沖縄産海洋生物の含有する生物活性テルペノイドの化学的研究

Chemical study on bioactive terpenoids from Okinawan marine organisms.

Introduction

Marine organisms have proven to be valuable sources of structurally unique and biologically active secondary metabolites. Due to a geographical location, the Okinawa Islands have abundant marine invertebrates: sponges, octocorals (soft corals and gorgonians), ascidians (tunicates or sea squirts), bryozoans, molluscs (snails, nudibranchs, and sea hares), and zoanthus corals. Okinawa is one of the best places to search for novel marine secondary metabolites and plays a potential role in marine natural product chemistry.

In the course of study on chemical study on bioactive terpenoids from Okinawan marine organisms, we have investigated the soft corals, sponge and isolated and characterized forty-one terpenoids including seventeen new compounds.

Methods

Samples were collected around Okinawa Island, Japan. Different chromatographic techniques were used for isolation and purification of compounds. The structures were elucidated by spectroscopic analysis (1D and 2D NMR, IR and MS) and by comparison of their NMR data with those of reported values. The antimicrobial activity was carried out through paper disc diffusion method and cytotoxicity was examined using HTC116 cells or fertilized sea urchin eggs and anti-inflammatory effect in RAW264.7 macrophage cells.

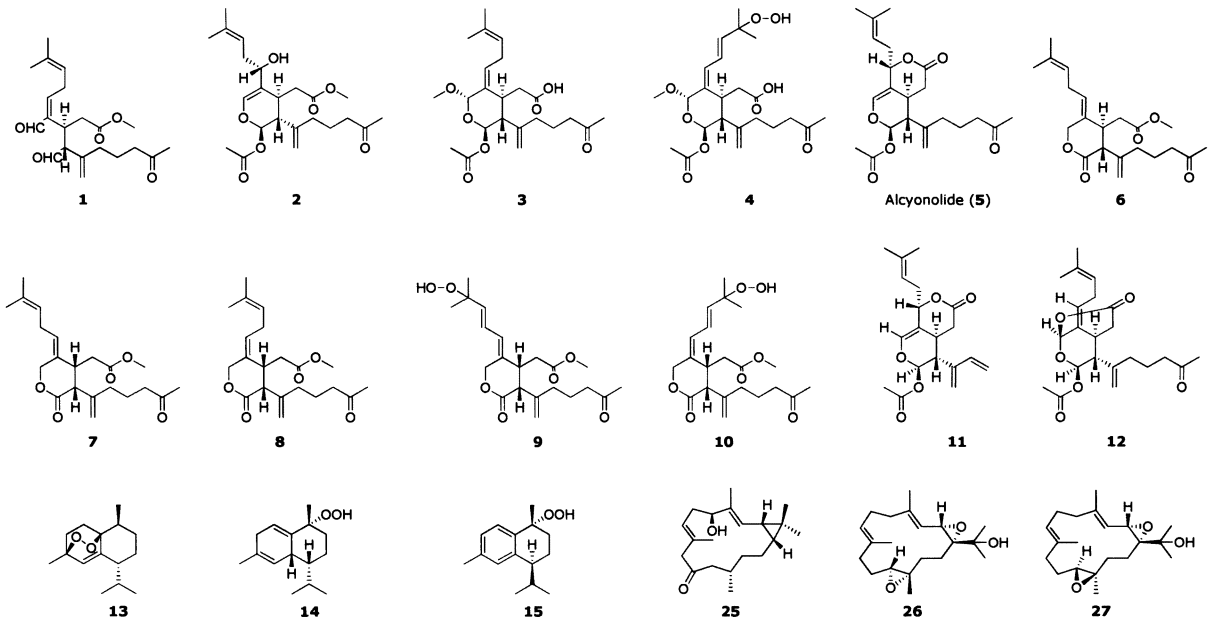


Figure: Structures of new compounds and known alcyonolide (**5**).

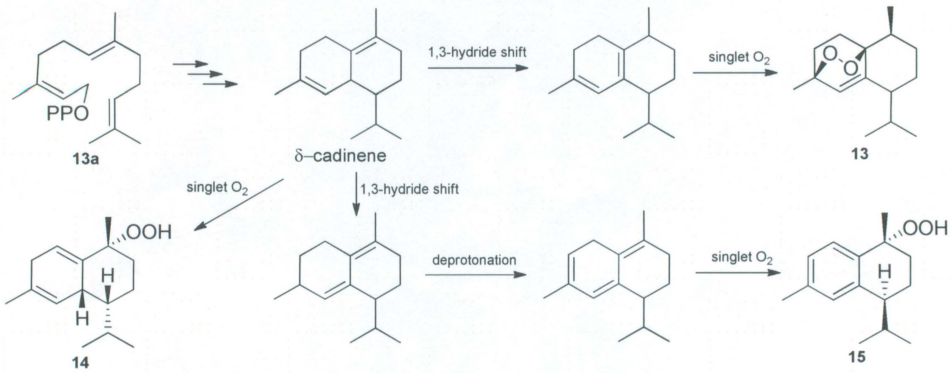
Results and Discussion

The soft corals *Cespitularia* sp. (3.0 kg and 2.9 kg) were collected from Zamami Island in March, 2011 and April, 2012. The first collection was extracted with acetone and partitioned between water and ethyl acetate. The ethyl acetate extract, which showed 80% inhibition of the first cleavage of fertilized sea urchin eggs at 20 μg/mL, was partitioned between aqueous MeOH and hexane. The aqueous MeOH extract was purified by column chromatography followed by normal-phase HPLC gave four new compounds **1**–**4** and the known alcyonolide (**5**). The extraction and fractionation of the second collection were conducted without using methanol to give seven new compounds **6**–**12** and alcyonolide (**5**).

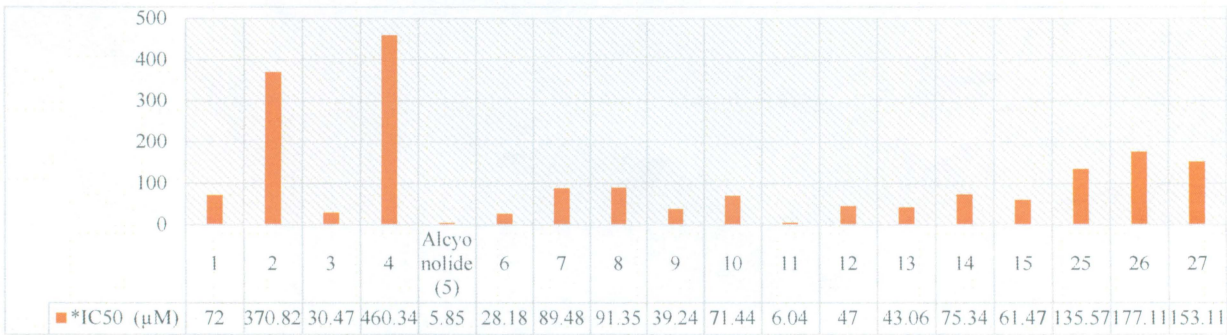
The soft coral *Sinularia* sp. (190.0 g) and *Lobophytum* sp. (220.0 g) were collected from Irabu Island, Okinawa in March 2013 and extracted with acetone. The acetone extract was partitioned between ethyl acetate and water. The ethyl acetate extract of *Sinularia* sp., inhibited the growth of *Staphylococcus aureus* and ethyl acetate extract of *Lobophytum* sp., inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*. Fractionation of the ethyl acetate part of *Sinularia* sp., by column chromatography followed by normal phase HPLC purification gave three new

compounds **13–15** and nine known terpenoids (**16–24**) and ethyl acetate part of *Lobophytum* sp. gave three new metabolites **25–27** and eight known cembrane-type terpenoids (**28–35**).

The sponge *Dysidea* sp. (2.6 kg, wet weight) was collected from Tarama Island, Okinawa in March 2013 and extracted with acetone and partitioned between H_2O and EtOAc. Fractionation of the ethyl acetate part by column chromatography followed by normal phase HPLC purification gave six known cyclic/spiro sesquiterpenes (**36–41**).



Scheme. Plausible biogenetic pathway of the isolated compounds (**13–15**).



*IC_{50}: 50 % cells were killed activity due to treatment compounds

Figure: Cytotoxicity on HCT116 cells of new compounds and known alcyonolide (**5**).

Conclusion

A total of forty-one terpenoids, which have diverse characteristics for carbon skeletons and cytotoxicities, were isolated from Okinawan marine organisms. Among those, seventeen compounds were new.

Twelve diterpenoids were isolated from the soft coral *Cespitularia* sp., including eleven new bioactive diterpenes (**1–4** and **6–12**) and a major known alcyonolide (**5**). The carbon framework of the isolated compounds (**1–12**) is corresponding to a seco-type variety of xenicin and dialdehyde **1** is a precursor-like compound of alcyonolide (**5**). A *Sinularia* sp. contained three new endoperoxy and hydroperoxy cadinene-type sesquiterpenoids (**13–15**), six known sesquiterpenoids (**16–21**) and three known cembrane diterpenoids (**22–24**). The biogenetic pathways of three new compounds (**13–15**) were proposed. Three new diterpenes (**25–27**) and including a rare casbene-type diterpene (**25**) were isolated from the Okinawan soft coral *Lobophytum* sp. together with eight known cembrane-type diterpenes (**28–35**). Six known cyclic/spiro sesquiterpenes (**36–41**) were isolated from the Okinawan sponge *Dysidea* sp.

The isolated metabolites **1–15**, and **25–27** showed cytotoxicity against HCT116 colon cancer cells with IC_{50s} in the range of 5.85–460 μM. Alcyonolide (**5**) was the most cytotoxic (5.85 μM). These compounds also showed anti-inflammatory activity in LPS/IFN-γ-stimulated inflammatory RAW 264.7 macrophage cells.

Compounds **13–15**, **25–29** and **36–41** were evaluated for antibacterial and/or antifungal activity. Compound **29** showed good antibacterial activity against *Escherichia coli* and compounds **36**, **38** and **41** showed potent antifungal activity against *Trichophyton rubrum* which is one of the most common causes of chronic tinea pedis commonly known as athlete's foot.