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Bioactive compounds from marine sponges

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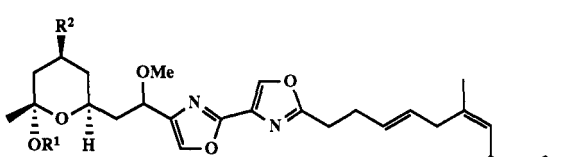
Abstract: Several marine sponges from Okinawan waters have been examined for bioactive compounds. Further investigation with *Polyfibrospongia* sp. which have already yielded novel metabolites gave new hennoxazoles (**6-9**), of which **7** was moderately cytotoxic. *Echinoclathria* sp. furnished new pyridine alkaloids (**10-12**), two of which showed immunosuppressive activity. A sponge identified to be *Dysidea herbacea* afforded cytotoxic 20,24-dimethyldeoxoscalarin-3-one (**14**) along with known diterpenes. Two cytotoxic dimers (**15,16**) of a sesquiterpene have been isolated from *Halichondria* sp. Mytiloxanthin derivatives (**18,19**) were the major pigments of *Phakellia stelliderma*. A new species of the family Niphatidae gave highly cytotoxic polyacetylenes (**20-26**).

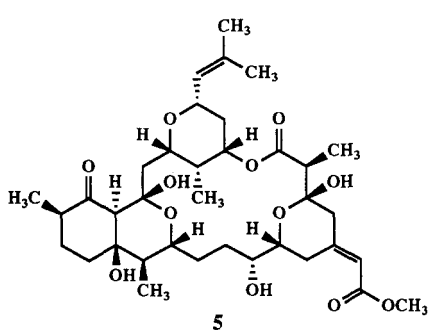
Extensive research on marine natural products over the past two decades has revealed that marine sponges are most prolific sources of novel and diverse metabolites (1). Since we started screening for bioactive compounds in 1985, we have examined a number of sponges from Okinawan waters and found a variety of interesting compounds having antitumor, antiviral or other biological activities. Structurally these compounds were complex alkaloids, macrolides, acetogenins, and terpenoids (2). In this paper we report more recent results on our search of bioactive compounds from Okinawan marine sponges.

Nitrogenous Compounds

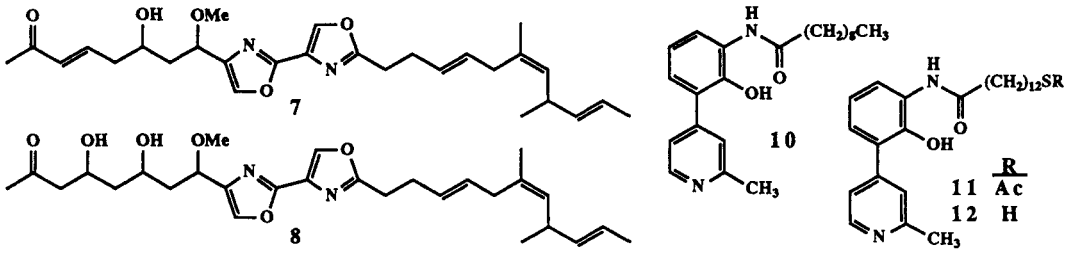
We recently reported antiviral alkaloids, hennoxazoles A-D (**1-4**) and antitumor macrolide, miyakolide (**5**), from a single species of the genus *Polyfibrospongia* (3,4). Further investigation of the sponge furnished four additional alkaloids, hennoxazoles E-G (**6-8**) and hennoxazole A acetate (**9**). The structures of the new congeners were secured by spectroscopic correlation with the previous compounds. Compound **9** was identical with an acetylation product of **1**. Hennoxazole G (**8**) was characterized as its diacetate. The ketones **7** and **8** may either be precursors to or hydrolysis products from the ketal congeners. Among the new hennoxazoles **7** was most cytotoxic at a level of IC_{50} 2 μg/mL against L1210.



1 $R^1 = CH_3$ $R^2 = OH$
2 $R^1 = CH_2CH_3$ $R^2 = OH$
3 $R^1 = CH_2CH_2CH_2CH_3$ $R^2 = OH$
4 $R^1 = CH_3$ $R^2 = H$
6 $R^1 = H$ $R^2 = OH$
9 $R^1 = CH_3$ $R^2 = OCOCH_3$



5



New pyridine alkaloids, echinoclathrines A-C (**10**-**12**) have been isolated from a new species of the genus *Echinoclathria* (5). The structures determined by spectroscopic analysis including 2D-NMR study contained the same 2-methyl-(3-amino-2-hydroxyphenyl)pyridine moiety and differ at the side chains. Echinoclathrine A (**10**) has a decanoyl group, while **11** and **12** have a tridecanoyl chain with -SAc and -SH, respectively. Fig. 1 shows HMBC correlation for **10** and ^{13}C NMR assignment for **10** and **11**. Acetylation of **12** gave a product identical with **11**. Echinoclathrines are a new class of pyridine alkaloids from marine sources. In addition to weak cytotoxicity, **10** and **11** showed immunosuppressive activity in the mixed lymphocyte reaction assay.

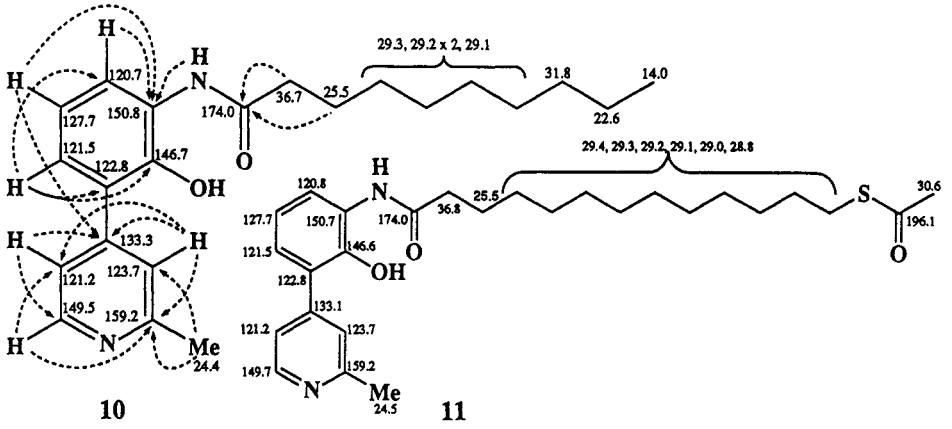
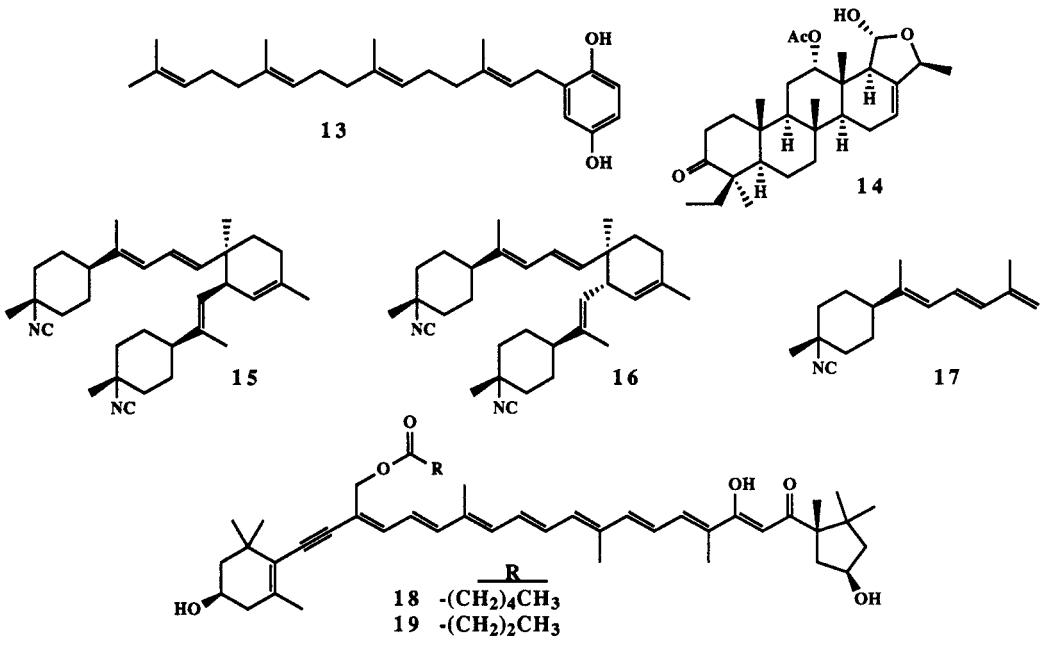


Fig. 1 Assignment of ^{13}C NMR data and HMBC correlation for echinoclathrine A (**10**)

Cytotoxic Terpenoids

An extract of a sponge which was later identified to be *Dysidea herbacea* showed high activity in a cytotoxicity assay. Five constituents have been isolated and identified. Four of them were found to be known diterpenes, ambliofuran (6), 3,7,11,15-tetramethyl-6,10,14-hexadecatrienoic acid, 2-tetraprenyl-1,4-benzoquinol (**13**), and 4-hydroxy-3-tetraprenylbenzoic acid (7). The fifth compound was new and characterized to be 20,24-dimethyldeoxoscalarin-3-one (**14**) by spectroscopic analysis including COSY and COLOC experiments. The relative stereochemistry of **14** was secured by NOE study as shown in Fig. 2. Although the diterpenes are among the classes of compounds found in *Dysidea* spp., the bishomosesterterpene skeleton of **14** is typical to the metabolites of some species of *Carteriospongia* (*Phyllospongia*) but not to *D. herbacea* (8). Except for ambliofuran which was weakly cytotoxic, four other compounds were moderately cytotoxic against P388, A-549 human lung carcinoma, and HT-29 human colon adenocarcinoma cells. The quinol **13** was most active at the level of IC_{50} 0.3 μg/mL.

Chromatography of an acetone extract of *Halichondria* sp., collected in Kerama, Okinawa, yielded nine sesquiterpenes of theonellin (9) class (bisabolene skeleton) and dimers. Among the compounds dimers **15** and **16** were cytotoxic, and **16** also showed weak immunosuppressive activity. The structure of these dimers were established by 2D-NMR studies. The dimers are regarded to be the products of Diels Alder reaction of **17** which was also isolated.



Recent report (10) on the antitumor effect of some marine carotenoids such as halocynthiaxanthin prompted us to examine carotenoid pigments often encountered during separation of some sponge extracts. Chromatography of an intensely colored extract from the bright orange sponge *Phakellia stelliderma* afforded two red pigments, 19'-hexanoyloxymytiloxanthin (**18**) and 19'-butanoyloxymytiloxanthin (**19**). The skeletal structure of these pigments was established by comparing spectral data with those reported for mytiloxanthin (11). The acyloxy substituents were determined by comparison of the ^{13}C NMR data with those of corresponding acids. The positions of the substituents was secured by the correlation observed between H-19' (δ 4.84) and C-8 (δ 95.6) in a COLOC spectrum of **18**. In *in vitro* assay with P388 **18** showed IC_{50} 10 μg/mL.

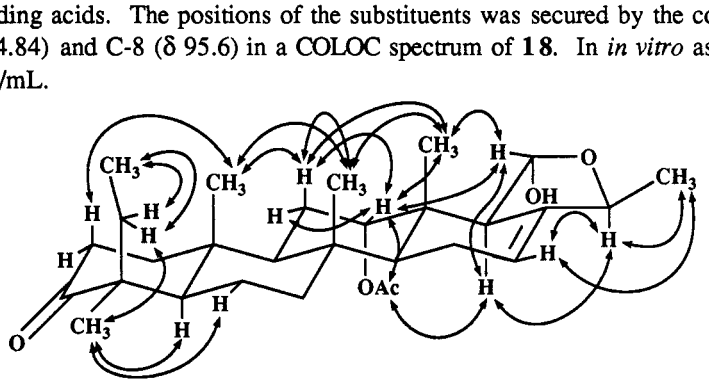
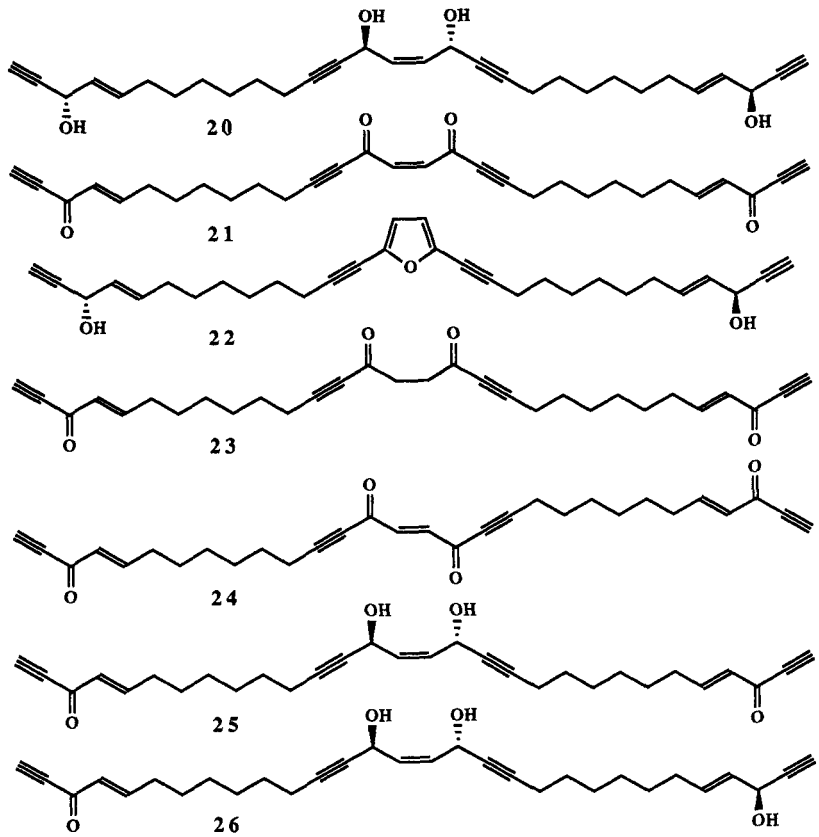


Fig. 2 NOE observation for **14**

Polyacetylenes

Polyacetylenes, petrosynol (**20**) and petrosynone (**21**), have been reported from a sponge, *Petrosia* sp. (12). When we examined a lipophilic extract of a sponge identified to be a new species of the family Niphatidae (5), we obtained five new polyacetylenes as minor constituents along with **20** and **21**. Spectroscopic analysis and correlation with **20** and **21** defined the structures of the new compounds which were designated as petrofuran (**22**), $[\alpha]_D$ +19°($CHCl_3$); 15,16-dihydropetrosynone (**23**); 15*E*-petrosynone (**24**); 3,28-diketopetrosynol (**25**), $[\alpha]_D$ +93° ($CHCl_3$); and 3-ketopetrosynol (**26**), $[\alpha]_D$ +93° ($CHCl_3$). Since the optical rotation ($[\alpha]_D$ +119°(c 0.48, $CHCl_3$)) of the present sample of **20** is in the same range with that (+107° (c 0.37, $CHCl_3$)) reported for **20**, we assume the configuration of the present sample to be the same with that of petrosynol, the absolute configuration of which has been known (12). We tentatively

assign the configurations of **22**, **25**, and **26** as shown. Except for **22** which has not yet been tested, all the acetylenes were highly cytotoxic against P388, A-549, HT-29, and MEL-28 melanoma cells.



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