

Award Accounts

The Chemical Society of Japan Award for Young Chemists for 2002

Bioorganic Studies on Marine Natural Products with Bioactivity, Such as Antitumor Activity and Feeding Attractance

Kiyotake Suenaga

Department of Chemistry, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8571

Received August 29, 2003; E-mail: suenaga@chem.tsukuba.ac.jp

This account describes bioorganic studies on marine natural products with bioactivity, such as antitumor activity and feeding attractance. Chemical studies on the following bioactive compounds of marine origin have been carried out: 1) feeding attractants for the starfish *Acanthaster planci*, 2) potent toxins from shellfish of the genus *Pinna* that cause food poisoning, and 3) a variety of cytotoxic and antitumor compounds from marine invertebrates including sea hares, *Dolabella auricularia* and *Aplysia kurodai*. Their isolation, structure elucidation, synthesis, and some aspects of their biological activities are summarized.

The discovery of bioactive natural products and their chemical studies are becoming increasingly important not only in the field of chemistry but also in a wide range of related fields such as biology and medicinal sciences. Natural bioactive substances such as tetrodotoxin[1] and okadaic acid[2] often serve as useful probes to lead to breakthroughs in a variety of life-science fields. New bioactive substances are essential for further advance in the life sciences, but it is impossible to design them artificially. They can only be found in nature. Marine organisms have attracted considerable attention as a rich source of new bioactive substances, and a large number of bioactive substances have been found from this source.[3,4] First, in a search for the compounds responsible for interesting biological phenomena, such as feeding attractance for a kind of starfish and shellfish poisoning, the active compounds were isolated and their structures were elucidated. Second, sea hares, which are shell-less, slow-moving marine invertebrates, are postulated to have chemical defense compounds, and therefore they are expected to contain a great variety of bioactive compounds, including some that are cytotoxic. The isolation, structure determination, and synthesis of these cytotoxic principles have thus been carried out. In this account, we describe the results of bioorganic studies on bioactive natural products isolated from marine invertebrates, such as sea urchin, coral, shellfish, and sea hares.

1. Feeding Attractants for the Crown-of-Thorns Starfish *Acanthaster planci*

Coral reefs are being increasingly recognized as important sources of potential medicines, including unique antimicrobial and anticancer agents.[5] However, coral reefs are rapidly disappearing. Although many factors such as overfishing, pollution, typhoons and global warming play roles in the destruction of coral reefs, a major reason of destruction is starfish predation. In the tropical zones of the Indo-Pacific Oceans, the crown-of-thorns starfish *Acanthaster planci* destroys coral reefs by predation. Since the early 1960s, the predation of extensive areas of coral reefs by *A. planci* has been documented,[6] but it has been unclear how corals attract *A. planci*. Once coral reefs are damaged, more than 10 years are required for their recovery. Elucidation of the feeding attractants for *A. planci* is a matter of great importance. Several attempts to identify the feeding attractants of starfish have been made since the 1970s,[7] but the active principles have not yet been identified.

It was recently found that the viscera of the sea urchin *Toxopneustes pileolus* contained feeding attractants for this starfish. A jelly prepared from the ethanol extract of the sea urchin attracted *A. planci* in an aquarium with inversion of its stomach, which is a characteristic feeding behavior of the starfish. Based on this behavior and attractive activity, isolation of the feeding attractants for the starfish was attempted from the sea urchin. The crude EtOH extract of the sea urchin (1.9 kg) was concentrated to give an aqueous residue. The EtOAc-soluble material obtained from the aqueous residue was partitioned between hexane and 90% methanol. The hexane layer was purified by three-step chromatography to give arachidonic acid (**1**, 110 mg) and α-linolenic acid (**2**, 5.5 mg) (Fig. 1) as active princi-

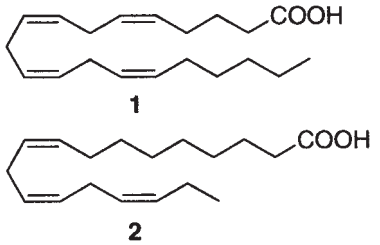


Fig. 1.

Published on the web March 12, 2004; DOI 10.1246/bcsj.77.443

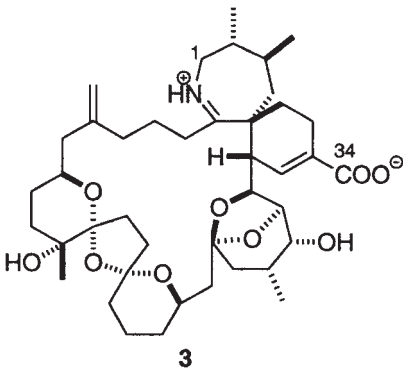
ples.[8] Authentic specimens of **1** and **2** also exhibited attractive activity for the starfish at concentrations similar to those of the compounds obtained from the sea urchin. Thus, the attractants for the starfish from the sea urchin were established to be **1** and **2**. The attractive activity of **1** was equal to that of **2**, and this activity was not increased by mixing them. Other unsaturated fatty acids such as eicosapentaenoic acid, docosahexaenoic acid, and linoleic acid showed no activity even at concentrations 20-fold higher than those of **1** and **2**.

Subsequently, the constituents of corals were examined to determine whether or not the corals contained the feeding attractants described above. Some coral *Montipora sp*. (30 g), which is commonly eaten by *A. planci*, was extracted with ethanol, and the extract was purified by monitoring the attractive activity for the starfish. This gave arachidonic acid (**1**, 1 mg) as an active substance.[8] Another active substance was also isolated from the aqueous phase; based on spectral data, this was found to be *N*,*N*,*N*-trimethylglycine (IUPAC name: trimethylammonioacetate). Its attractive activity was equal to those of **1** and **2**, but gradually disappeared, whereas the activities of **1** and **2** were maintained for many hours. Moore and Huxley reported that *N*,*N*,*N*-trimethylglycine exhibited feeding-inducing activity in a random test of several kinds of known compounds, such as amino acids, and suggested that corals may contain *N*,*N*,*N*-trimethylglycine.[9] In this study, the presence of *N*,*N*,*N*-trimethylglycine was confirmed as an active substance in corals.

Experiments to trap the starfish *A. planci* in the sea were also conducted. Seven starfish were caught in nine days using a 1.5 m^2 trap containing α-linolenic acid (**2**) as an attractant.[8] These results should be useful in future studies to protect coral reefs from starfish predation.

2. Shellfish Toxins from Japanese Bivalves

2.1 Pinnatoxins B and C. Poisoning by the ingestion of shellfish is a serious health problem. Several shellfish toxins with novel chemical structures and potent bioactivities, such as saxitoxin,[10] okadaic acid,[2] brevetoxins,[11] azaspiracid,[12] gymnodimine,[13] neosurugatoxin,[14] and spirolides,[15] have been isolated and structurally characterized. However, people still suffer shellfish poisoning of unknown origin.

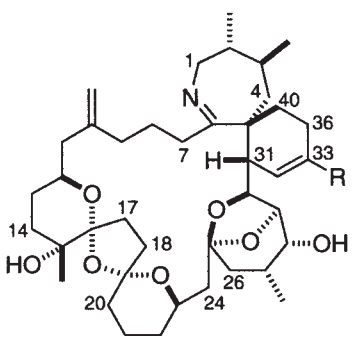


3

Fig. 2.

Human intoxication resulting from the intake of adductor muscle of shellfish of the genus *Pinna* frequently occurs in Japan and China. In Japan, 2766 people were reportedly affected by food poisoning due to *Pinna pectinata* between 1975 and 1991.[16] Chinese investigators reported that the toxic extract from *P. attenuata* activated the Ca^{2+} channel.[17] Uemura and co-workers reported the isolation and structural determination of pinnatoxins A (**3**, Fig. 2)[18] and D,[19] major toxic components in *P. muricata*. However, there was a small fraction containing the most potent toxic component(s) in *P. muricata*, purification of which was very difficult. To identify these toxins, large-scale extraction and separation experiments were carried out.

The 80% EtOH extract of viscera (21 kg) of *P. muricata* was partitioned between EtOAc and H_2O. The aqueous fraction was successively chromatographed on TSK G-3000S polystyrene gel (50% EtOH), Sephadex LH-20 (MeOH), DEAE Sephadex A-25 (0.02 M phosphate buffer), reversed-phase MPLC (ODS, MeCN–H_2O–TFA), and reversed-phase HPLC (ODS, MeOH–H_2O–TFA) guided by acute toxicity against mice. Final purification was achieved by reversed-phase HPLC (ODS, MeOH–H_2O–TFA) to give a 1:1 mixture of pinnatoxins B (**4**) and C (**5**) (0.3 mg, LD_{99} 22 μg/kg mice, ip)[20] (Fig. 3). The molecular formulas of both **4** and **5** were determined to be



4: R = $^{34}CH(COO^{\ominus})(NH_3^{\oplus})$ 34*S* isomer

5: R = $^{34}CH(COO^{\ominus})(NH_3^{\oplus})$ 34*R* isomer

6: R = $^{34}CH(OH)-S-CH(COO^{\ominus})(NH_3^{\oplus})$

7: R = $^{34}CH(HO)-S-CH(NH_3^{\oplus})(COO^{\ominus})$ diastereomer at C34

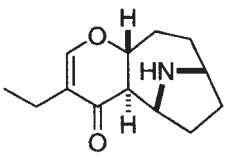
8: R = $^{34}CH(HO)-S-CH(NH_3^{\oplus})(COO^{\ominus})$ diastereomer at C34

Fig. 3.

$C_{42}H_{64}N_2O_9$ by ESIMS, which corresponds to an increase of 29 mass units (CH_3N) compared to that of pinnatoxin A (**3**). A detailed analysis of DQF-COSY and HOHAHA spectra disclosed five partial structures (C1–C4, C7–C14, C17–C18, C20–C24, and C26–C33–C36–C40). In the NMR spectra, the similarity of the chemical shifts and ^1H–^1H coupling constants of **4** and **5** to those of **3** suggested that **4** and **5** consist of the same polyether macrocycles as **3**. The presence of an α-amino acid function was revealed by characteristic NMR signals. Furthermore, positive ion ESI MS/MS of **4** and **5** showed the same series of prominent fragment ions due to the carbocyclic moiety as were observed in pinnatoxin A (**3**). Based on these results, the gross structures of pinnatoxins B and C were determined to be those shown in formulas **4** and **5**. Their relative stereostructures were determined by spectroscopic analysis and chemical correlation with the known homolog, pinnatoxin A (**3**). The absolute stereostructures of pinnatoxins B and C were suggested to be those shown in formulas **4** and **5**, respectively considering their potent toxicities.[21]

2.2 Pteriatoxins. The Okinawan bivalve *Pteria penguin*, which has large adductor muscles, produces pearls, but is not used as food. Since the presence of toxins was expected, extraction and separation experiments were carried out on this bivalve. The 75% EtOH extract of viscera (82 kg) of the bivalves was partitioned between EtOAc and H_2O. The aqueous fraction was purified by 11-step chromatography guided by acute toxicity against mice to give pteriatoxin A (**6**, 20 μg, LD_{99} 100 μg/kg mice, ip) and a 1:1 mixture of pteriatoxins B (**7**) and C (**8**) (8 μg, LD_{99} 8 μg/kg mice, ip).[22] Since the toxic symptoms and ^1H NMR spectra of pteriatoxins A, B, and C resemble those of pinnatoxins, pteriatoxins were thought to be pinnatoxin analogs. Their gross structures were elucidated in nanomolar quantities by detailed analyses of 2D NMR (COSY and HOHAHA) and ESI-MS/MS to be pinnatoxin analogs containing a cysteine moiety, as shown in formulas **6**, **7** and **8** (Fig. 3). Considering the chemical shifts and coupling patterns in the ^1H NMR spectra, we conclude that the stereochemistries of the carbocycles of pteriatoxins and pinnatoxins may be superimposed on each other.

2.3 Pinnamine. In continuing studies on pinnatoxins, the presence of another toxin that causes characteristic toxic symptoms, such as scurrying around, was revealed. This toxic fraction, which was more polar than that containing pinnatoxins, was subjected to a series of chromatography guided by acute toxicity against mice to give pinnamine (**9**, LD_{99} 0.5 mg/kg).[23] The gross structure of **9** (Fig. 4) was established by spectroscopic analysis. The absolute stereostructure of **9** was determined based on NOESY, coupling constants in NMR spectra, and the CD spectrum, and was confirmed by enantioselective synthesis by Kigoshi and co-workers.[24] The structure and toxic symptoms of pinnamine resemble those of anatoxin-a,[25] which has been called Very Fast Death Factor, and atropine,[26] a representative suppressor of the parasympathetic nervous system.



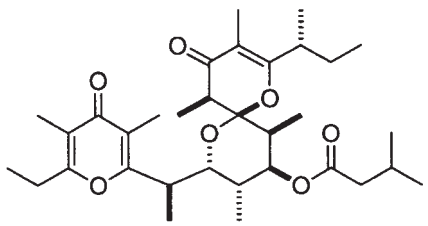
9

Fig. 4.

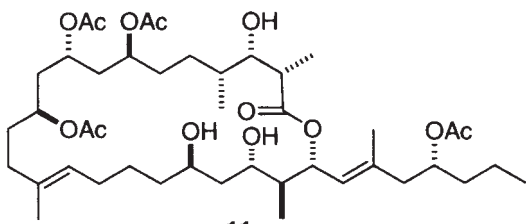
3. Cytotoxic and Antitumor Compounds of Marine Invertebrate Origin: Aurilide from the Sea Hare *Dolabella auricularia*

Over the past three decades, a large number of bioactive substances have been isolated and characterized from marine organisms such as algae, sponges, tunicates, coelenterates, bryozoans, and molluscs.[3,4] Many of them were produced by symbiotic microorganisms and were probably transferred to those invertebrates by the food chain. Therefore, molluscs that feed on a variety of marine algae as well as sponges in symbiosis with a large number of microbes are expected to be rich sources of bioactive substances. The examination of bioactive constituents of marine invertebrates such as sea hares, sponges, and tunicates resulted in the isolation and structure determination of about 30 kinds of cytotoxic and antitumor compounds:[27] the cytotoxic compounds include auripyrone A (**10**),[27a] dolabelide C (**11**),[27c] and attenol A (**12**)[27g] (Fig. 5). The enantioselective total synthesis of some of these compounds has been achieved.[28] In this section, chemical studies on the cytotoxic and antitumor compound aurilide are described as a typical example.

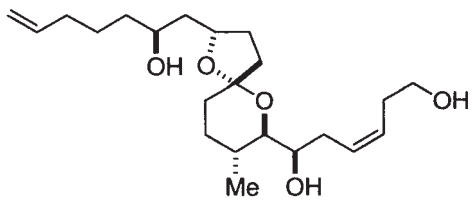
The sea hare *Dolabella auricularia* (Aplysiidae) is known to be a rich source of cytotoxic and/or antitumor peptides and other unique metabolites.[29,30] Aurilide (**13**, Fig. 6) was isolated as a minute constituent of the sea hare *D. auricularia* collected off the coast of the Shima peninsula, Mie prefecture, Japan (0.5 mg from 262 kg of sea hare).[27b] The procedure for isolating aurilide



10



11



12

Fig. 5.

(**13**), which involves two-step solvent partition and subsequent 10-step chromatographic separation, is shown in Scheme 1. The gross structure of aurilide (**13**) was determined based on an interpretation of NMR spectral data, and the absolute stereochemistry of the peptide moiety was determined by chiral HPLC analysis of acid hydrolysates of aurilide (**13**). The absolute stereochemistry of a new dihydroxylated fatty acid part in aurilide (**13**) was determined by enantioselective synthesis of four possible diastereomers of fragment **14** (Fig. 6) derived from aurilide (**13**) and subsequent comparison of their spectral data (^{1}H NMR and CD) to those of the natural fragment **14**. Thus, the absolute stereostructure of aurilide was determined to be a 26-membered cyclodepsipeptide, as depicted in **13**.[27b]

To confirm the stereostructure and to obtain **13** in adequate quantities for biological and pharmaceutical studies, the enantioselective synthesis of aurilide (**13**) was carried out (Scheme 2).[28d] Starting from (4*S*, 5*R*)-4-methyl-5-phenyl-3-propionyl-2-oxazolidinone, the protected dihydroxy carboxylic acid **15** was synthesized by using an anti-selective aldol reaction[31] and a vinylogous Mukaiyama aldol reaction[32] as key steps. The synthesis of pentapeptide **16** commenced with an *N*-methylglycine derivative in a stepwise manner. The coupling reaction between protected dihydroxy carboxylic acid **15** and pentapeptide **16**, followed by esterification with *N*-methylalanine derivative, gave seco amino acid **17**. Macrolactamization of **17** followed by removal of the protecting group afforded aurilide **13** (3.9% overall yield), which was found to be identical

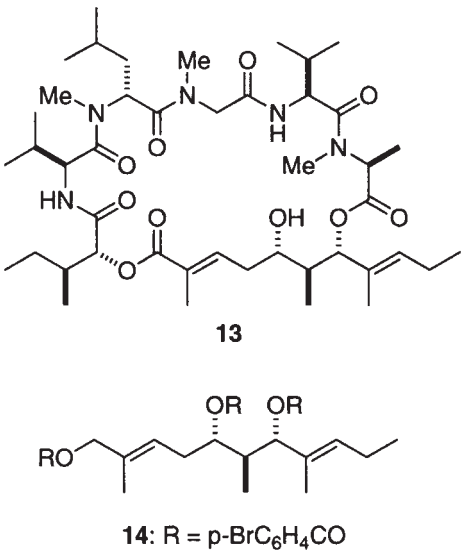
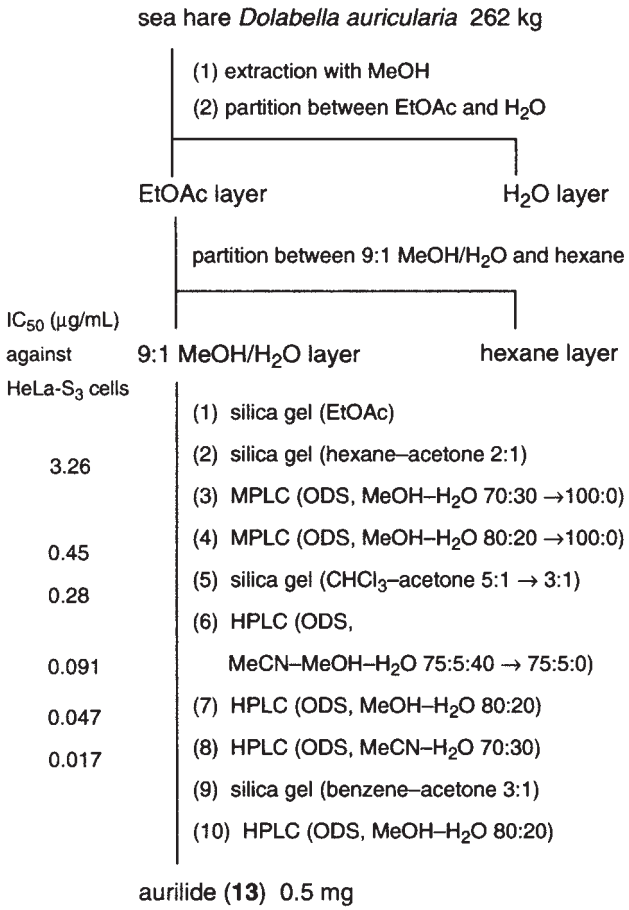
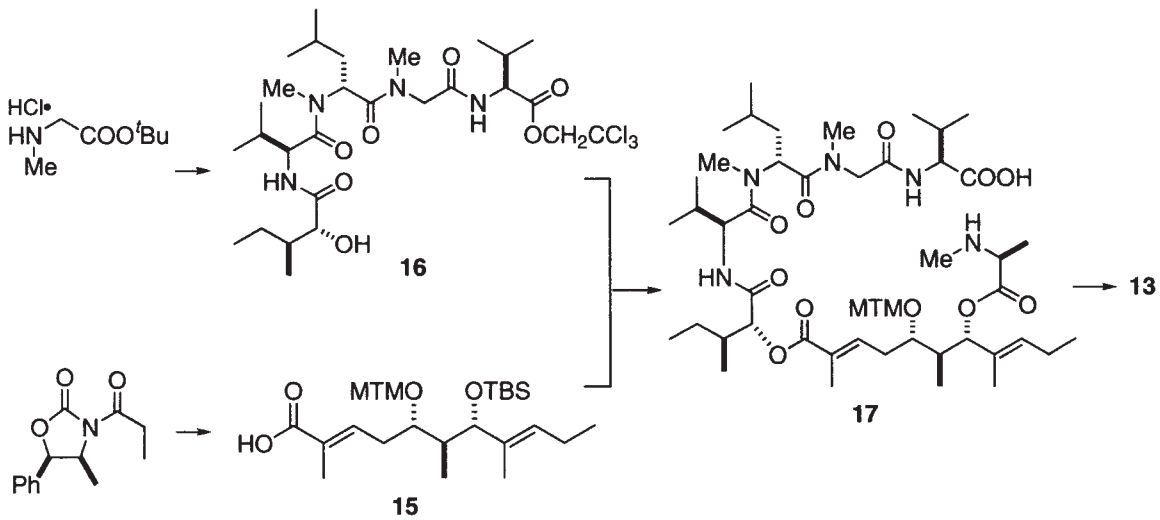


Fig. 6.



Scheme 1. Isolation procedure of aurilide (**13**).



Scheme 2. Outline of the synthesis of aurilide (**13**).

to the natural product in all respects. Thus, the stereostructure of aurilide was unambiguously confirmed to be as shown in **13**. Although aurilide (**13**) was isolated from a strongly cytotoxic fraction of the extract of the sea hare (Scheme 1), the scarcity of the natural sample had previously prevented an evaluation of its cytotoxicity. Thus, the cytotoxicity of aurilide was evaluated using the synthetic sample, which was found to exhibit potent cytotoxicity against HeLa S_3 cells with an IC_{50} of 0.011 μg/mL. When the improved enantioselective synthesis of **13** was used, an overall yield of 12% was realized,[33] and the synthetic sample was obtained on a gram scale. The availability of an ample amount of **13** by synthesis enabled us to perform various biological and pharmaceutical studies of aurilide (**13**). The NCI's human cancer cell panel showed that aurilide (**13**) exhibited a high level of cytotoxicity, and that **13** is selectively active against lung, ovarian, renal and prostate cancer cell lines. Aurilide (**13**) met the criteria of NCI's hollow–fiber assay[34] with high scores, which is a preliminary in vivo test for assessing the potential anticancer activity of cytotoxic compounds. Recently, Takahashi and co-workers achieved a solid-phase library synthesis of aurilide (**13**) and related analogs.[35]

4. Cytotoxic and Antitumor Compounds, Aplyronines from the Sea Hare *Aplysia kurodai*

Aplyronines A (**18**), B (**19**), and C (**20**) in Fig. 7 are a group of three related macrolides that were isolated from the sea hare *Aplysia kurodai* (Aplysiidae) collected off the coast of the Shima peninsula, Mie prefecture, Japan.[36] Aplyronines A (**18**), B (**19**), and C (**20**) exhibited potent cytotoxicities against HeLa S_3 cells with IC_{50} values of 0.48, 3.11, and 21.2 ng/mL, respectively. Although aplyronine A (**18**) exhibits strong cytotoxicity and exceedingly potent antitumor activities in vivo (Test/Control 545% against P388 murine leukemia, 556% against Lewis lung carcinoma, 398% against Ehrlich carcinoma, 255% against colon 26 carcinoma, and 201% against B16 melanoma), the scarcity of the natural sample has prevented the further evaluation of this compound as a potential therapeutic agent. While the *N*,*N*,*O*-trimethylserine group in **18** has been shown to play an important role in its strong cytotoxicity based on a comparison of aplyronines A (**18**) and C (**20**), more detailed studies on the structure–cytotoxicity relationships in **18** are needed. Under the background described above, the enantioselective synthesis of aplyronines and related compounds was carried out.[28a–c]

An outline of the synthesis of aplyronine A (**18**) is shown in Scheme 3. Starting from chiral *N*-propionyl oxazolidinone **21** and ent-**21**, three segments **22**, **23**, and **24** possessing four contiguous chiral centers were prepared using the Evans aldol reaction[37] and Sharpless epoxidation reaction[38] as key steps. The sulfone **22** was alkylated with iodide **25** and then coupled with sulfone **26** by the Julia olefination reaction[39] that constructed an *E*-trisubstituted olefin to afford C5–C20 segment **27**, while two segments, **23** and **24**, were coupled to give C21–C34 segment **28**, which corresponds to the side chain moiety of aplyronine A (**18**). A Julia olefination reaction between two segments, **27** and **28**, followed by four-carbon homologation afforded seco acid **29**. Lactonization of **29** under Yamaguchi–Yonemitsu conditions[40,41] yielded a 24-membered macrolactone. After construction of the terminal *N*-methyl-*N*-vinylformamide and subsequent introduction of two amino acids, this macrolactone was transformed into aplyronine A (**18**). The overall yield of the synthesis based on the longest linear sequence (47 steps) was 0.35%. While this is the first total synthesis of aplyronine A (**18**), synthetic studies of **18** have been carried out by several groups.[42] The synthesis of aplyronine A (**18**) unambiguously established its absolute stereostructure and made it possible to supply amounts necessary for further studies of the biological and pharmacological properties of **18**. The syntheses of aplyronines B (**19**) and C (**20**) were also achieved, which established their stereostructures.[43] Recently, the total synthesis of scytophycin C[44] and mycalolide A,[45] which are structurally related to aplyronines, has been reported.

The target biomolecules of aplyronine A (**18**) were investigated: **18** did not interact with DNA, tubulin, or cell cycle-reg-

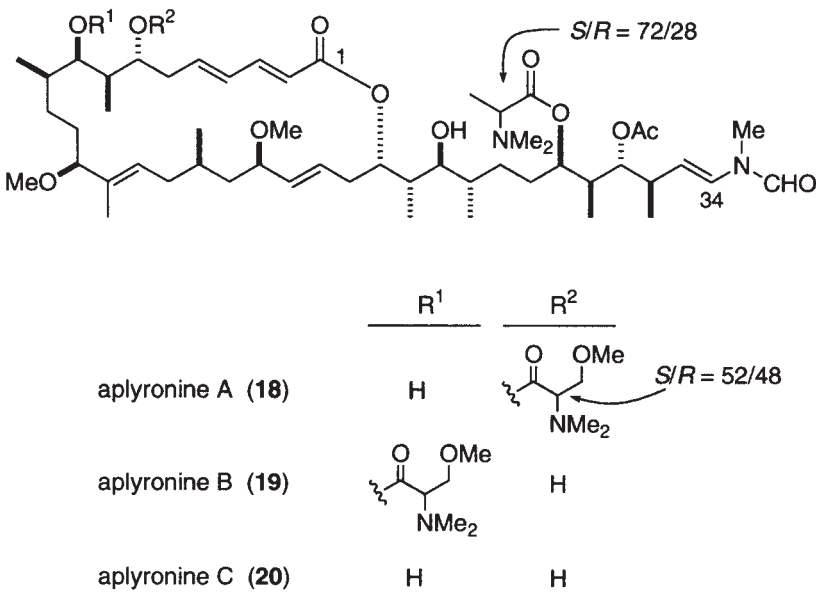
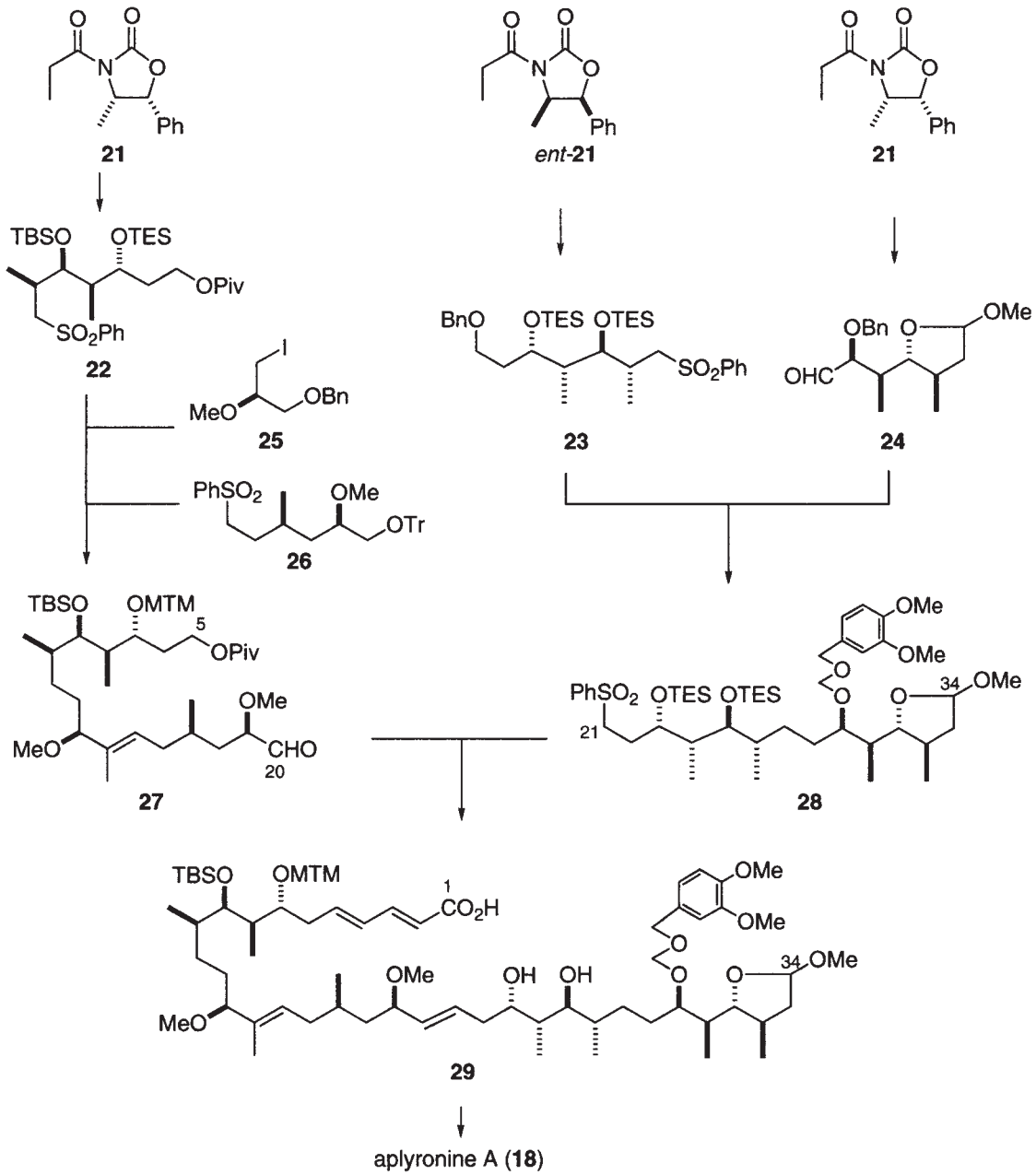
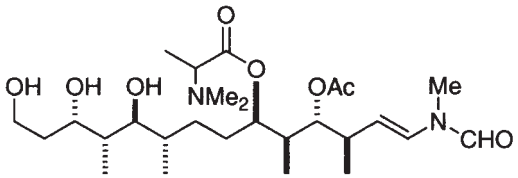


Fig. 7.

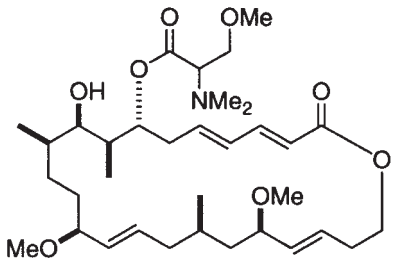


Scheme 3. Outline of the synthesis of aplyronine A (**18**).

ulating enzymes, but did interact with actin. Actin is a major protein in the cytoskeleton. Aplyronine A (**18**) not only binds to G-actin in a molecular ratio of 1:1 to inhibit the polymerization of G-actin to F-actin, but also depolymerizes F-actin to G-actin by severing.[46] To date, very few antitimor substances that interact with actin have been reported. Thus, aplyronine A (**18**) is considered to be a new type of antitumor substance with regard to its mode of action. Although many proteins that interact with actin have been known, only a few compounds with low molecular weights that interact with actin have been reported: these include cytochalasins, phalloidin, and recently discovered marine macrolides such as latrunculins,[47] scytophycins,[48] goniodomin,[49] mycalolide B,[50] swinholide A,[51] and bistheonelide.[52] To investigate the structure–bioactivity relationships of aplyronines, 16 kinds of artificial analogs and related compounds were synthesized, and their cytotoxicities and actin-depolymerizing activities were evaluated.[28c,53] Results revealed that not only the presence of the side-chain of aplyronine A (**18**) but also its length are crucial for strong cytotoxicity. The *N*,*N*,*O*-trimethylserine moiety, two hydroxy groups, and the conjugate diene moiety are also important for the cytotoxicity of **18**. Either an *N*-methyl-*N*-vinylformamide group or an *N*,*N*-dimethylalanine group is necessary for the strong cytotoxicity of aplyronine analogs. As to actin-depolymerizing activity, both the presence of the side-chain of aplyronine A (**18**) and its length are essential, as is true with cytotoxicity. Interestingly, analog **30** (Fig. 8), which consists of only the side chain part of aplyronine A (**18**), exhibited strong actin-depolymerizing activity, considering the size of the molecule, whereas the macrolactone without the side chain (**31**, Fig. 8) is completely inac-



30



31

Fig. 8.

tive. Analog **30** should be useful in future studies on the interaction between actin and aplyronines. Interestingly, the functional groups of aplyronine A (**18**) such as amino acid residues, two hydroxy groups, the conjugate diene moiety, and the *N*-methyl-*N*-vinylformamide group had little effect on actin-depolymerizing activity, in contrast to their remarkable influence on cytotoxicity. Considering the difference in the structure–activity relationship between cytotoxicity and actin-depolymerizing activity, we think it is unlikely that these two bioactivities directly correlate with each other. Further studies on the interaction between aplyronines and actin are needed at the molecular level.

In summary, bioorganic studies on marine natural products with bioactivity, such as antitumor and feeding attractance activity, have been performed. The following research results were obtained. The feeding attractant for the starfish *A. planci* was identified as arachidonic acid, which was found in corals. This is a scientific answer to the question "why do corals attract starfish?" In chemical studies on food poisoning due to the shellfish *Pinna* sp., pinnatoxins B and C, the most toxic components in the pinnatoxin series, were purified and structurally characterized from an Okinawan bivalve. Isolation and structure determination of three potent toxins, pteriatoxins A, B, and C, from the Okinawan bivalve *Pteria penguin* were also achieved at a nanomolar scale. The cytotoxicity-guided investigation of bioactive constituents of marine invertebrates such as sea hares, sponges, and tunicates has resulted in the isolation and structure determination of about 30 cytotoxic and antitumor compounds. As an example, the isolation, structure determination, and enantioselective synthesis of aurilide, a 26-membered cyclodepsipeptide from the sea hare *Dolabella auricularia*, were described together with studies on its cytotoxic and antitumor activities. While these substances are generally obtained in minute amounts from animals, we can now obtain sufficient quantities for further biological and medicinal studies by chemical synthesis in some cases. Enantioselective syntheses of aplyronine A, a potent antitumor compound that interacts with actin, its congeners, aplyronines B and C, and analogs were achieved, and the structure–activity relationships regarding their cytotoxicity and actin-depolymerizing activity were investigated. The synthetic analog that only consists of the side-chain part of aplyronine A turned out to have actin-depolymerizing activity. This is the first demonstration of the molecular structure necessary for actin-depolymerizing activity.

I wish to thank Professor Emeritus K. Yamada and Professor D. Uemura (Nagoya University) for their advice and guidance. I am also grateful to all of the collaborators whose names appear in the references for their efforts in this work. This study was supported in part by Grants-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science and Technology, and by Research Aid of Inoue Foundation for Science.

References

1 a) T. Goto, Y. Kishi, S. Takahashi, and Y. Hirata, *Tetrahedron Lett.*, **5**, 779 (1964). b) K. Tsuda, C. Tamura, R. Tachikawa, K. Sasaki, K. O. Amakasu, M. Kawamura, and S. Ikuma, *Chem. Pharm. Bull.*, **12**, 634 (1964). c) R. B. Woodward, *Pure Appl. Chem.*, **9**, 49 (1964).

2 a) K. Tachibana, P. J. Scheuer, Y. Tsukitani, H. Kikuchi, D. Van Engen, J. Clardy, Y. Gopichand, and F. J. Schmitz, *J. Am. Chem. Soc*, **103**, 2469 (1981). b) T. Yasumoto, M. Murata, Y. Oshima, M. Sano, G. K. Matsumoto, and J. Clardy, *Tetrahedron*, **41**, 1019 (1985).

3 D. J. Faulkner, *Tetrahedron*, **33**, 1421 (1977).

4 a) D. J. Faulkner, *Nat. Prod. Rep.*, **1**, 251 (1984). b) D. J. Faulkner, *Nat. Prod. Rep.*, **1**, 551 (1984). c) D. J. Faulkner, *Nat. Prod. Rep.*, **3**, 1 (1986). d) D. J. Faulkner, *Nat. Prod. Rep.*, **4**, 539 (1987). e) D. J. Faulkner, *Nat. Prod. Rep.*, **5**, 613 (1988). f) D. J. Faulkner, *Nat. Prod. Rep.*, **7**, 269 (1990). g) D. J. Faulkner, *Nat. Prod. Rep.*, **8**, 97 (1991). h) D. J. Faulkner, *Nat. Prod. Rep.*, **9**, 323 (1992). i) D. J. Faulkner, *Nat. Prod. Rep.*, **10**, 497 (1993). j) D. J. Faulkner, *Nat. Prod. Rep.*, **11**, 355 (1994). k) D. J. Faulkner, *Nat. Prod. Rep.*, **12**, 223 (1995). l) D. J. Faulkner, *Nat. Prod. Rep.*, **13**, 75 (1996). m) D. J. Faulkner, *Nat. Prod. Rep.*, **14**, 259 (1997). n) D. J. Faulkner, *Nat. Prod. Rep.*, **15**, 113 (1998). o) D. J. Faulkner, *Nat. Prod. Rep.*, **16**, 155 (1999). p) D. J. Faulkner, *Nat. Prod. Rep.*, **17**, 7 (2000). q) D. J. Faulkner, *Nat. Prod. Rep.*, **18**, 1 (2001). r) D. J. Faulkner, *Nat. Prod. Rep.*, **19**, 1 (2002). s) J. B. Blunt, B. R. Copp, M. H. G. Munro, P. T. Northcote, and M. R. Prinsep, *Nat. Prod. Rep.*, **20**, 1 (2003).

5 a) G. M. Cragg and D. J. Newman, *Cancer Invest.*, **17**, 153 (1999). b) B. H. Long, J. M. Carboni, A. J. Wasserman, L. A. Cornell, A. M. Casazza, P. R. Jensen, T. Lindel, W. Fenical, and C. R. Fairchild, *Cancer Res.*, **58**, 1111 (1998).

6 C. Birkeland and J. S. Lucas, "*Acanthaster planci*: Major Management Problem of Coral Reefs," CRC Press (1990).

7 a) R. F. G. Ormond, A. C. Campbell, S. H. Head, R. J. Moore, P. R. Rainbow, and A. P. Saunders, *Nature*, **246**, 167 (1973). b) A. R. S. Collins, *J. Exp. Mar. Biol. Ecol.*, **17**, 69 (1975).

8 T. Teruya, K. Suenaga, T. Koyama, Y. Nakano, and D. Uemura, *J. Exp. Mar. Biol. Ecol.*, **266**, 123 (2001).

9 R. J. Moore and C. J. Huxley, *Nature*, **263**, 407 (1976).

10 a) E. J. Schantz, V. E. Ghazarossian, H. K. Schnoes, F. M. Strong, J. P. Springer, J. O. Pezzanite, and J. Clardy, *J. Am. Chem. Soc.*, **97**, 1238 (1975). b) V. E. Ghazarossian, E. J. Schantz, H. K. Schnoes, and F. M. Strong, *Biochem. Biophys. Res. Commun.*, **59**, 1219 (1974).

11 a) Y.-Y. Lin, M. Risk, S. M. Ray, D. Van Engen, J. Clardy, J. Golik, J. C. James, and K. Nakanishi, *J. Am. Chem. Soc.*, **103**, 6773 (1981). b) Y. Shimizu, H. N. Chou, H. Bando, G. Van Duyne, and J. Clardy, *J. Am. Chem. Soc.*, **106**, 514 (1986). c) H. Ishida, A. Nozawa, K. Totoribe, N. Muramatsu, H. Nukaya, K. Tsuji, K. Yamaguchi, T. Yasumoto, H. Kasper, N. Berkett, and T. Kosuge, *Tetrahedron Lett.*, **36**, 725 (1995). d) A. Morohashi, M. Satake, K. Murata, H. Naoki, H. F. Kaspar, and T. Yasumoto, *Tetrahedron Lett.*, **36**, 8995 (1995). e) K. Murata, M. Satake, H. Naoki, H. F. Kaspar, and T. Yasumoto, *Tetrahedron*, **54**, 735 (1998).

12 M. Satake, K. Ofuji, H. Naoki, K. J. James, A. Furey, T. McMahon, J. Silke, and T. Yasumoto, *J. Am. Chem. Soc.*, **120**, 9967 (1998).

13 a) T. Seki, M. Satake, L. Mackenzie, H. F. Kaspar, and T. Yasumoto, *Tetrahedron Lett.*, **36**, 7093 (1995). b) M. Stewart, J. W. Blunt, M. H. G. Munro, W. T. Robinson, and D. J. Hannah, *Tetrahedron Lett.*, **38**, 4889 (1997).

14 a) T. Kosuge, K. Tsuji, K. Hirai, K. Yamaguchi, T. Okamoto, and Y. Iitaka, *Tetrahedron Lett.*, **22**, 3417 (1981). b) T. Kosuge, K. Tsuji, and K. Hirai, *Chem. Pharm. Bull.*, **30**, 3255 (1982).

15 a) T. Hu, J. M. Curtis, Y. Oshima, M. A. Quilliam, J. A. Walter, W. M. Watson-Wright, and J. L. C. Wright, *J. Chem. Soc., Chem. Commun.*, **1995**, 2159. b) T. Hu, J. M. Curtis, J. A. Walter, and J. L. C. Wright, *Tetrahedron Lett.*, **37**, 7671 (1996).

16 a) T. Otofuji, A. Ogo, J. Koishi, K. Matsuo, H. Tokiwa, T. Yasumoto, K. Nishihara, E. Yamamoto, M. Saisho, Y. Kurihara, and K. Hayashida, *Food Sanit. Res.*, **31**, 76 (1981). b) Department of Public Health, Fukuoka Prefecture, *Food Sanit. Res.*, **26**, 11 (1976).

17 S. Z. Zheng, F. L. Huang, S. C. Chen, X. F. Tan, J. B. Zuo, J. Peng, and R. W. Xie, *Zhongguo Haiyang Yaowu (Chin. J. Mar. Drugs)*, **33**, 33 (1990).

18 a) D. Uemura, T. Chou, T. Haino, A. Nagatsu, S. Fukuzawa, S. Z. Zheng, and H. S. Chen, *J. Am. Chem. Soc.*, **117**, 1155 (1995). b) T. Chou, O. Kamo, and D. Uemura, *Tetrahedron Lett.*, **37**, 4023 (1996).

19 T. Chou, T. Haino, M. Kuramoto, and D. Uemura, *Tetrahedron Lett.*, **37**, 4026 (1996).

20 N. Takada, N. Umemura, K. Suenaga, T. Chou, A. Nagatsu, T. Haino, K. Yamada, and D. Uemura, *Tetrahedron Lett.*, **42**, 3491 (2001).

21 Kishi's group achieved the total synthesis of pinnatoxin A (**3**) and *ent*-**3**. They reported that only natural pinnatoxin A was toxic. J. A. McCauley, K. Nagasawa, P. A. Lander, S. G. Mischke, M. A. Semones, and Y. Kishi, *J. Am. Chem. Soc.*, **120**, 7647 (1998).

22 N. Takada, N. Umemura, K. Suenaga, and D. Uemura, *Tetrahedron Lett.*, **42**, 3495 (2001).

23 N. Takada, M. Iwatsuki, K. Suenaga, and D. Uemura, *Tetrahedron Lett.*, **41**, 6425 (2000).

24 H. Kigoshi, N. Hayashi, and D. Uemura, *Tetrahedron Lett.*, **42**, 7469 (2001).

25 J. P. Devlin, O. E. Edwards, P. R. Gorham, N. R. Hunter, R. K. Pike, and B. Stavric, *Can. J. Chem.*, **55**, 1367 (1977).

26 R. L. Cahen and K. Tvede, *J. Pharmacol. Exp. Ther.*, **105**, 166 (1952).

27 a) K. Suenaga, H. Kigoshi, and K. Yamada, *Tetrahedron Lett.*, **37**, 5151 (1996). b) K. Suenaga, T. Mutou, T. Shibata, T. Itoh, H. Kigoshi, and K. Yamada, *Tetrahedron Lett.*, **37**, 6771 (1996). c) K. Suenaga, T. Nagoya, T. Shibata, H. Kigoshi, and K. Yamada, *J. Nat. Prod.*, **60**, 155 (1997). d) K. Suenaga, T. Shibata, N. Takada, H. Kigoshi, and K. Yamada, *J. Nat. Prod.*, **61**, 515 (1998). e) K. Suenaga, S. Kokubo, C. Shinohara, T. Tsuji, and D. Uemura, *Tetrahedron Lett.*, **40**, 1945 (1999). f) N. Takada, H. Sato, K. Suenaga, H. Arimoto, K. Yamada, K. Ueda, and D. Uemura, *Tetrahedron Lett.*, **40**, 6309 (1999). g) N. Takada, K. Suenaga, K. Yamada, S. Z. Zheng, H. S. Chen, and D. Uemura, *Chem. Lett.*, **1999**, 1025. h) S. Kokubo, K. Suenaga, C. Shinohara, T. Tsuji, and D. Uemura, *Tetrahedron*, **56**, 6435 (2000). i) S. Kokubo, K. Yogi, M. J. Uddin, T. Inuzuka, K. Suenaga, K. Ueda, and D. Uemura, *Chem. Lett.*, **2001**, 176. j) M. J. Uddin, S. Kokubo, K. Suenaga, K. Ueda, and D. Uemura, *Heterocycles*, **54**, 1039 (2001). k) N. Takada, M. Watanabe, A. Yamada, K. Suenaga, K. Yamada, K. Ueda, and D. Uemura, *J. Nat. Prod.*, **64**, 356 (2001). l) N. Takada, R. Watanabe, K. Suenaga, K. Yamada, and D. Uemura, *J. Nat. Prod.*, **64**, 653 (2001). m) H. Kigoshi, T. Itoh, T. Ogawa, K. Ochi, M. Okada, K. Suenaga, and K. Yamada, *Tetrahedron Lett.*, **42**, 7461 (2001). n) M. J. Uddin, S. Kokubo, K. Ueda, K. Suenaga, and D. Uemura, *J. Nat. Prod.*, **64**, 1169 (2001). o) M. J. Uddin, S. Kokubo, K. Ueda, K. Suenaga, and D. Uemura, *Chem. Lett.*, **2002**, 1028. p) T. Teruya, S. Nakagawa, T. Koyama, K. Suenaga, M. Kita, and D. Uemura, *Tetrahedron Lett.*, **44**, 5171 (2003).

28 a) H. Kigoshi, M. Ojika, K. Suenaga, T. Mutou, J. Hirano, A. Sakakura, T. Ogawa, M. Nisiwaki, and K. Yamada, *Tetrahedron Lett.*, **35**, 1247 (1994). b) H. Kigoshi, M. Ojika, T. Ishigaki, K. Suenaga, T. Mutou, A. Sakakura, T. Ogawa, and K. Yamada, *J. Am. Chem. Soc.*, **116**, 7443 (1994). c) H. Kigoshi, K. Suenaga, T. Mutou, T. Ishigaki, T. Atsumi, H. Ishiwata, A. Sakakura, T. Ogawa, M. Ojika, and K. Yamada, *J. Org. Chem.*, **61**, 5326 (1996). d) T. Mutou, K. Suenaga, T. Fujita, T. Itoh, N. Takada, K. Hayamizu, H. Kigoshi, and K. Yamada, *Synlett*, **1997**, 199. e) H. Sone, K. Suenaga, Y. Bessho, T. Kondo, H. Kigoshi, and K. Yamada, *Chem. Lett.*, **1998**, 85. f) K. Suenaga, K. Araki, T. Sengoku, and D. Uemura, *Org. Lett.*, **3**, 527 (2001). g) K. Araki, K. Suenaga, T. Sengoku, and D. Uemura, *Tetrahedron*, **58**, 1983 (2002).

29 G. R. Pettit, "The Dolastatins," in "Progress in the Chemistry of Organic Natural Products," ed by W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, and Ch. Tamm, Springer-Verlag, Wien (1997), Vol. 70, pp. 1–79.

30 K. Yamada and H. Kigoshi, *Bull. Soc. Chem. Jpn.*, **70**, 1479 (1997).

31 a) M. A. Walker and C. H. Heathcock, *J. Org. Chem.*, **56**, 5747 (1991). b) B. C. Raimundo and C. H. Heathcock, *Synlett*, **1995**, 1213.

32 a) T. Mukaiyama, K. Banno, and K. Narasaka, *J. Am. Chem. Soc.*, **96**, 7503 (1974). b) I. Paterson, J. D. Smith, and R. A. Ward, *Tetrahedron*, **51**, 9413 (1995).

33 Unpublished result.

34 M. G. Hollingshead, M. C. Alley, R. F. Camalier, B. J. Abbott, J. G. Mayo, L. Malspeis, and M. R. Grever, *Life Sci.*, **57**, 131 (1995).

35 T. Takahashi, H. Nagamiya, T. Doi, P. G. Griffiths, and A. M. Bray, *J. Comb. Chem.*, **5**, 414 (2003).

36 a) K. Yamada, M. Ojika, T. Ishigaki, T. Yoshida, H. Ekimoto, and M. Arakawa, *J. Am. Chem. Soc.*, **115**, 11020 (1993). b) M. Ojika, H. Kigoshi, T. Ishigaki, and K. Yamada, *Tetrahedron. Lett.*, **34**, 8501 (1993). c) M. Ojika, H. Kigoshi, T. Ishigaki, M. Nisiwaki, I. Tsukada, K. Mizuta, and K. Yamada, *Tetrahedron Lett.*, **34**, 8505 (1993). d) M. Ojika, H. Kigoshi, T. Ishigaki, I. Tsukada, T. Tsuboi, T. Ogawa, and K. Yamada, *J. Am. Chem. Soc.*, **116**, 7441 (1994).

37 D. A. Evans, J. Bartroli, and T. L. Shih, *J. Am. Chem. Soc.*, **103**, 2127 (1981).
38 T. Katsuki and K. B. Sharpless, *J. Am. Chem. Soc.*, **102**, 5974 (1980).
39 M. Julia and J.-M. Paris, *Tetrahedron Lett.*, **14**, 4833 (1973).
40 J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, and M. Yamaguchi, *Bull. Chem. Soc. Jpn.*, **52**, 1989 (1979).
41 M. Hikota, Y. Sakurai, K. Horita, and O. Yonemitsu, *Tetrahedron. Lett.*, **31**, 6367 (1990).
42 a) I. Paterson, C. J. Cowden, and M. D. Woodrow, *Tetrahedron Lett.*, **39**, 6037 (1998). b) I. Paterson, M. D. Woodrow, and C. J. Cowden, *Tetrahedron Lett.*, **39**, 6041 (1998). c) J. A. Marshall and B. A. Johns, *J. Org. Chem.*, **65**, 1501 (2000). d) I. Paterson, S. B. Blakey, and C. J. Cowden, *Tetrahedron Lett.*, **43**, 6005 (2002). e) M. A. Calter and X. Guo, *Tetrahedron*, **58**, 7093 (2002).
43 K. Suenaga, T. Ishigaki, A. Sakakura, H. Kigoshi, and K. Yamada, *Tetrahedron Lett.*, **36**, 5053 (1995).
44 a) I. Paterson, C. Watson, K.-S. Yeung, P. A. Wallace, and R. A. Ward, *J. Org. Chem.*, **62**, 452 (1997). b) I. Paterson, K.-S. Yeung, C. Watson, R. A. Ward, and P. A. Wallace, *Tetrahedron*, **54**, 11935 (1998). c) I. Paterson, C. Watson, K.-S. Yeung, R. A. Ward, and P. A. Wallace, *Tetrahedron*, **54**, 11955 (1998). d) R. Nakamura, K. Tanino, and M. Miyashita, *Org. Lett.*, **5**, 3579 (2003). e) R. Nakamura, K. Tanino, and M. Miyashita, *Org. Lett.*, **5**, 3583 (2003).
45 a) P. Liu and J. S. Panek, *J. Am. Chem. Soc.*, **122**, 1235 (2000). b) J. S. Panek and P. Liu, *J. Am. Chem. Soc.*, **122**, 11090 (2000).
46 S. Saito, S. Watabe, H. Ozaki, H. Kigoshi, K. Yamada, N. Fusetani, and H. Karaki, *J. Biochem.*, **120**, 552 (1996).
47 M. Coue, S. L. Brenner, I. Spector, and E. D. Korn, *FEBS Lett.*, **213**, 316 (1987).
48 G. M. Patterson, C. D. Smith, L. H. Kimura, B. A. Britton, and S. Carmeli, *Cell Motil. Cytoskeleton*, **24**, 39 (1993).
49 K. Furukawa, K. Sakai, S. Watanabe, K. Maruyama, M. Murakami, K. Yamaguchi, and Y. Ohizumi, *J. Biol. Chem.*, **268**, 26026 (1993).
50 S. Saito, S. Watabe, H. Ozaki, N. Fusetani, and H. Karaki, *J. Biol. Chem.*, **269**, 29710 (1994).
51 M. R. Bubb, I. Spector, A. D. Bershadsky, and E. D. Korn, *J. Biol. Chem.*, **270**, 3463 (1995).
52 S. Watabe, S. Wada, S. Saito, S. Matsunaga, N. Fusetani, H. Ozaki, and H. Karaki, *Cell Struct. Funct.*, **21**, 199 (1996).
53 a) K. Suenaga, N. Kamei, Y. Okugawa, M. Takagi, A. Akao, H. Kigoshi, and K. Yamada, *Bioorg. Med. Chem. Lett.*, **7**, 269 (1997). b) H. Kigoshi, K. Suenaga, M. Takagi, A. Akao, K. Kanematsu, N. Kamei, Y. Okugawa, and K. Yamada, *Tetrahedron*, **58**, 1075 (2002).

Kiyotake Suenaga was born in Aizu Wakamatsu, Fukushima, Japan in 1969. He received his B. Sc. in 1992 and Ph. D. in 1997 from Nagoya University under the direction of Professor K. Yamada. In 1995, he became a research associate of Nagoya University and moved to School of Pharmaceutical Science, University of Shizuoka in 2001. He was appointed as an assistant professor at University of Tsukuba in 2003. His current research interests lie in natural products chemistry: isolation, structure elucidation, synthesis, and biological function.