

Review

Cytotoxic substances from two species of Japanese sea hares: chemistry and bioactivity

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Abstract: From the sea hares belonging to two genera, *Aplysia* and *Dolabella*, a variety of new cytotoxic substances were isolated as minute constituents: their chemical structures were determined and their cytotoxicity was evaluated. Regarding the highly cytotoxic substances, further chemical and biological studies were performed that included their asymmetric chemical synthesis and elucidation of biological characteristics such as antitumor activity.

Keywords: cytotoxic substances, sea hare, isolation, chemical structure, chemical synthesis, antitumor activity

1. Introduction

A large number of bioactive substances have been isolated and characterized from marine organisms such as microorganisms, algae, sponges, tunicates, coelenterates, echinoderms, bryozoans, and mollusks.¹⁾ Sea hares are shell-less and slow-moving marine mollusks that feed on a variety of algae and sponges. Sea hares, which are not eaten by other marine animals, have been postulated to have chemical defense substances. The poisonous properties of the sea hare secretions were already known in the Roman times. Sea hares have been known to be a rich source of bioactive substances, which have generally been isolated as minute constituents of sea hares and are considered to be of dietary origin and/or to be produced by symbiotic microbes.²⁾

Since the notable report on the isolation of aplysin, a bromosquiterpene, by Yamamura and Hirata,³⁾ chemical constituents of the sea hare of the genus *Aplysia* were examined extensively to yield

various compounds, most of which were halogenated. The Pettit group investigated the bioactive constituents of the sea hare of the genus *Dolabella* collected in the Indian Ocean, resulting in the isolation of fifteen novel peptide- and depsipeptide-type compounds named dolastatins 1–15, most of which were cytostatic and antineoplastic.⁴⁾

We intensively examined the cytotoxic constituents of the sea hares of two species, *Aplysia kurodai* and *Dolabella auricularia*, collected off the coast of the Shima peninsula, Mie prefecture, Japan, and isolated a number of cytotoxic substances. In the present article, the authors comprehensively describe the research results of chemistry (structure, synthesis, etc.) and bioactivity (cytotoxicity, antitumor activity, target biomolecule, etc.) regarding thirty-two cytotoxic substances isolated from the two species of sea hares, *A. kurodai* and *D. auricularia* mentioned above: these results were obtained by the authors' group during the period between 1990 and 2008. Furthermore, this article includes the related research results up to 2009 that were reported by a number of domestic and overseas research groups. Previously, the reviews published by the authors (1997⁵⁾ and 2000⁶⁾) described the chemical and bioactive aspects of the compounds obtained from these two species of sea hares: the references up to 1998 were covered. Another review published in 2009⁷⁾ dealt with only aplyronines isolated from *A. kurodai* and covered the references up to 2007. Besides the aforemen-

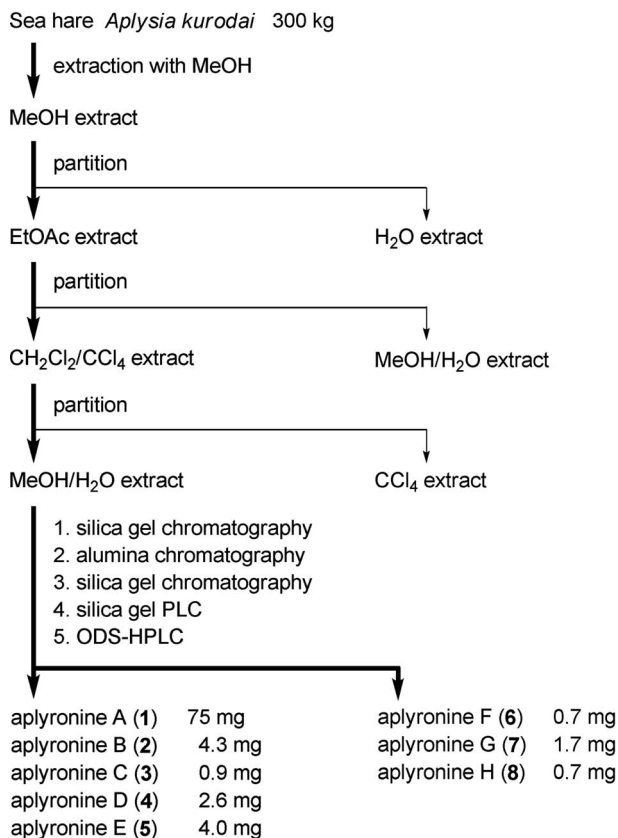
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Scheme 1. Isolation procedure for aplyronines A-H (1–8).

tioned reviews by the authors, a review by Kamiya and co-workers is available regarding the bioactive compounds obtained from these sea hares.⁸⁾

2. Cytotoxic substances from the sea hare *Aplysia kurodai*

2.1. Aplyronine A and aplyronines B–H.

2.1.1. Isolation. Originally, aplyronine A (1) was isolated by eight-step chromatographic separation of the lipophilic extract of the sea hare *A. kurodai* guided by cytotoxicity assay using human tumor (HeLa S₃) cells.^{9),10)} Subsequently, we developed a more efficient method for the isolation of 1, which enabled us to isolate not only a considerable quantity of 1 but also seven minor congeners, aplyronines B (2) and C (3)^{9),10)} and D–H (4–8)^{6),7)} (Scheme 1).

2.1.2. Structures. The gross structure of aplyronine A (1) was elucidated on the basis of detailed spectral (IR, NMR, and mass) analysis.^{9),10)} The absolute stereostructure of 1, possessing seventeen asymmetric centers, was determined by NMR spectral analysis and the asymmetric synthesis of the five fragments obtained from chemical degradation

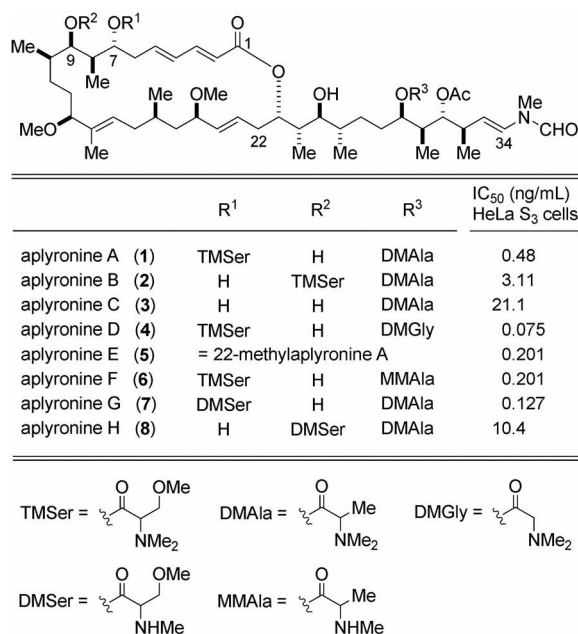
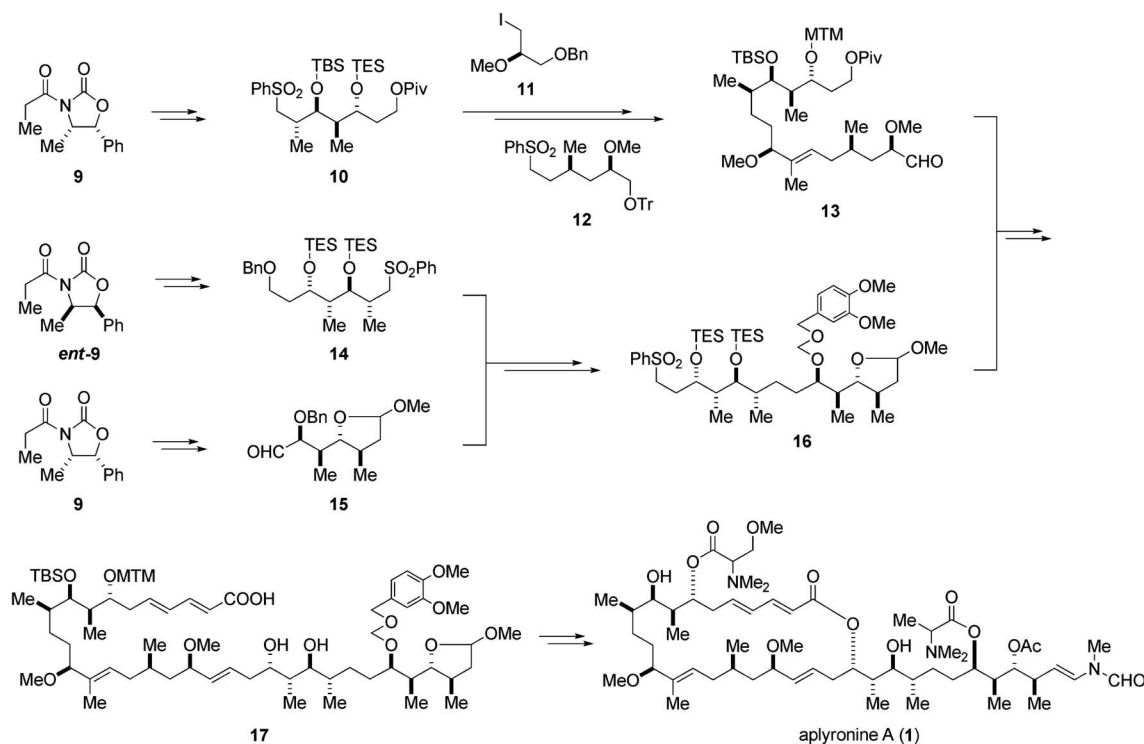


Fig. 1. Structures and cytotoxicity of aplyronines A-H (1–8).

of aplyronine A (1).^{7),9)–13)} Aplyronine A (1) was revealed to be an inseparable mixture of four diastereomers as to two amino acid esters: this was confirmed by the asymmetric chemical synthesis of 1 as a diastereomeric mixture of the amino acid esters with the same ratios as in the case of natural 1.^{14)–16)}

The structural elucidation of aplyronines B–H (2–8) was performed by means of the spectral analysis, which led us to deduce their structures (Fig. 1).^{6),7),9),10)} Among them, the structures of aplyronines B (2) and C (3) were confirmed by the asymmetric chemical synthesis of 2 and 3, respectively.^{16),17)} The structural diversity of aplyronines except for aplyronine E (5) originates in variation of the amino acid residues as well as difference in their locations.

2.1.3. Asymmetric chemical synthesis of aplyronines A–C. The authors' group achieved the asymmetric chemical synthesis of aplyronine A (1) (Scheme 2), which brought on the following results.^{14)–16)} First, the asymmetric synthesis of 1 unambiguously confirmed its absolute stereostructure. Secondly, while the supply of 1 from the natural source was scarce, the synthesis provided a sufficient amount of 1, which enabled further biological tests. Thirdly, the structure-activity relationships of 1 were examined by employing the analogs and intermediates obtained in the course of the synthesis of 1. The asymmetric chemical synthesis of aplyronines



Scheme 2. Outline of the asymmetric chemical synthesis of aplyronine A (1).

B (2) and C (3) was also performed.^{16,17)} Although the efforts have been made to synthesize aplyronines worldwide, chemical synthesis of aplyronines has not been reported so far except for our synthesis.

2.1.4. Cytotoxicity. Aplyronines A–H (1–8) exhibit strong cytotoxicity against HeLa S₃ cells *in vitro* (Fig. 1).^{6,7)} Five among eight aplyronines (1, 4, 5, 6, 7) are particularly strong cytotoxins. The structural feature common to these five aplyronines is the presence of the methylated serine ester moiety at C7 of the macrolide part of the molecule: aplyronine A (1) is more cytotoxic (*ca.* 50 times) than aplyronine C (3) that lacks the methylated serine ester group. Furthermore, the location of the methylated serine ester group in the molecule affects the cytotoxicity: aplyronine A (1) is considerably more cytotoxic than aplyronine B (2) or aplyronine H (8).

2.1.5. Antitumor activity. Aplyronine A (1) exhibited potent antitumor activity *in vivo* against five tumor cell lines^{6,7,9)} (Table 1). The activity was dose-dependent. Particularly, the antitumor activity against P388 leukemia, Lewis lung carcinoma and Ehrlich carcinoma is noteworthy. It is remarkable that all the mice employed for the testing survived

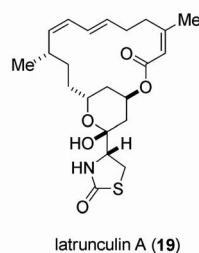
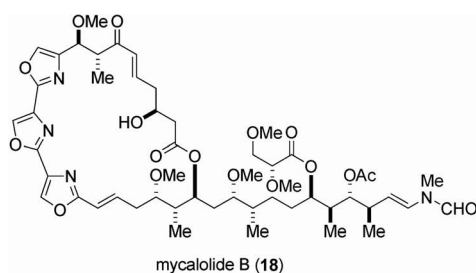
after the end of the experimental term (60 days) in the case of Lewis lung carcinoma. Aplyronine A (1) is regarded as a candidate for the development of antitumor agents.¹⁸⁾

2.1.6. The target biomolecule: actin. The target biomolecule of aplyronine A (1) was found to be actin, which is one of the most abundant and common proteins in the cytoskeleton.¹⁹⁾ Aplyronine A (1) was shown to form a 1:1 complex with globular actin (G-actin, monomer), and to inhibit polymerization of G-actin to fibrous actin (F-actin, polymer). Aplyronine A (1) proved to depolymerize F-actin to G-actin by severing. Mycalolide B (18),^{20,21)} a cytotoxic substance isolated from a marine sponge by Fusetani and co-workers, was reported to depolymerize F-actin by severing and to form a 1:1 complex with G-actin.²²⁾ The side chain portion of aplyronine A (1) was deduced to participate in its binding toward actin, because of the structural similarity concerning the side chain part for both compounds, aplyronine A (1) and mycalolide B (18). Latrunculins [e.g., latrunculin A (19)] were the first actin-binding substances isolated from a marine source.^{23,24)} Since then, a considerable number of cytotoxic marine

Table 1. Antitumor activity of aplyronine A (**1**)

Tumor	Route ^{a)}	Dose (mg/kg/day)	Median survival time (days)	Test/Control (%)	Number of survivors after 60 days
P388 leukemia	i.p.	0.08	59.9	545	4 / 6
		0.04	46.0	418	2 / 6
		0.02	17.3	157	0 / 6
		0.01	14.4	131	0 / 6
		–	11.0	100	0 / 7
	Controls	–	11.0	100	0 / 7
Colon C26 carcinoma	i.p.	0.08	40.0	255	0 / 6
		0.04	39.0	248	1 / 6
		0.02	25.0	159	1 / 6
		0.01	21.0	134	0 / 6
		–	15.7	100	0 / 10
	Controls	–	15.7	100	0 / 10
Lewis lung carcinoma	i.p.	0.08	9.3	86	0 / 6
		0.04	60.1	556	6 / 6
		0.02	59.9	555	4 / 6
		0.01	29.0	269	2 / 6
		–	10.8	100	0 / 8
	Controls	–	10.8	100	0 / 8
B16 melanoma	i.p.	0.08	10.1	43	0 / 6
		0.04	46.8	201	0 / 6
		0.02	43.0	185	0 / 6
		0.01	35.0	152	0 / 6
		–	23.3	100	1 / 9
	Controls	–	23.3	100	1 / 9
Ehrlich carcinoma	i.p.	0.08	12.0	80	0 / 6
		0.04	59.7	398	2 / 6
		0.02	33.0	220	1 / 6
		0.01	21.8	145	0 / 6
		–	15.0	100	0 / 8
	Controls	–	15.0	100	0 / 8

^{a)} Schedule: i.p. days 1–5. Aplyronine A (**1**) was dissolved in DMSO (0.08 mg/mL) and then diluted with a physiological solution of NaCl.



natural products that interact with actin have been identified. For details regarding actin-targeting natural products, reviews are available.^{25),26)}

2.1.7. Structure-activity relationships. Bioactivity of eighteen synthetic analogs and natural aplyronines was examined.^{16),27),28)} Selected data about cytotoxicity and actin depolymerizing activity of these compounds are illustrated in Fig. 2.

(i) Structure-cytotoxicity relationship. In order for aplyronine A (**1**) to reveal strong cytotoxicity, the combination of the macrolide ring part and the side chain part is essential, because either the analog **21** (the macrolide moiety alone) or the analog **23** (the side chain moiety alone) showed extremely weak cytotoxicity (Fig. 2). Regarding the functional groups in **1**, the presence of the trimethylserine ester,

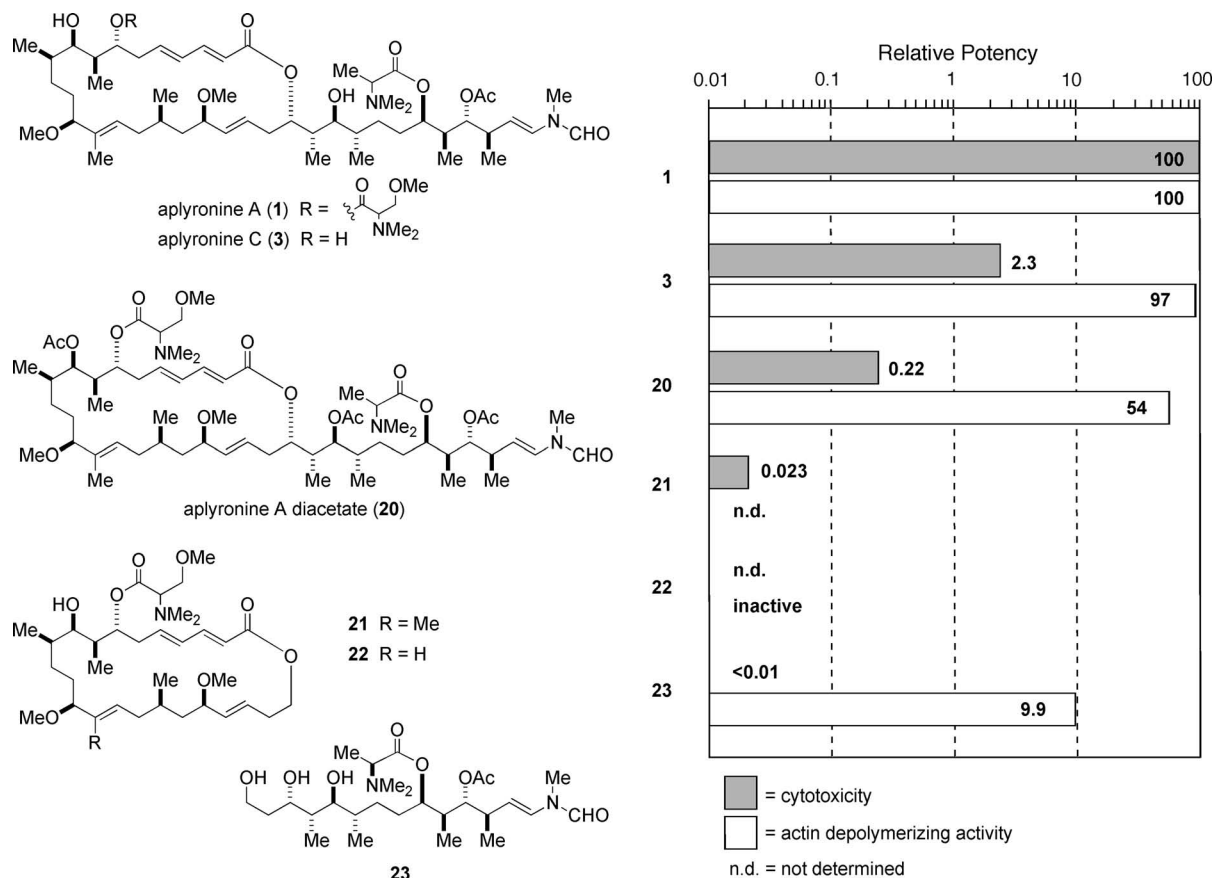
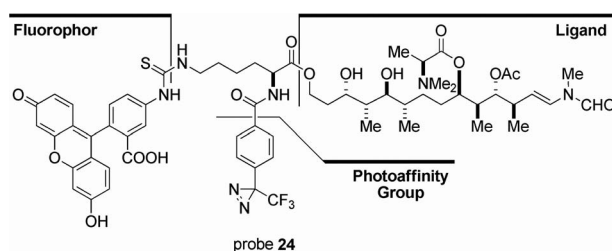


Fig. 2. Structure-activity relationships of aplyronine A (**1**) and its analogs.

the conjugated diene, and two hydroxyl groups was necessary for the strong cytotoxicity of **1**.

(ii) Structure-actin depolymerizing activity relationship. The side chain moiety of **1** was shown to play a key role in the actin depolymerizing activity, because the synthetic analog **23** only consisting of the side chain portion of **1** revealed relatively strong activity and the synthetic analog **22** without the side chain was totally inactive (Fig. 2). Further chemical evidence about the interaction between aplyronine A (**1**) and actin was obtained by photoaffinity labeling experiments, which employed probe **24** containing the side chain portion of **1**.²⁹ Since aplyronine A (**1**) competitively inhibited the binding of the probe **24** to actin, the probe **24** proved to bind to actin specifically at the binding site of aplyronine A (**1**).

(iii) Relation between cytotoxicity and actin depolymerizing activity. As described above, the side chain portion of aplyronine A (**1**) is essential for both cytotoxicity and actin depolymerizing activity. On the other hand, cytotoxicity of **1** is markedly



influenced by the trimethylserine ester, the conjugated diene and two hydroxyl groups, whereas these functional groups affect actin depolymerizing activity to a quite small extent.

2.1.8. Structure of the actin-aplyronine A complex. The three-dimensional structure of the actin-aplyronine A (**1**) complex (referred to as the AA complex) was determined by X-ray crystallographic analysis, providing details about the molecular interactions between actin and aplyronine A (**1**) (Fig. 3).³⁰

Aplyronine A (**1**) binds to the hydrophobic cleft

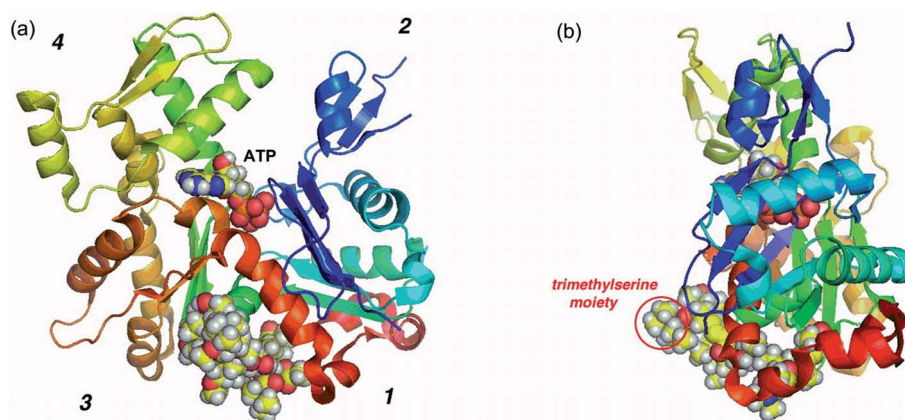


Fig. 3. (a) Structure of the actin-aplyronine A (**1**) complex; (b) the same complex rotated by *ca.* 90°.

composed of subdomains 1 and 3 of actin by intercalating the side chain of **1** into actin. The hydrophobic interactions between the cleft of actin and aplyronine A (**1**) play a key role in the actin depolymerizing activity. The macrolide part of aplyronine A (**1**) binds to subdomain 3 of actin in the AA complex: the conformation of the macrolide part of **1** within subdomain 3 is stabilized by the interactions involving a hydrogen bond between the hydroxyl group at C9 of **1** and Glu334 of actin. One important feature of the structure of the AA complex is that the trimethylserine moiety protrudes toward the bulk solvent region from the molecular surface of actin and does not interact with actin (Fig. 3(b)).

These structural characteristics are considered to be associated with the cytotoxicity of aplyronine A (**1**). Actually, the cytotoxicity of aplyronine C (**3**) (which lacks the trimethylserine moiety) and aplyronine A diacetate (**20**) (which has no hydroxyl group at C9) is weak (Fig. 2).

The cytotoxic analogs of aplyronine A (**1**) necessarily possess actin depolymerizing activity, whereas the analogs having actin depolymerizing activity are not necessarily cytotoxic. The above findings suggest that aplyronine A (**1**) first binds to actin to form the actin-aplyronine A (**1**) complex, which secondly binds to another biomolecule in order to reveal cytotoxicity: the protruding trimethylserine ester group of **1** would play an important role in the second stage.

2.2. Aplaminones. Three cytotoxic alkaloids, aplaminone (**25**), neoplaminone (**26**) and neoplaminone sulfate (**27**) were isolated from the sea hare *A. kurodai*, and their structures were elucidated by the spectral analysis (Fig. 4).³¹⁾ The absolute stereo-

structures of **25** and **26** were determined by the chemical method.³²⁾ These substances showed cytotoxicity against HeLa S₃ cells *in vitro* [IC₅₀ (μg/mL): 0.28 for **25**, 1.6×10^{-7} for **26**, and 0.51 for **27**].

3. Cytotoxic substances from the sea hare *Dolabella auricularia*

We performed the bioassay-guided fractionation of the methanol extract of the sea hare *Dolabella auricularia* and isolated various cytotoxic substances, most of which were minute constituents of the sea hare: some of them were obtained in sub-milligram quantities. Cytotoxicity of these compounds was evaluated and listed in Table 2. Among the isolated substances, aurilide (**28**), dolastatin H (**29**), isodolastatin H (**30**) and dolicolide (**31**) were highly cytotoxic, further studies of which were performed in detail.

3.1. Aurilide. Aurilide (**28**) was isolated as a trace constituent: 0.5 mg from 262 kg of the sea hare.³³⁾ The isolation procedure involved two-step solvent partition and subsequent ten-step chromatographic separation. The gross structure of aurilide (**28**) was determined by the spectral analysis, and the absolute stereostructure of the peptide part was elucidated by chiral HPLC analysis of the component amino acids and isoleucic acid obtained by acid hydrolysis of **28**. The absolute stereostructure of a new dihydroxy fatty acid in **28** was elucidated by the asymmetric chemical synthesis. Thus, the absolute stereostructure of aurilide was determined to be a 26-membered cyclodepsipeptide as shown in **28** (Fig. 4).³³⁾ The asymmetric chemical synthesis of aurilide (**28**) was achieved, which not only confirmed its stereostructure but also supplied the ample

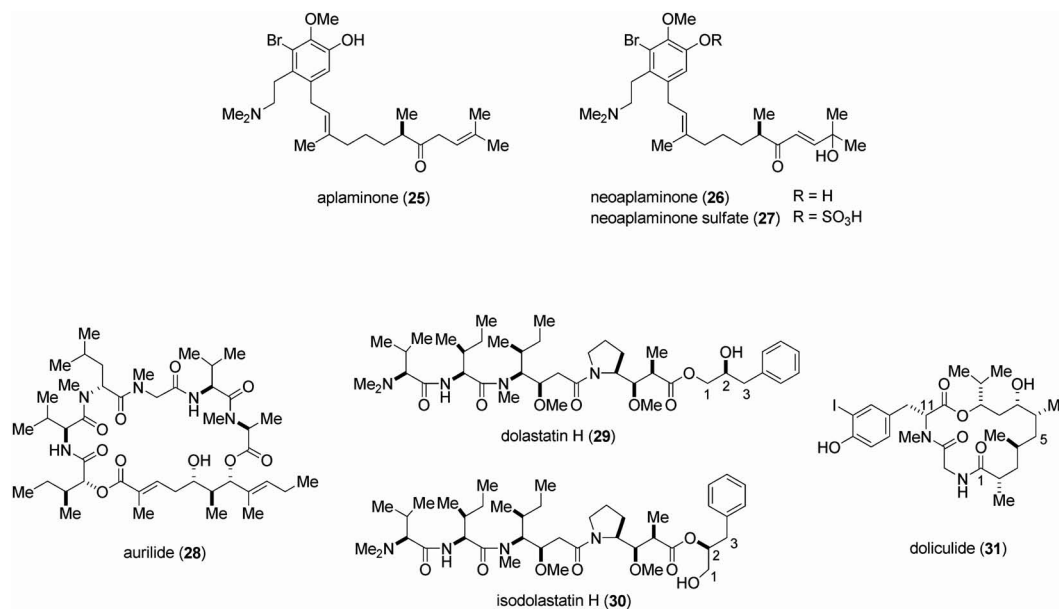


Fig. 4. Cytotoxic substances (25-27) from the sea hare *A. kurodai* and highly cytotoxic substances (28-31) from the sea hare *D. auricularia*.

Table 2. Cytotoxicity of substances from the sea hare *D. auricularia*

Substance	IC ₅₀ (μg/mL) HeLa S ₃ cells
aurilide (28)	0.011
dolastatin H (29)	0.0022
isodolastatin H (30)	0.0016
doliculide (31)	0.005
dolastatin C (32)	17.0
dolastatin D (33)	2.2
dolastatin E (34)	22.0
dolastatin G (35)	1.0
isodolastatin G (36)	5.3
dolastatin I (37)	12.0
dolabellin (38)	6.1
auriside A (39)	0.17
auriside B (40)	1.2
auripyrone A (41)	0.26
auripyrone B (42)	0.48
dolabelide A (43)	6.3
dolabelide B (44)	1.3
dolabelide C (45)	1.9
dolabelide D (46)	1.5
aurilol (47)	4.3
auriculol (48)	6.7

amount of **28** on a gram scale.³⁴⁾ The scarcity of the natural sample of **28** prevented the evaluation of its cytotoxicity. Thus, cytotoxicity of aurilide (**28**) was estimated by employing the synthetic sample and was shown to be strong against HeLa S₃ cells with an IC₅₀ value of 11 ng/mL (Table 2). Interestingly, 6-*epi*-aurilide obtained by chemical synthesis was almost non-cytotoxic (IC₅₀ > 4 μg/mL).³⁵⁾ The NCI's human cancer cell panel indicated that aurilide (**28**) showed a high level of cytotoxicity (the mean panel GI₅₀ concentration was 0.12 μg/mL), and that **28** was selectively active against lung, ovarian, renal and prostate cancer cell lines.³⁵⁾ Aurilide (**28**) was not cytotoxic but cytostatic against human leukemia cell lines. Aurilide (**28**) showed unusually high *in vivo* antitumor activity in the NCI's hollow-fiber assays, but did not have significant antitumor activity owing to toxicity in the *in vivo* human tumor xenograft tests.

Solid phase library synthesis of aurilide (**28**) and its analogs was achieved by Takahashi, Doi and co-workers in 2003.³⁶⁾ The synthesis of the aurilide analogs was performed and the structure-cytotoxicity relationship was investigated in 2008.³⁷⁾

Aurilides B and C were isolated from a marine cyanobacterium and their stereostructures were determined by Gerwick and co-workers in 2006.³⁸⁾ Struc-

turally, aurilides B and C are closely related to aurilide (**28**). Cytotoxicity of aurilides B and C against various cancer cell lines was evaluated.³⁸⁾

In 2004, Nakao, Scheuer and co-workers isolated kulokekahilide-2, a cytotoxin structurally related to aurilide (**28**), from a Hawaiian marine mollusk and elucidated its structure,³⁹⁾ which was revised by Kimura and co-workers in 2008.⁴⁰⁾

3.2. Dolastatin H and isodolastatin H.

Dolastatin H (**29**) and isodolastatin H (**30**) were isolated in trace amounts: 0.3 mg each from 33 kg of the sea hare.⁴¹⁾ On the basis of the spectroscopic analysis, these compounds were shown to be peptide esters that were closely related to dolastatin 10 (Fig. 4).⁴²⁾ The structural feature of these compounds is that 3-phenylpropan-1,2-diol is attached through the ester linkage to the C-terminus of a tetrapeptide containing unusual amino acids. The asymmetric chemical synthesis of dolastatin H (**29**) and isodolastatin H (**30**) was executed, which unambiguously determined the absolute stereostructures of both compounds, **29** and **30**.⁴¹⁾ Owing to the scarcity of the natural samples, cytotoxicity of **29** and **30** was evaluated by employing the synthetic samples. Dolastatin H (**29**) and isodolastatin H (**30**) showed strong cytotoxicity against HeLa S₃ cells with IC₅₀ values of 2.2 and 1.6 ng/mL, respectively (Table 2). The C2 epimers of **29** and **30** are less cytotoxic (IC₅₀: 20 and 29 ng/mL, respectively) than **29** and **30** themselves.⁴¹⁾ These findings suggest that cytotoxicity of dolastatins H (**29**) and isodolastatin H (**30**) is slightly affected by the C-terminal stereostructures of these peptide esters. The *in vivo* antitumor activity of **29** and **30** was examined. While no significant activity was shown for dolastatin H (**29**), isodolastatin H (**30**) exhibited antitumor activity with a T/C value of 141% against P388 leukemia (intraperitoneal tumor inoculation-intravenous drug administration). This antitumor activity for **30** is a little weaker than that for dolastatin 10, a well-known potent antitumor agent (T/C, 155% against P388 leukemia).

Scheuer and co-workers isolated malevamide D, an isodolastatin H (**30**) analog, from a marine cyanobacterium and elucidated its structure in 2002.⁴³⁾ Malevamide D demonstrated cytotoxicity against four cancer cell lines in the subnanomolar range.⁴³⁾

3.3. Dolicolide. Dolicolide (**31**) was isolated as a minute constituent: 8.2 mg from 33 kg of the sea hare. The structure including stereochemistry was elucidated by means of the spectroscopic and chemi-

cal methods to be a cyclodepsipeptide of mixed peptide-polyketide biogenesis (Fig. 4).⁴⁴⁾ The asymmetric chemical synthesis of dolicolide (**31**) was achieved, which unambiguously confirmed its absolute stereostructure.^{45),46)} Dolicolide (**31**) was shown to be highly cytotoxic (Table 2). Dolicolide (**31**) phosphate showed antitumor effect to a considerable extent against Lu-61 xenograft in nude mice. In the course of the asymmetric chemical synthesis of **31**, analogs of dolicolide (**31**) were synthesized, which were utilized to examine the structure-activity relation of **31**. One example is the finding that 11-*epi*-dolicolide was 1000-fold less cytotoxic than dolicolide (**31**): this fact suggested that the conformation of the 16-membered dilactam-lactone ring would be important for cytotoxicity, because the conformation of dolicolide (**31**) was significantly different from that of 11-*epi*-dolicolide.

Dolicolide (**31**) is structurally related to cyclodepsipeptides such as geodiamolides^{47),48)} and jaspamide (jaspalakinolide).^{49),50)}

The asymmetric chemical synthesis of dolicolide (**31**) was also achieved by the Ghosh group in 2001⁵¹⁾ and by the Hanessian group in 2004.⁵²⁾ Hamel, Ghosh and co-workers reported that dolicolide (**31**) arrested cells at the G₂/M phase of the cell cycle by interfering with normal actin assembly.⁵³⁾

3.4. Peptides and depsipeptides. In addition to the strong cytotoxins (**28–31**) described above, we isolated cytotoxic peptides and depsipeptides as minute constituents of the sea hare *D. auricularia* as follows (Fig. 5).

Dolastatin C (**32**) is a linear depsipeptide, the structure of which was elucidated by chemical and spectral methods and was confirmed by the asymmetric chemical synthesis.⁵⁴⁾ Dolastatin C (**32**) was shown to be weakly cytotoxic (Table 2). Dolastatin D (**33**) is a cyclodepsipeptide. The structure of **33** was determined by chemical and spectral means and was confirmed by the asymmetric chemical synthesis.⁵⁵⁾ Dolastatin D (**33**) exhibited moderate cytotoxicity (Table 2). Dolastatin E (**34**) is a cyclic hexapeptide that contains a thiazoline moiety labile to be epimerized.⁵⁶⁾ The gross structure of **34** was elucidated by spectral data,⁵⁶⁾ and the stereostructure was determined by the asymmetric chemical synthesis.⁵⁷⁾ Dolastatin E (**34**) showed weak cytotoxicity (Table 2).

Dolastatin G (**35**) is a 35-membered depsipeptide that contains two new fatty acids.⁵⁸⁾ The stereo-

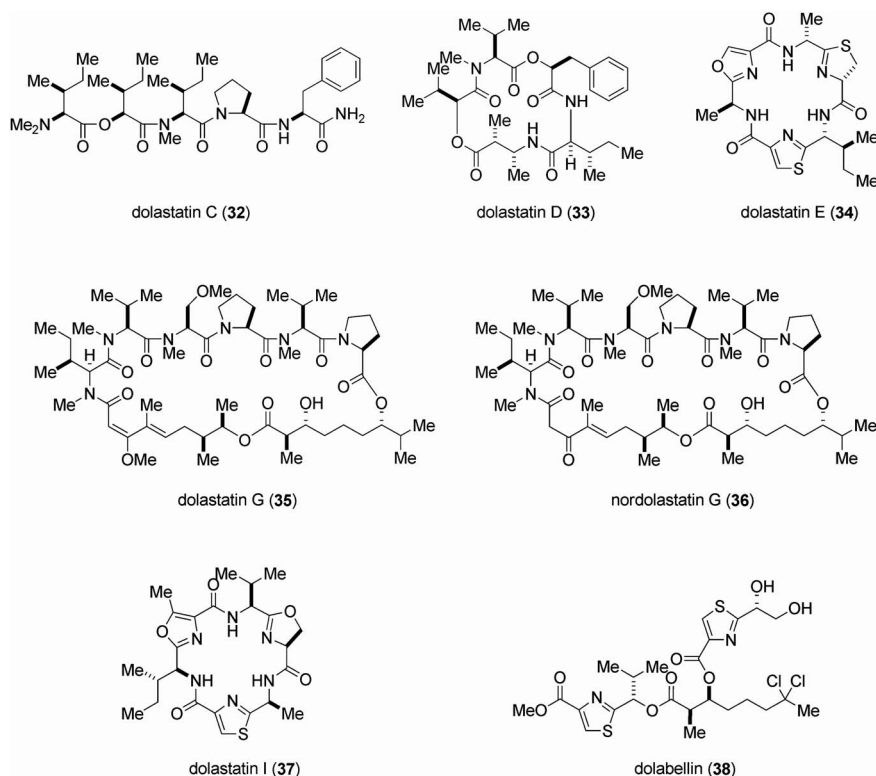


Fig. 5. Cytotoxic peptides and depsipeptides from the sea hare *D. auricularia*.

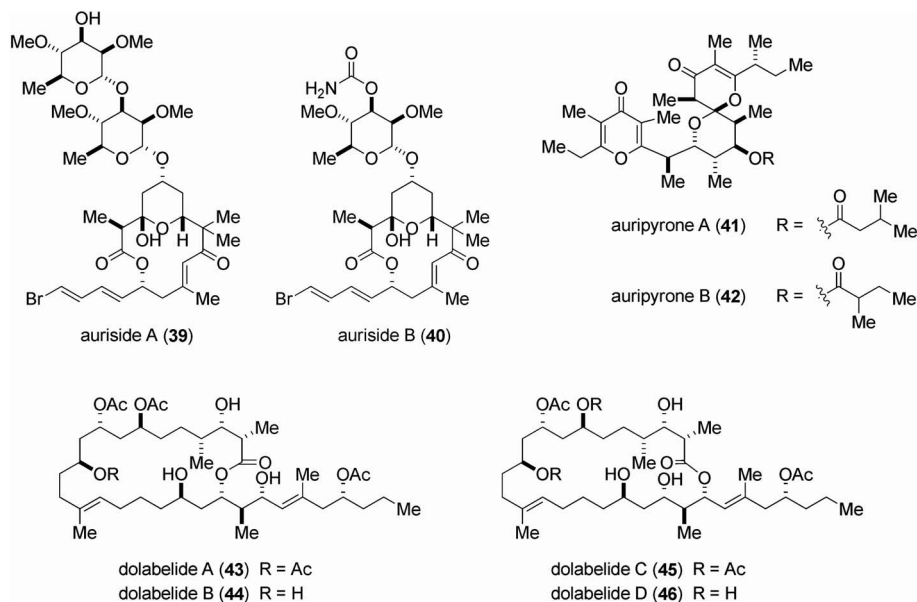
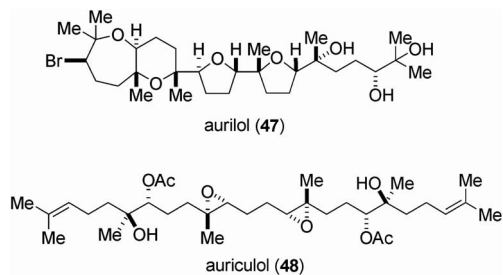
structure of **35** was established by spectral data and the chemical method⁵⁸⁾ and was confirmed by the asymmetric chemical synthesis.⁵⁹⁾ Nordolastatin G (**36**) is a congener of dolastatin G (**35**): its structure was disclosed by chemical correlation with dolastatin G (**35**) and by asymmetric chemical synthesis.^{58),59)} Both compounds (**35**, **36**) were shown to be moderately cytotoxic (Table 2). Moore, Paul and co-workers isolated lyngbyastatin 2 and norlyngbyastatin 2, analogs of dolastatin G (**35**) and nordolastatin G (**36**), from a marine cyanobacterium and elucidated their structures by the NMR spectroscopic method in 1999.⁶⁰⁾ Dolastatin I (**37**) is a cyclic hexapeptide, the structure of which was determined by spectral analysis:⁶¹⁾ the stereostructure was established by the chiral HPLC analytical method and was confirmed by the asymmetric chemical synthesis.^{61),62)} Cytotoxicity of **37** was weak (Table 2). Dolabellin (**38**) is a bithiazole substance. Its gross structure was elucidated by spectral analysis, and subsequently the stereostructure was determined by means of the chemical method, which was confirmed by the asymmetric chemical synthesis.⁶³⁾ Cytotoxicity of **38** was shown to be moderate (Table 2).

Gerwick, Scheuer and co-workers isolated hectochlorin, a potent stimulator of actin assembly, from a marine cyanobacterium and determined its structure in 2002.⁶⁴⁾ Structurally, hectochlorin is closely related to dolabellin (**38**).

3.5. Polyketides. As minute constituents of the sea hare *D. auricularia*, we isolated the following cytotoxic polyketides (Fig. 6).

Aurisides A (**39**) and B (**40**) were separated in sub-milligram quantities: 0.8 mg of **39** and 0.7 mg of **40** from 278 kg of the sea hare.⁶⁵⁾ First, their gross structures were elucidated to be macrolide glycosides by virtue of spectroscopic analysis, and secondly, their stereostructures were determined on the basis of spectral analysis in conjunction with chemical degradation experiments.⁶⁵⁾ Whereas cytotoxicity of auriside A (**39**) was shown to be relatively strong, auriside B (**40**) was moderately cytotoxic (Table 2). Paterson and co-workers achieved the asymmetric chemical synthesis of aurisides A (**39**) and B (**40**) in 2005.⁶⁶⁾ We executed the asymmetric chemical synthesis of both substances (**39**, **40**) in 2006.^{67),68)}

Two closely related polypropionates, auripyrones A (**41**) and B (**42**) were isolated as minute con-

Fig. 6. Cytotoxic polyketides from the sea hare *D. auricularia*.Fig. 7. Cytotoxic terpenoids from the sea hare *D. auricularia*.

stituents: 1.0 mg of **41** and 1.7 mg of **42** from 452 kg of the sea hare.⁶⁹⁾ The gross structures of both substances (**41**, **42**) were determined by spectral analysis. Relative stereochemistry of **41** and **42** except for the ester part of **42** was established by the NMR spectral analysis. Auripyrones A (**41**) and B (**42**) were shown to be relatively strong cytotoxins (Table 2). The Perkins group performed the asymmetric chemical synthesis of auripyronone A (**41**) in 2006, which established the absolute stereostructure.⁷⁰⁾ Subsequently, in 2009, the Jung group achieved the chemical synthesis of auripyronone A (**41**).⁷¹⁾

Dolabelides A (**43**), B (**44**), C (**45**) and D (**46**) are closely related macrolides with moderate cytotoxicity (Table 2), and their stereostructures were determined by a combination of the chemical and spectroscopic means.^{72),73)} Leighton and co-workers

achieved the asymmetric chemical synthesis of dolabelide D (**46**) in 2006.⁷⁴⁾

3.6. Terpenoids. Concerning cytotoxic terpenoids, two substances were isolated as minute constituents of the sea hare (Fig. 7).

Aurilol (**47**) is a bromotriterpene, and the structure including the absolute stereochemistry of the five asymmetric centers among ten was determined by spectroscopic and chemical means.⁷⁵⁾ Subsequently, Morimoto and co-workers elucidated the complete stereostructure of **47** and performed the asymmetric chemical synthesis of **47** in 2005.⁷⁶⁾ Auriculol (**48**) is a highly oxygenated squalene, and the stereostructure was determined by means of the spectroscopic and chemical methods.⁷⁷⁾ Both aurilol (**47**) and auriculol (**48**) exhibited moderate cytotoxicity (Table 2).

4. Concluding remarks

From the sea hares of two genera, *Aplysia* and *Dolabella*, a variety of new cytotoxic substances were isolated in minute amounts: their chemical structures were determined and their cytotoxicity against tumor cells was evaluated. Furthermore, chemical and biological studies were performed in detail concerning the highly cytotoxic substances such as aplyronine A (**1**), aurilide (**28**), dolastatin H (**29**) and isodolastatin H (**30**), some of which exhibited promising *in vivo* antitumor activity. Since the supply of cytotoxic substances from the natural source

was scarce, chemical synthesis of these substances was achieved, which, in some cases, provided the ample amounts necessary for the elucidation of their biological characteristics.

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Profiles

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