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Bioactive products from marine micro- and macro-organisms*

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Abstract: A novel 37-membered macrolides, theonezolides A ~ C, and two novel manzamine-related alkaloids, nakadomarin A and ma'eganedin A, were isolated from Okinawan marine sponges, while three new polyhydroxyl compounds, luteophanols A ~ C, were obtained from a marine dinoflagellate. The structures were elucidated on the basis of the spectroscopic data. The absolute stereochemistry of the C-4 ~ C-17 fragment of theonezolides A ~ C was established by synthetic studies, and a plausible biogenetic path of nakadomarin A was proposed.

Marine invertebrates such as sponges and tunicates and marine micro-organisms such as dinoflagellates and fungi have proven to be a good source of compounds with intriguing structures and interesting biological activities.

THEONEZOLIDES A ~ C

Theonezolides A, B, and C [1,2] are novel cytotoxic 37-membered macrolides isolated from the Okinawan marine sponge *Theonella* sp., having unique bioactivity of induction of rabbit platelet shape change and aggregation [3]. Theonezolides A ~ C contain 23 chiral centers, among which the absolute configuration of one chiral center at each terminal position (C-75, C-73, and C-77 of theonezolides A ~ C, respectively) was determined as all *R* on the basis of synthesis of their ozonolysis products [2]. As to the C-4 ~ C-17 fragment, which was commonly obtained by ozonolysis of the three macrolides, the relative configurations of two 1,3-diol type moieties (14-OAc/16-OMe and 8-OAc/10-OAc) were investigated on the basis of preparation of model compounds and spectral comparisons with the natural product-derived specimen [4]. As a result, the two 1,3-diol type moieties were both suggested as *syn*, and four structures (**a** and **b** and their enantiomers) remain to be likely out of 16 possibilities. Two possible diastereomers (**a** and **b**) were synthesized as optically active forms and comparison of their spectral and optical data with those of the natural specimen (C-4~C-17) led to establish the absolute configuration of four chiral centers contained in the C-4~C-17 fragment as 8*S*, 10*R*, 14*S*, and 16*S* (Scheme 1) [5].

NAKADOMARIN A AND MA'EGANEDIN A

Recently a series of unique polycyclic alkaloids with intricate skeletons have been isolated from marine Haplosclerid sponges, among which the representative alkaloids, manzamines A and B, are characterized by a penta- or tetracyclic nitrogen-containing ring system bound to a β-carboline, respectively. These unusual ring systems have attracted great interest as one of the most challenging targets for total synthesis or biosynthetic studies. Our continuing search for biogenetic precursors of manzamines A~C resulted in the isolation of several novel alkaloids, ircinals A and B, keramaphidins B and C, and keramamine C from an *Amphimedon* sponge, in which ircinals and keramaphidin B correspond to tetra-and pentacyclic biogenetic precursors of manzamines A and B, respectively, proposed by Baldwin & Whitehead [6].

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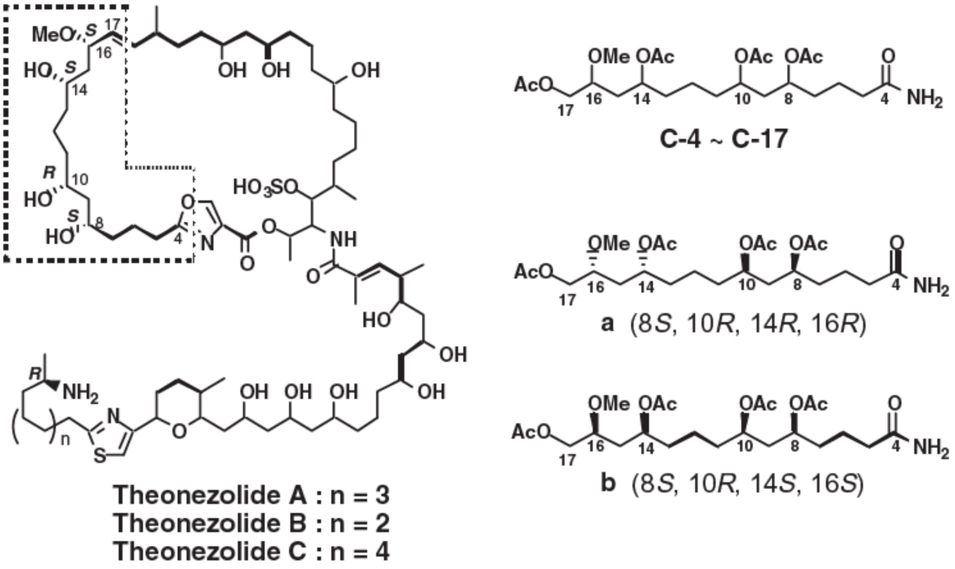
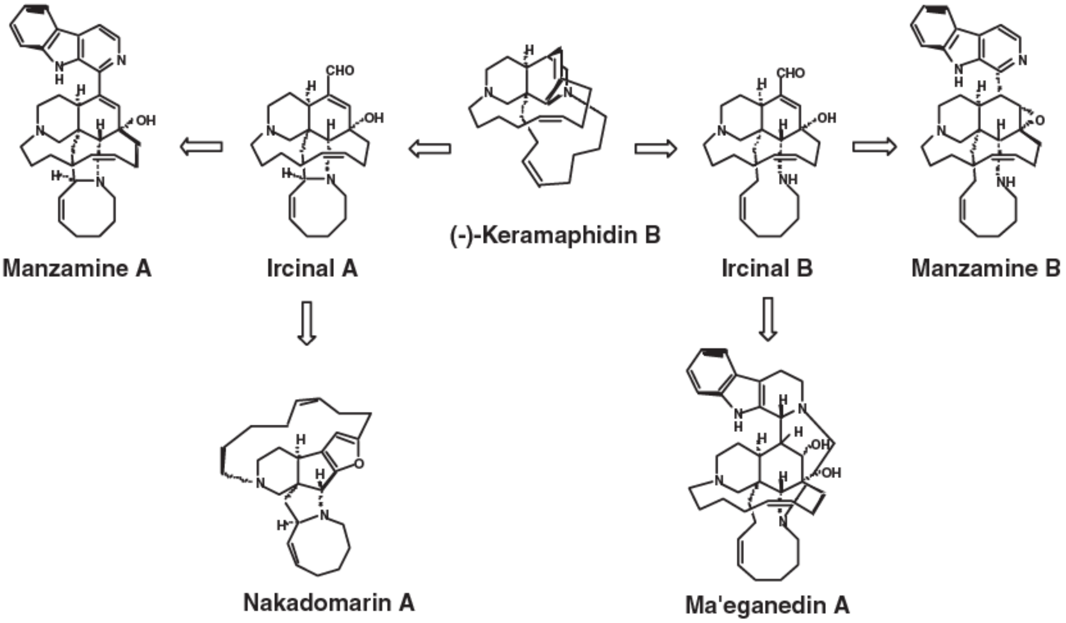


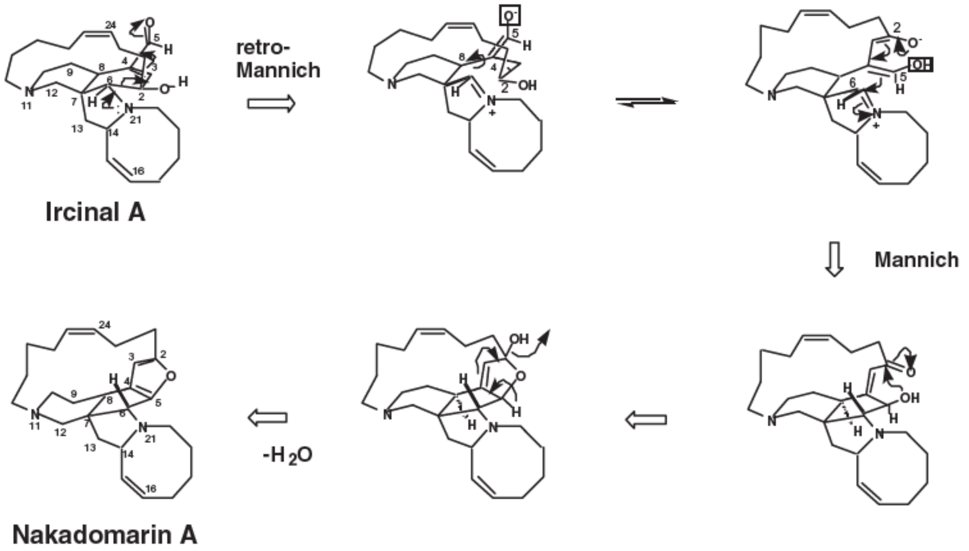
Scheme 1

Further investigation of biogenetically related compounds to manzamines from another *Amphimedon* sponge led to the isolation of nakadomarin A [7], a novel furan-containing hexacyclic alkaloid consisting of an unprecedented 8/5/5/5/15/6 ring system. The unique structure containing a furan ring was elucidated on the basis of the spectroscopic data. The relative stereochemistry was deduced from the NOE data and proton couplings, and a plausible biogenetic path of nakadomarin A through ircinal A was proposed. Nakadomarin A showed cytotoxicity against murine lymphoma L1210 cells, inhibitory activity against cyclin dependent kinase 4, and anti-microbial activity against *Trichophyton mentagrophytes* and *Corynebacterium xerosis* (Schemes 2 and 3).

On the other hand, another new type manzamine alkaloid, ma’eganedin A [8] with a methylene carbon bridge between N-2 and N-27, was also isolated from the same sponge, and the structure including absolute stereochemistry was elucidated on the basis of spectroscopic data. Ma’eganedin A exhibited



Scheme 2

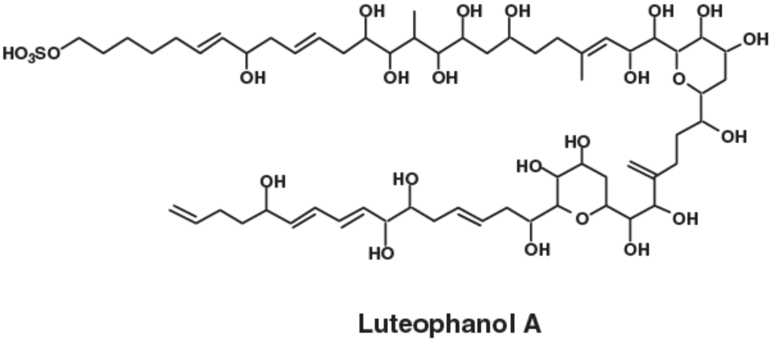


Scheme 3

anti-bacterial activity against *Sarcina lutea*, *Bacillus subtilis*, and *Corynebacterium xerosis,* and showed cytotoxicity against murine leukemia L1210 cells.

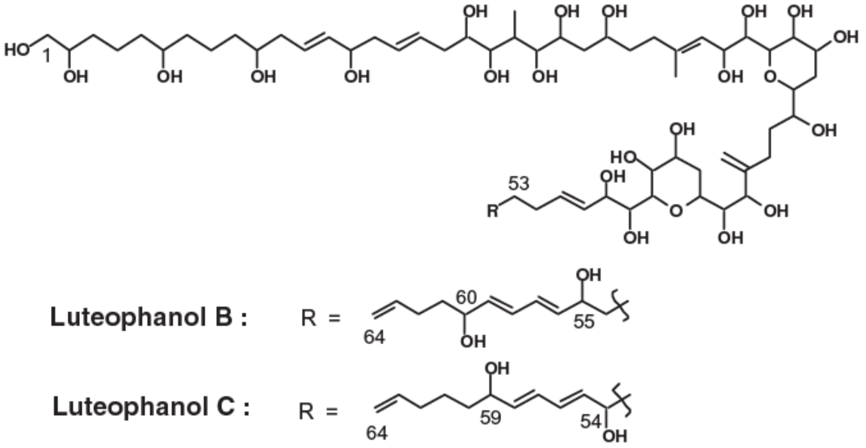
LUTEOPHANOLS A ~ C

During our continuing search for structurally unique compounds from marine dinoflagellates, we previously isolated a series of cytotoxic macrolides, amphidinolides A ~ H and J ~ S [9], from dinoflagellates *Amphidinium* sp., some (B, C, G, H, and N) of which were potent cytotoxic (IC_{50}, 0.006 ~ 0.00006 μg/mL). The absolute stereochemistries (B, J and L) were investigated by syntheses of the degradation products with $NaIO_4$ or O_3 (Scheme 4).



Scheme 4

Recently we investigated another strain of *Amphidinium* sp., which was separated from the inside cells of the Okinawan marine acoel flatworm *Pseudaphanostoma luteocoloris*, and isolated a new polyhydroxyl linear carbon-chain compound, luteophanol A, $C_{60}H_{102}O_{25}S$ (*m/z* 1254) [10]. The structure was elucidated by extensive analyses of 2D NMR spectra as well as FABMS/MS data. Luteophanol A possesses a sulfate ester, two tetrahydropyrans, and 19 hydroxyl groups on C_{57}-linear aliphatic chain with one exomethylene and two methyl branches. The central portion (the C-15 ~ C-42 moiety) of luteophanol A is structurally common to that of amphidinols, potent anti-fungal metabolites previously isolated from dinoflagellates *Amphidinium* sp. Luteophanol A, however, possesses different structural features from those of amphidinols in both ends of the molecule. Particularly, amphidinols comprise a hydrophobic polyene portion in one end of the molecule, whereas the corresponding portion of luteophanol A contains three hydroxyl groups (C-47, C-48, and C-53) with no conjugated triene, which may make this side of molecule less hydrophobic. Luteophanol A showed anti-bacterial activity against *Staphylococcus aureus*, *Sarcina lutea*, and *Bacillus subtilis* (Scheme 5).



Scheme 5

Further investigation of extracts of the same dinoflagellate led to the isolation of two new polyhydroxyl compounds, luteophanols B and C [11], possessing two tetrahydropyran rings and 23 hydroxy groups on a C_{64}-linear aliphatic chain with one exo-methylene and two methyl branches. The structures of luteophanols B and C were elucidated by detailed analyses of two-dimensional NMR data containing HMBC, HMQC-RELAY, CH_2-selected E-HSQC, and CH_2-selected E-HSQC-TOCSY. Pharmacological activities of these compounds are under investigation.

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