-C_{25}), 33.69 (C_{15}, C_{16}), 37.60 (C_4, C_{27}), 62.23 (C_3, C_{28}), 72.83 (C_1, C_{30}), 80.87 (C_{13}, C_{18}), 85.09 (C_2, C_{29}), 85.88 (C_{12}, C_{19}).

5) I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, **113**, 4092 (1992).

6) **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31-1.40 (16H, m), 1.45 (4H, m, 5-, 26-H_2), 1.57 (4H, quint, *J*=7.1 Hz, 10-, 21-H_2), 1.70 (4H, m, 4-, 27-H_2), 1.98 (2H, brs, OH), 2.35 (4H, t, *J*=7.1 Hz, 11-, 20-H_2), 2.46 (2H, d, *J*=2.0 Hz, 1-, 30-H), 2.88 (4H, s, 15-, 16-H_2), 4.36 (2H, td, *J*=4.4, 2.0 Hz, 3-, 28-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.96 (C_{11}, C_{20}), 24.91 (C_5, C_{26}), 27.58 (C_{10}, C_{21}), 28.76, 28.84, 29.08, 29.22 (C_6-C_9, C_{22}-C_{25}), 37.60 (C_4, C_{27}), 38.93 (C_{15}, C_{16}), 62.26 (C_3, C_{28}), 72.83 (C_1, C_{30}), 80.54 (C_{12}, C_{19}), 85.01 (C_2, C_{29}), 95.31 (C_{13}, C_{18}), 185.47 (C_{14}, C_{17}).

7) **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29-1.44 (12H, m), 1.57 (4H, quint, *J*=7.1 Hz, 10-, 21-H_2), 1.74 (2H, brs, OH), 2.07 (4H, dd, *J*=14.2, 6.8 Hz, 6-, 25-H_2), 2.36 (4H, t, *J*=7.1 Hz, 11-, 20-H_2), 2.56 (2H, d, *J*=2.0 Hz, 1-, 30-H), 2.88 (4H, s, 15-, 16-H_2), 4.83 (2H, ddd, *J*=6.4, 2.0, 1.0 Hz, 3-, 28-H), 5.60 (2H, ddt, *J*=15.1, 6.4, 1.5 Hz, 4-, 27-H), 5.89 (2H, dtd, *J*=15.1, 6.8, 1.5 Hz, 5-, 26-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.93 (C_{11}, C_{20}), 27.50 (C_{10}, C_{21}), 28.44, 28.53, 28.59 (C_7-C_9, C_{22}-C_{24}), 38.92 (C_{15}, C_{16}), 62.72 (C_3, C_{28}), 73.98 (C_1, C_{30}), 80.57 (C_{12}, C_{19}), 83.33 (C_2, C_{29}), 95.21 (C_{13}, C_{18}), 128.65 (C_4, C_{27}), 134.09 (C_5, C_{26}), 185.47 (C_{14}, C_{17}).

8) N. C. Gonnella, K. Nakanishi, V. S. Martin, and K. B. Sharpless, *J. Am. Chem. Soc.*, **104**, 3775 (1982).

9) **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26-1.36 (28H, m), 1.40 (2H, m, 25-H_2), 1.53, 1.62 (1H each, m, 6-H_2), 1.74 (1H, brs, OH), 2.01 (4H, td, *J*=7.2, 4.6 Hz, 14-, 17-H_2), 2.32 (4H, ddd, *J*=8.2, 6.9, 1.2 Hz, 26-H_2), 3.05 (1H, d, *J*=2.1 Hz, 30-H), 3.12 (1H, d, *J*=2.4 Hz, 1-H), 4.67 (1H, dt, *J*=8.2, 6.6 Hz, 5-H), 5.34 (2H, t, *J*=4.6 Hz, 15-, 16-H), 5.43 (1H, ddt, *J*=10.9, 2.1, 1.2 Hz, 28-H), 5.52 (1H, ddd, *J*=10.9, 2.4, 0.6 Hz, 3-H), 5.98 (1H, dd, *J*=10.9, 8.2 Hz, 4-H), 6.00 (1H, dt, *J*=10.9, 8.2 Hz, 27-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.12 (C_{25}), 27.28 (C_{14}, C_{17}), 28.78, 29.22, 29.35, 29.46, 29.58, 29.64, 29.81 (C_7-C_{13}, C_{18}-C_{24}), 30.30 (C_{26}), 36.67 (C_6), 70.12 (C_5), 79.66 (C_1), 81.12 (C_{30}), 80.65 (C_2), 82.71 (C_{29}), 108.05 (C_{28}), 108.85 (C_3), 129.94 (C_{15}, C_{16}), 146.22 (C_{27}), 147.60 (C_4).

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