## BIOACTIVE MARINE METABOLITES XX<sup>1</sup>. PETROSYNOL AND PETROSYNONE, ANTIMICROBIAL C30 POLYACETYLENES FROM THE MARINE SPONGE PETROSIA SP.: DETERMINATION OF THE ABSOLUTE CONFIGURATION

N. Fusetani, \* T. Shiragaki, S. Matsunaga, and K. Hashimoto Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo (Japan)

Abstract: An antimicobial  $C_{30}$  polyacetylene alcohol and its tetraketo analog have been isolated from the marine sponge <u>Petrosia</u> sp. and their structures including absolute configuration determined by spectral and chemical methods.

Polyacetylenes have been known from several marine sponges and nudibranchs that prey on sponges.<sup>2</sup> In the course of our search for bioactive metabolites from Japanese marine invertebrates, we found that the crude ethanol extract of a marine sponge, genus Petrosia, was inhibitory against bacteria and fungi. The bioassay-directed purification of the ethanol extract has led to isolation of petrosynol and petrosynone, which were identified as the  $C_{3\Omega}$ polyacetylene alcohol 1 and its tetraketo analog 2, respectively. The absolute configuration of the former compound has been determined by the exciton chirality method. $^{3,4}$ 

The ether-soluble portion of the ethanol extract of the frozen sponge (3.3 kg), which had been collected by SCUBA (-15 m) in Hachijo-jima Island, Izu Archipelago, was fractionated by medium pressure chromatography on silica gel with benzene/ethyl acetate. The antifungal fraction was purified by reversed-phase HPLC (ODS, 70% MeOH) to afford 520 mg of  ${f l}$  as a colorless oil. The antibacterial fraction was repeatedly fractionated on silica gel with isooctane/i-PrOH (9:1) followed by silica gel HPLC (benzene-ethyl acetate, 19:1) to yield 30 mg of 2 as a yellowish oil.

Petrosynol (1) was not only inhibitory against Mortierella ramannianus but also active in the starfish egg asssay ( $IC_{QQ}$ ,  $l \mu g/mL$ ). It was identical in every respect to the polyacetylene alcohol which we had previously isolated from another Petrosia sponge.<sup>5</sup> However, the absolute configuration of four hydroxyl-bearing carbons and the geometry of the central double bond remained to be determined. Since our compound is optically active  $[\alpha]^{23}$  n +107° (c 0.37, CHCl<sub>3</sub>) and symmetrical, the following eight stereostructures are possible: (35, 14S, 15Z, 17S, 28S), (3S, 14R, 15Z, 17R, 28S), (3S, 14S, 15E, 17S, 28S), (3S, 14R, 15E, 17R, 28S), and the corresponding enantiomers. In order to determine the absolute configuration, we prepared the 3-benzoate  $(3)^6$  and 14-benzoate  $(4)^6$  by treating the alcohol with 1.2 eq. of benzoyl chloride and catalytic amounts of dimethylaminopyridine in pyridine, followed by HPLC separation.

Both benzoates revealed negative first Cotton effects at 228 nm(  $\Delta \epsilon$  -1.14° for 3 and  $\Delta \epsilon$ -3.7° for 4 ), indicating S configuration for C-3, C-14, C-17, and C-28.4 In view of the geometry of the  $\Delta^{15,16}$  double bond, introduction of a benzoate at C-14 made it possible to distinguish between C-15 and C-16 proton signals (\$ 5.73, dd, J=10.6, 8.4 Hz, 15-H; 5.82, dd, 10.6, 7.5 Hz, 16-H); thus Z geometry was secured for the  $\Delta^{15,16}$  double bond. Therefore, the



structure of I is (35, 145, 175, 295)-triaconta-1, 12, 18, 29-tetrayne-4E, 15Z, 26E-trien-3, 14, 17, 28-tetraol.

Petrosynone (2) showed antimicrobial activity against <u>Bacillus subtilis</u>. The UV [ $\lambda_{max}$ (MeOH) 261 nm ( $\epsilon$  6700)], IR (3250, 2250, 2140, and 1645 cm<sup>-1</sup>), EI mass (<u>m/z</u> 456, M<sup>+</sup>), and NMR  $spectra^7$  strongly suggested that 2 must be the tetraketo analog of 1. This was confirmed by chemical conversion of 1 to 2 with MnO<sub>2</sub> in CH<sub>2</sub>Cl<sub>2</sub>.

This is the first report of the complete elucidation of the stereochemistry of polyacetylenes isolated from marine organisms. Biosynthesis and roles of these acetylenes in sponges are interesting problems.

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- 1. Part 19. N. Fusetani, M. Sugano, S. Matsunaga, and K. Hashimoto, Experientia, submitted.
- D. J. Faulkner, <u>Nat. Prod. Rep</u>, 1984, 1, 551, 1986, 3, 1.
  N. C. Gonnella, K. Nakanishi, V. S. Martin, and K. B. Sharpless, <u>J. Am. Chem. Soc.</u>, 1982, <u>104</u>, 3775; N. Harada and K. Nakanishi, Circular Dichroic Spectroscopy---Exciton Coupling Grand Charles M. Markanish, Grand M. Grand M. Grand M. S. Charles Couperline Couperlin
- Tetrosia in this report should be corrected to Petrosia. 3: FABMS  $\underline{m/z}$  674 (MH + diethanolamine)<sup>+</sup>; IR (neat) 3400, 3250, 2250, 2140, 1730, 1600,
- 6. 1580, 1490 cm<sup>-1</sup>; UV (MeOH)  $\lambda_{max}$  230 nm ( $\epsilon$  11000); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.07 (2H, d), 7.59 (1H, t), 7.45 (2H, t), 6.09 (1H, ddt, 14.3, 1.0, 7.3, 5-H), 6.08 (1H, br d, 7, 3-H), 5.67 (1H, dd, 14.3, 7, 4-H). 4: FABMS m/z 674 (MH + diethanolamine)<sup>+</sup>; 1R (neat) 3400, 3250, 2250, 2140, 1730, 1600, 1580, 1490 cm  $^{-1};$  UV (MeOH)  $\lambda_{\rm max}$  230 nm (c 10000);  $^1{\rm H}$  NMR (400 MHz, CDCl<sub>3</sub>) & 8.07 (2H, d), 7.56 (1H, t), 7.44 (2H, t), 6.59 (1H, br d, 8.4, 14-H), 5.82 (1H, dd, 10.6, 7.5, 16-H), 5.73 (1H, dd, 10.6, 8.4, 15-H), 5.30 (1H, br d, 7.5, 17-H).
- 7. 2: <sup>1</sup>H NMR (400 MHz, CDCl<sub>2</sub>) § 7.14 (1H, dt, 15.8, 7.0, 5-H), 6.96 (1H, s, 15-H), 6.08 (1H, d, 15.8, 4-H), 3.14 (1H, s, 1-H), 2.36 (1H, t, 7.0, 5-H), 2.21 (1H, dt, 7.0, 7.0, 6-H), 1.6-1.1 (8H, m, 7-10-H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 177.6 s (C-3, C-14), 155.2 d (C-5), 142.4 d (C-15), 132.0 d (C-4), 97.4 s (C-2), 79.8 s (C-13), 79.1 d (C-1), 32.5 t (C-6), 28.6 t, 27.7 t, 27.4 t (C-7-10), 19.0 t (C-11).

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