Short Paper

Furospongolide, an Antifouling Substance from the Marine Sponge *Phyllospongia papyracea* against the Barnacle *Balanus amphitrite1**

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In oceanic subtidal and intertidal zones, fouling is a common phenomenon of serious economical and ecological significance. A variety of sessile organisms such as sponges, barnacles, and tunicates are involved in fouling. In theory, these invertebrates themselves are also subject to fouling by other invertebrate larvae and algae. In reality, however, many of these sessile animals remain surprisingly clean despite tremendous opportunities to be fouled by other organisms. There is a speculation that these organisms possess defense systems, and possibly excrete antifouling substances.¹⁻³ Under this hypothesis, we conducted a screening of antifouling substances in sponges using the assay of barnacle settling inhibition, and discovered a strong settling-inhibitor in the marine sponge *Phyllospongia papyracea*.

The sponge *P. papyracea* was collected in July 1989 off Kuchinoerabu Island, Kagoshima, Japan. The frozen specimen (200 g wet wt.) was ground and extracted with methanol. The extract was fractionated into *n*-hexane, carbon tetrachloride, chloroform, and 60% methanol fractions according to the Kupchan procedure.⁴ The two fractions of *n*-hexane and carbon tetrachloride showed activity. From the former fraction, fatty acids were isolated as active components.⁵ The latter fraction (200 mg) was separated by HPLC on TSK ODS-120T (0.76×60 cm) with a gradient of 70–85% acetonitrile in water by monitoring antifouling activity using the barnacle *Balanus amphitrite* larvae according to the previously reported method.⁵

Typically, a sample dissolved in a volatile solvent was applied around the circumference of a petri dish (Corning 35 mm), on which more than 90% of the larvae settled. After evaporation of the solvent, 5 ml of sterilized sea water and twenty cyprids were introduced. The petri dish was incubated at 25°C under dark conditions for two days. After counting attached and unattached larvae under a dissecting microscope, the settlement ratio was calculated as the percentage of total larvae which were attached to the dish. The purified compound (5.3 mg) inhibited 100% and 13% of the barnacle settlement at 100 and 50 µg/dish (ca. 20 and 10 µg/cm²), respectively. No lethal activity was observed during the two-day observation period at a level of 100 µg/dish.

The active compound had a molecular formula C₂₁H₂₈O₃ [HR-EIMS: (M⁺) *m/z* 328.2039, *d*+0.01 mmu]. The spectral data suggested the presence of an α,β-unsaturated γ-lactone (IR band at 1750 and 1780 cm⁻¹; ¹³C-NMR δ 174.3, 115.3, and 170.5)⁶ and a substituted furan (fragment ion in EIMS at *m/z* 67; ¹³C-NMR δ 142.5, 125.6, 110.0, and 138.8).⁷ These data together with 2D NMR experiments

including COSY, HMBC and NOESY led to the structure 1, furospongolide, previously reported from the Red Sea sponge *Dysidea herbacea*.⁸

The ¹H- and ¹³C-NMR spectral assignments are as follows: ¹H-NMR (CDCl₃, 400 MHz): δ 7.32 (1H, brs, H-1), 6.27 (1H, brs, H-2), 7.19 (1H, brs, H-4), 2.44 (2H, t, *J*=7 Hz, H-5), 2.23 (2H, q, *J*=7 Hz, H-6), 5.15 (1H, t, *J*=7 Hz, H-7), 1.58 (6H, s, H-9 and 14), 1.99 (2H, t, *J*=7 Hz, H-10), 2.08 (2H, q, *J*=7 Hz, H-11), 5.10 (1H, t, *J*=7 Hz, H-12), 2.03 (2H, t, *J*=8.5 Hz, H-15), 1.68 (2H, quin, *J*=8.5 Hz, H-16), 2.34 (2H, t, *J*=8.5 Hz, H-17), 5.83 (1H, s, H-19), 4.72 (2H, s, H-21). ¹³C-NMR (CDCl₃, 100 MHz): δ 142.5 (d, C-1), 110.0 (d, C-2), 125.6 (s, C-3), 138.8 (d, C-4), 25.0 (t, C-5), 28.3 (t, C-6), 123.9 (d, C-7), 135.5 (s, C-8), 16.0 (q, C-9), 39.5 (q, C-10), 26.4 (t, C-11), 125.6 (t, C-12), 133.4 (s, C-13), 15.7 (q, C-14), 38.8 (t, C-15), 25.2 (t, C-16), 27.8 (t, C-17), 170.5 (s, C-18), 115.3 (d, C-19), 174.3 (s, C-20), 73.0 (t, C-21).

C₂₁-Furanoterpenes mainly occur in the genera *Spongia*, *Hippospongia*, and *Phyllospongia*.⁹ As to the physiological function of these furanoterpenes in sponges, there are only few reports seen in the literature. One example is kurospongins from the Okinawan sponge, *Spongia* sp., which was reported to have a fish-deterrent activity.¹⁰ In an earlier paper, we reported the isolation of a mixture of fatty acids from *P. papyracea* as potent antifouling components.⁵ The barnacle anti-settling activity shown by 1 is stronger than the standard fatty acids mixture (Serdary Research Lab Inc.) which showed 50% inhibition at a concentration of 20 µg/cm². It is likely that the organism utilizes a combination of compounds, i.e. the furanoterpenoid together with fatty acids, to defend itself from fouling.

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