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Accounts

Bioactive Compounds from the Sea Hares of Two Genera: *Aplysia* and *Dolabella*

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This account describes the chemistry of the bioactive compounds isolated from the sea hares belonging to two genera: *Aplysia* and *Dolabella*. The bioactive compounds are classified as i) polyketides, ii) terpenes, iii) peptides and depsipeptides, and iv) others. Special emphasis is placed on the chemistry of cytotoxic compounds such as aplyronines and dolastatins that were obtained in our laboratory: Their isolation, structural elucidation, synthesis, and selected bioactivities data are summarized.

During the last three decades a large number of secondary metabolites exhibiting diverse bioactivities have been isolated and characterized from marine organisms such as cyanobacteria, algae, sponges, tunicates, coelenterates, bryozoans, and molluscs.[1,2] Sea hares of two genera, *Aplysia* and *Dolabella*, belong to the family Aplysiidae; they are shell-less and slow-moving marine molluscs that feed on marine algae. The sea hares are postulated to have chemical defense substances, which appear to deter predators from eating the sea hares. The poisonous properties of sea hare secretions were already recorded in the Roman times.[3] Since the notable report on the isolation of aplysin (**1**), a bromine-containing sesquiterpene (Chart 1), by Hirata and Yamamura in 1963,[4] investigations on the secondary metabolites of the sea hare of the genus *Aplysia* were carried out to yield a variety of compounds, most of which were halogenated. Intensive studies on the bioactive constituents of the Indian Ocean sea hare *Dolabella auricularia* were performed by Pettit, resulting in the isolation of the cell growth inhibitory and antineoplastic compounds designated dolastatins 1—15. We have examined the bioactive constituents of the two species of the sea hare, *Aplysia kurodai* and *Dolabella auricularia*, collected off the coast of the Shima peninsula, Mie prefecture, Japan for the past ten years and isolated a number of bioactive compounds, most of which were cytotoxic.

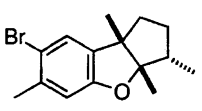

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Chart 1.

In this account, we describe the chemistry of the bioactive compounds isolated from the sea hares of two genera, *Aplysia* and *Dolabella*, with the emphasis on the research results obtained in our laboratory.

1. Bioactive Compounds from the Sea Hare of the Genus *Aplysia*

Since the discovery of aplysin (**1**),[4] chemical constituents of the sea hare of the genus *Aplysia* were intensively examined to obtain compounds with novel structures and various bioactivities; some of them were probably of dietary origin and/or might be produced by symbiotic microorganisms.

1.1 Polyketides. 1.1.1 Aplyronines. Originally aplyronine A (**2**) was isolated by eight-step chromatography of the lipophilic extract guided by the cytotoxicity against tumor (HeLa S_3) cells (Chart 2). A more efficient method for the isolation of **2** was developed to afford two minor congeners, aplyronines B (**3**) and C (**4**), in addition to **2** (Scheme 1).[5] Aplyronines A (**2**), B (**3**), and C (**4**) showed strong cytotoxicities against HeLa S_3 cells with IC_{50} values of 0.48, 3.11, and 21.2 ng mL^{-1}, respectively. It should be noted that aplyronine A (**2**) exhibited exceedingly potent antitumor activities in vivo (Table 1). The gross structure of **2** was determined on the basis of the spectral data. Although the NMR spectra of **2** were complicated by the doubled NMR signals for some protons and carbons arising from the restricted rotation about the *N*-methyl-*N*-vinylformamide terminus (2 : 1 ratio) and the presence of two scalemic amino acid portions (1.1 : 1 and 3 : 1 ratios for *N,N,O*-trimethylserine and *N,N*-dimethylalanine parts, respectively), detailed analysis of ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectra of **2** and its diacetate

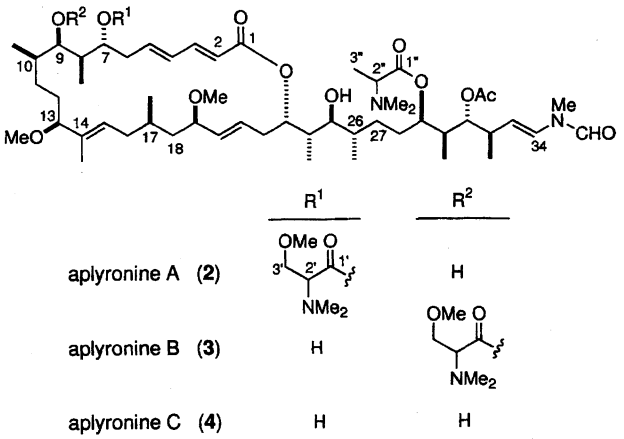
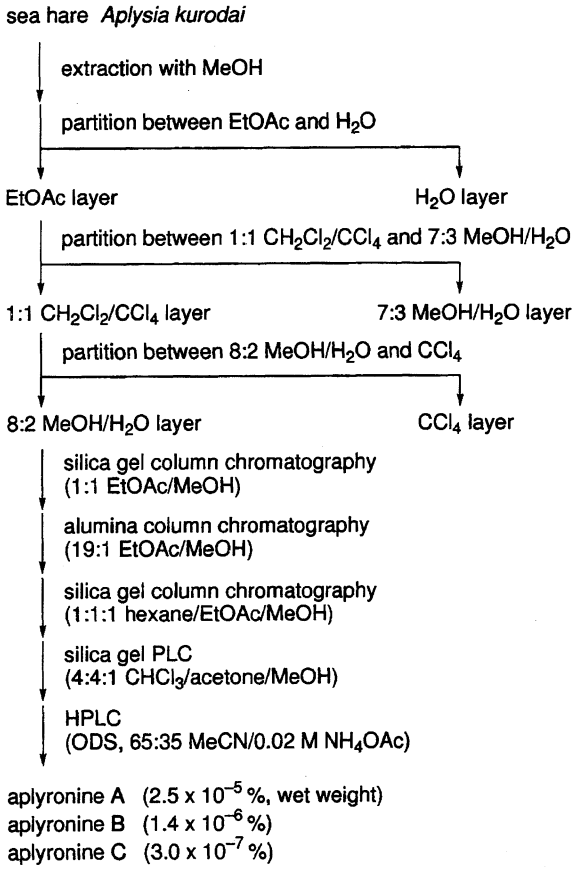


Chart 2.



Scheme 1. Isolation procudure for aplyronines A—C (**2**—**4**).

allowed construction of seven partial structures: C2–C9, C10–C13, C14–C17, C18–C26, C27–C34, C2′–C3′, and C2″–C3″. The HMBC spectra of **2** and its diacetate made it possible to connect these partial structures and led to the establishment of the gross structure of **2**.

The stereostructure of **2** was determined by the combination of NMR spectroscopy and the organic synthetic method. Chemical degradation of **2** afforded six products: the *p*-bromobenzoates, **5** and **6**, the C5–C14 fragments diastereomeric at C14, **7a** and **7b**, the C15–C20 fragment **8**, and the C21–C34 fragment **9** (Scheme 2).[6] The HPLC analysis of **5** and **6** using chiral columns showed that the ratios *S*/*R* for the trimethylserine part and for the dimethylalanine part were 52 : 48 and 72 : 28, respectively, and it was found that the ratios *S*/*R* varied with the animal samples employed, although the compounds with the *S* configuration were always predominant. Since the stereochemistry of the rigid dioxabicyclo[3.2.1]octane moiety in the C21–C34 fragment **9** was established by the coupling constants and NOEs in the ^{1}H NMR spectra of **9**, the relative stereostructure of four contiguous chiral centers (C29–C32) in **9** was determined to be *syn-anti-anti*.[7] The stereochemistry of the other four contiguous chiral centers (C23–C26) in **9** was determined as follows. The C21–C34 fragment **9** was converted into the corresponding acetonide **10** (Chart 3). The coupling constants of protons on the acetonide moiety and the chemical shifts of two secondary methyl groups around the acetonide moiety of **10** were compared with those of eight synthetic diastereomeric acetonides, **11a**—**11h**, (Fig. 1). The results led to the conclusion that the relative stereostructure of the C23–C26 part was *syn-anti-anti*.[7] In order to establish the relative stereochemistry between the C23–C26 and the C29–C32 parts in the natural C21–C34 fragment **9** and to determine the absolute stereostructure of **9**, two diastereomeric urethanes, **9a** and **9b** (Chart 4), were enantioselectively synthesized. The latter **9b** was found to be the enantiomer of the natural C21–C34 fragment **9**, thus establishing the abso-

Table 1. Antitumor Activities of Aplyronine A (**2**)

System	Dose	Test/Control
	mg kg^{-1} d^{-1} a)	%
P388 leukemia	0.08	545
Lewis lung carcinoma	0.04	556
Ehrlich carcinoma	0.04	398
Colon 26 carcinoma	0.08	255
B16 melanoma	0.04	201

a) On days 1, 2, 3, 4, 5, intraperitoneally (i.p.).

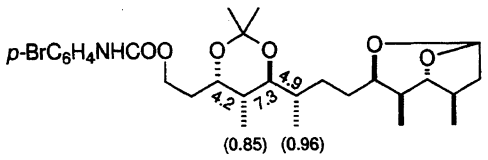


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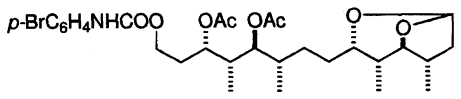
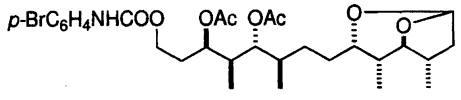
Chart 3.

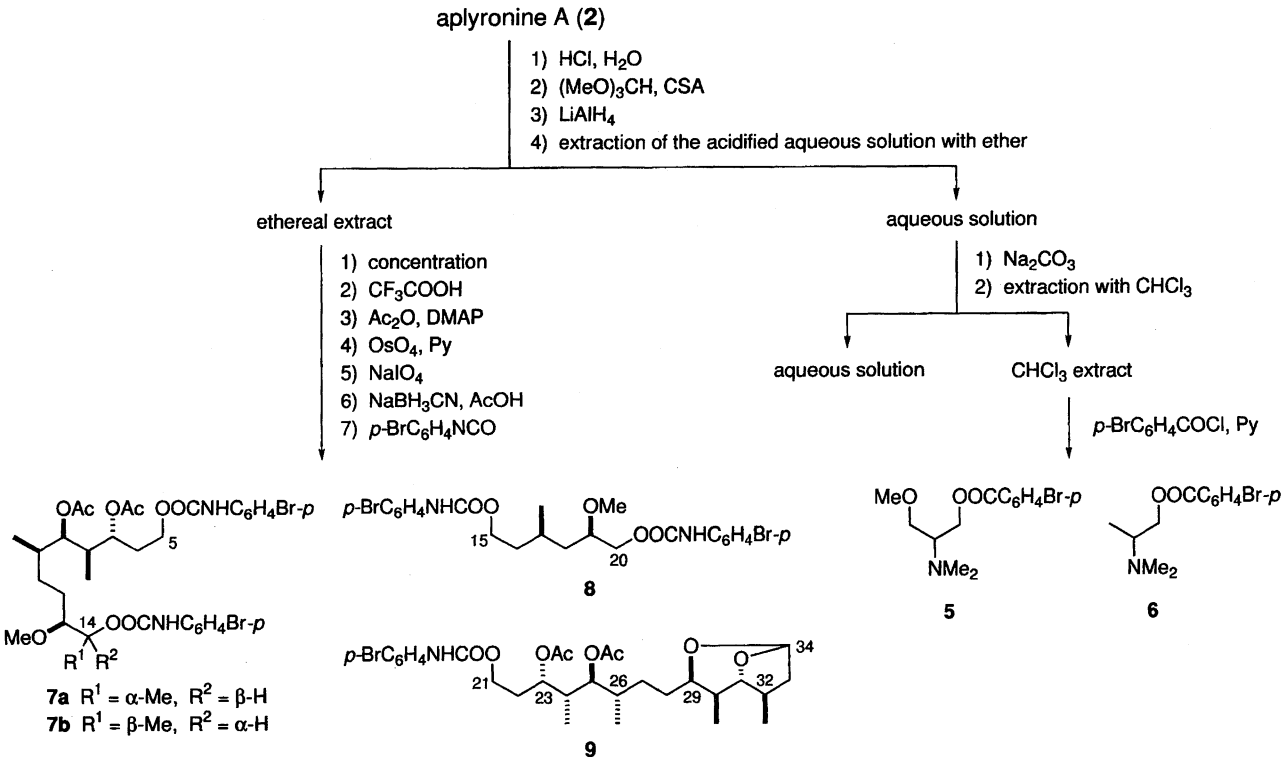


9a



9b

Chart 4.



Scheme 2. Degradation of aplyronine A (2).

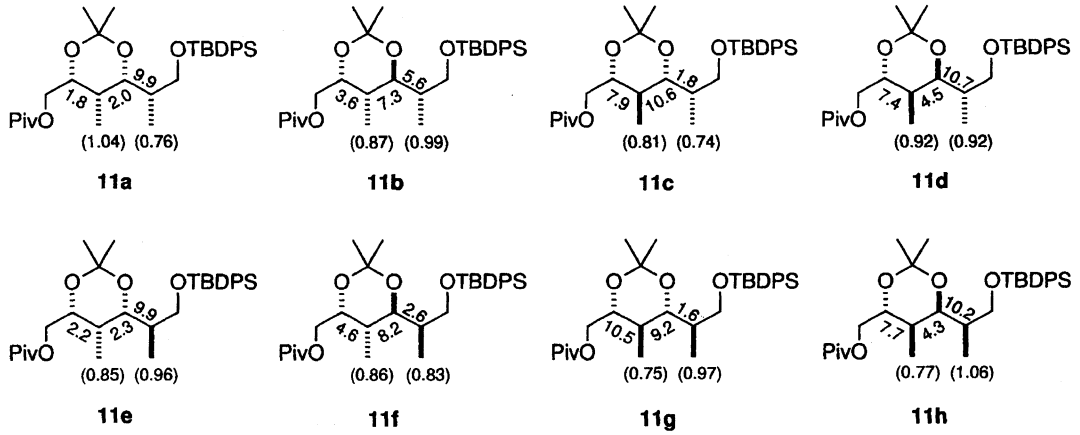
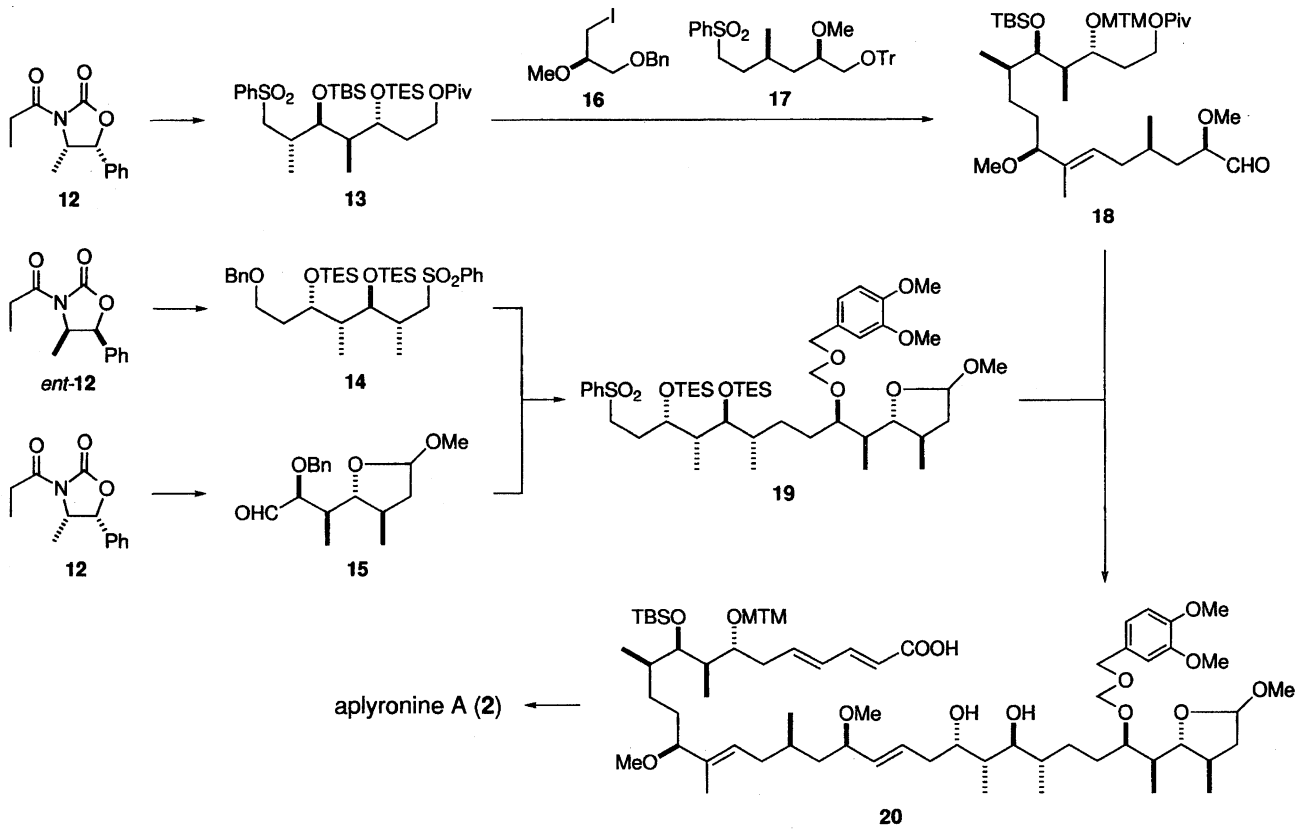


Fig. 1. Eight diastereomeric acetonides, **11a**—**11h**. Coupling constants and chemical shifts (in parentheses) are shown.

lute stereostructure of **9**.[7] The stereostructure of the C5–C14 fragment **7a** was determined by the same manner as employed in the case of **9**. The enantioselective synthesis of two possible diastereomers of **8** and their spectral comparison with natural **8** established the stereostructure of **8**. On the basis of the foregoing results, the absolute stereostructure of aplyronine A (**2**) was determined.[8]

The enantioselective synthesis of aplyronine A (**2**) was carried our in a convergent manner for the purposes of (i) confirming the stereostructure and the potent cytotoxicity and (ii) supplying **2** in quantities (Scheme 3).[9—11] The Evans aldol reaction and the Sharpless epoxidation reaction were employed as key reactions for the construction of three sets of the four contiguous chiral centers in **2**. Thus the three segments, **13**, **14**, and **15**, were prepared (Scheme 3). The carbon chain elongation of **13** was executed by using two segments, **16** and **17**, to afford segment **18**, while two segments, **14** and **15**, were coupled to yield segment **19**. Julia olefination between two segments, **18** and **19**, followed by four-carbon homologation provided seco acid **20**. Lactonization of **20** under Yamaguchi conditions yielded a macrolide, which, after construction of the terminal *N*-methyl-*N*-vinylformamide and subsequent introduction of two amino acids, led to aplyronine A (**2**). The overall yield of the synthesis, based on the longest linear sequence (47 steps), was 0.35%. The syntheses of aplyronines B (**3**) and C (**4**) were also achieved, which established their stereostructures unambiguously.[11,12]

Generally, the receptors for antitumor agents are considered to be the following three kinds of molecules: (i) DNA, (ii) microtubules, and (iii) cell cycle regulating enzymes.



Scheme 3. Outline of the synthesis of aplyronine A (**2**).

Aplyronine A (**2**) did not react with any of these molecules, but interacted with actin, the protein in cytoskeleton. Aplyronine A (**2**) inhibited polymerization of G-actin to F-actin and depolymerized F-actin to G-actin by severing.[13] Although a number of proteins that interact with actin have been known, there are only a few compounds of low molecular weights that interact with actin: The examples are cytochalasins, phalloidin, and recently discovered marine macrolides such as mycalolide B.[14] Interesting is the fact that the mode of action of aplyronine A (**2**) toward actin is different from those of cytochalasins and phalloidin. To investigate the structure-bioactivity relationships of **2**, fourteen artificial analogs were synthesized and their bioactivities were evaluated.[15] The presence and the length of the side chain portion of **2** proved to be crucial for both cytotoxicity and actin depolymerizing activity: For example, an artificial analog **21** was shown to be about 4400-fold less cytotoxic than **2** and to have no actin depolymerizing activity (Chart 5).

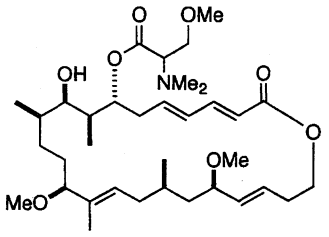
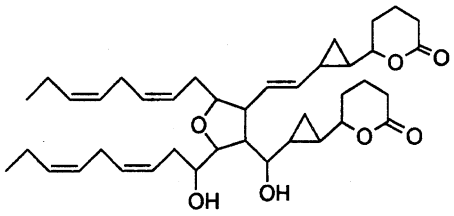


Chart 5.

1.1.2 Aplydilactone. From the lipophilic extract of the sea hare *A. kurodai* aplydilactone (**22**) was isolated by chromatographic separation (Chart 6).[16] The gross structure was elucidated on the basis of chemical and spectral means. This compound proved to be a fatty acid metabolite that is considered to be biosynthesized from two eicosapentaenoic acids via an unsymmetrical dimerization and oxidative cyclization to form lactones and cyclopropanes. Aplydilactone (**22**) exhibited the activity of activating phospholipase A_2 in vitro.

1.1.3 Halogenated C_{15} Cyclic Ethers. The compounds of this type isolated from the sea hare are conceivably of algal origin, since the compounds are present in the algae on which the animals feed. Dactylyne (**23**) is an acetylenic dibromochloro ether isolated from the sea hare *A. dactylomela* by Schmitz and its structure was determined by X-ray crystallography (Chart 6).[17] Dactylyne (**23**) prolongs pentobarbital hypnosis in mice by inhibiting pentobarbital metabolism.[18] Panacene (**24**) was isolated from the sea hare *A. brasiliana* by Meinwald and its structure was elucidated by spectral and synthetic means.[19] Brasilenyne (**25**) and *cis*-dihydrorhodophytin (**26**) were isolated from the sea hare *A. brasiliana* by Meinwald, Fenical, and Clardy, and their structures were determined by X-ray crystallography.[20] These three compounds, **24**, **25**, and **26**, were shown to be fish antifeedants. Recently, aplyparvunin (**27**) was isolated from the sea hare *A. parvula* by Higuchi and the structure was elucidated by X-ray crystallography.[21] This compound **27** was revealed to be ichthyotoxic.



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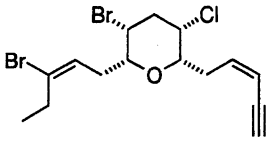

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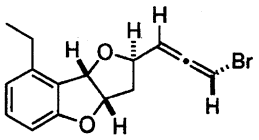

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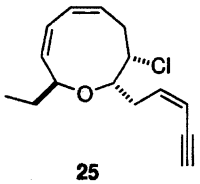

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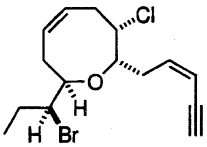

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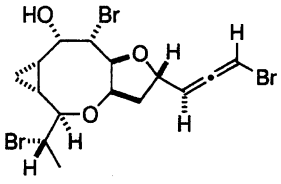

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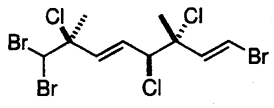
Chart 6.

1.2 Terpenes. Since the discovery of aplysin (**1**) in 1963,[4] a number of halogenated terpenes have been isolated from the sea hare of the genus *Aplysia*.

1.2.1 Monoterpenes. The first member of the halogenated monoterpene (**28**) was isolated from the sea hare *A. californica* by Faulkner and Clardy in 1973 and the structure of **28** was determined by X-ray crystallography (Chart 7).[22] Furthermore, the second halogenated monoterpene (**29**) was isolated and characterized.[23] The sea hare was shown to obtain these halogenated monoterpenes from the red alga *Plocamium* sp. Aplysiapyranoids A (**30**), B (**31**), C (**32**), and D (**33**) were isolated from the sea hare *A. kurodai* by Kakisawa; their structures were determined by X-ray crystallography and NMR spectroscopy.[24] These compounds **30**—**33** showed weak cytotoxicity. Aplysiaterpenoid A (**34**) was isolated from the sea hare *A. kurodai* by Komori; the structure was elucidated by X-ray crystallography.[25] This compound **34** exhibited moderate cytotoxicity (L1210, IC_{50} 10 μg mL^{-1}) as well as insecticidal activity. It is assumed that a sea hare utilizes the aforementioned halogenated monoterpenes as chemical defense substances.

1.2.2 Sesquiterpenes. Aplysistatin (**35**) was isolated



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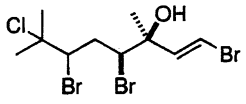

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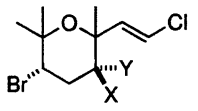

30 X = Br, Y = H; α-chlorovinyl
31 X = Br, Y = H; β-chlorovinyl
32 X = H, Y = Cl; α-chlorovinyl
33 X = H, Y = Cl; β-chlorovinyl

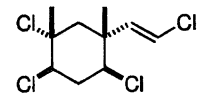

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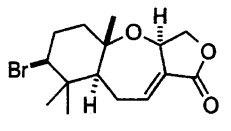
Chart 7.

from the sea hare *A. angasi* by Pettit; the structure was determined by X-ray crystallography (Chart 8).[26] Aplysistatin (**35**) represents a new type of sesquiterpene and was shown to be cytotoxic (P388, ED_{50} 2.7 μg mL^{-1}). From the sea hare *A. dactylomela* deodactol (**36**) was isolated, the structure of which was elucidated by X-ray crystallography.[27] This compound **36** is a halogenated bisabolane-type sesquiterpene and was shown to be cytotoxic (LE cell line, ED_{50} 10 μg mL^{-1}). Four brominated sesquiterpenes, cyclolaurenol (**37**), cyclolaurenol acetate (**38**), cupalaurenol (**39**), and cupalaurenol acetate (**40**), were isolated from *A. dactylomela* by Higa and their structures were elucidated by chemical and spectral means.[28] These compounds **37**—**40** were shown to be antibacterial, antifungal, and ichthyotoxic.

1.2.3 Diterpenes. Bromoobtusenediol (**41**) was isolated from the sea hare *A. dactylomela* collected in the Bahamas by Schmitz; the structure was determined by X-ray crystallography (Chart 9).[29] The compound **41** exhibited marginal cytotoxicity of an ED_{50} value of 4.5 μg mL^{-1} against KB cells. Furthermore, five brominated diterpenes represented by parguerol (**42**), deoxyparguerol (**43**), and isoparguerol (**44**) were isolated from the sea hare *A. dactylomela* collected in Puerto Rico by Schmitz; their structures were elucidated by chemical and spectral means.[30] They (**42**—**44**) were shown to be cytotoxic (P388, ED_{50} 0.38—4.6 μg mL^{-1}). These metabolites isolated from the genus *Aplysia* are assumed to be of algal origin.

1.2.4 Miscellaneous Terpenes. Three alkaloids, apl-



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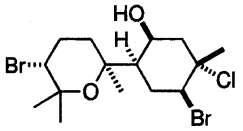

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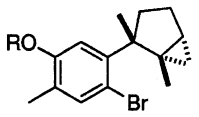
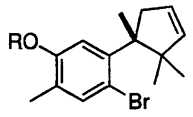
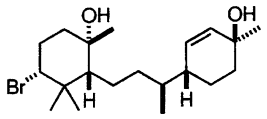


37 R = H
38 R = Ac



39 R = H
40 R = Ac

Chart 8.



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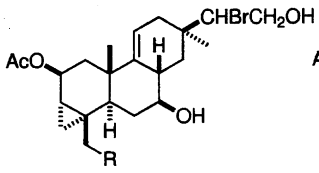

42 R = OH
43 R = H

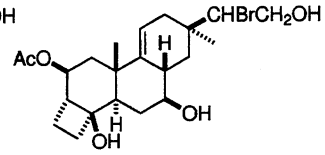

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Chart 9.

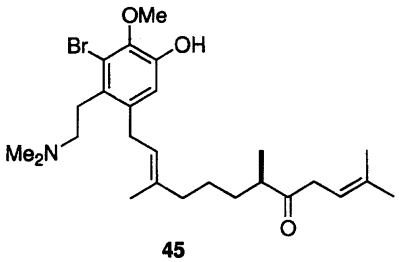
aminone (**45**), neoaplaminone (**46**), and neoaplaminone sulfate (**47**) were isolated from the sea hare *A. kurodai*; the structures were elucidated by spectral analysis and organic synthesis (Chart 10).[31,32] They (**45**—**47**) were shown to be strongly cytotoxic [HeLa S_3, IC_{50} ($\mu g\,mL^{-1}$): 0.28 for **45**, 1.6×10^{-7} for **46**, and 0.51 for **47**]. Aplykurodins A (**48**) and B (**49**) were isolated from the sea hare *A. kurodai* by Komori; their structures were determined by X-ray crystallography and spectral analysis.[33] The related compounds, 4-acetylaplykurodin B (**50**) and aplykurodinone B (**51**), isolated from the sea hare *A. fasciata* by Spinella were shown to be ichthyotoxic.[34]

1.3 Others. Polybrominated diphenyl ether (**52**) was isolated from the sea hare *A. dactylomela* as well as from green alga *Cladophora fascicularis* by Higa and was shown to exhibit antibacterial and antiinflammatory activities (Chart 10).[35] The structure was elucidated by chemical and spectral means.

2. Bioactive Compounds from the Sea Hare of the Genus *Dolabella*

Bioactive compounds have generally been isolated as very minute constituents of the sea hare and are assumed to be of dietary origin and/or to be produced by symbiotic microbes.

2.1 Peptides and Depsipeptides. 2.1.1 Dolastatins. Since 1965 Pettit has intensively examined the cell growth inhibitory and antineoplastic constituents of the Indian Ocean sea hare *D. auricularia*, resulting in the isolation of fifteen structurally unique peptide and depsipeptide type-substances termed dolastatins 1—15.[36—47] Most of them exhibited exceedingly strong cytotoxicities: For example, the ED_{50} value of dolastatin 10 was 0.045 ng mL^{-1} against P388 cells. Huge amounts of the sea hare were required to obtain dolastatins 1—15, since they were trace components of each animal. Among dolastatins 1—15, the gross structures of dolastatins 3 (**53**),[38] 10 (**54**),[39] 11 (**55**),[41] 12 (**56**),[41] 13 (**57**),[43] 14 (**58**),[44] and 15 (**59**)[45] were elucidated by chemical and spectral methods (Chart 11). The absolute stereostructures of dolastatins 3 (**53**),[38] 10 (**54**),[40] 11 (**55**),[42] and 15 (**59**)[46] were determined by their synthesis. Furthermore, the synthesis of dolastatin 10 (**54**) was achieved.[48] Dolastatin 10 (**54**) displayed unprecedented potency in experimental antineoplastic and tubulin assembly systems. Both dolastatins 10 (**54**) and 15 (**59**) are antitumor compounds and were reported to be in advanced preclinical development.[47,49] Dolastatin 10 (**54**) was shown to be powerfully effective at binding to tubulin, inhibiting polymerization.[50]



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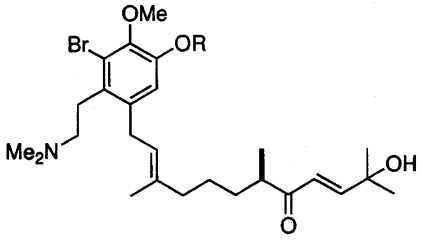
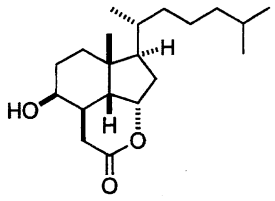


46 R = H
47 R = SO_3H



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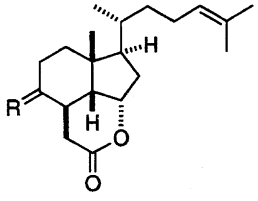
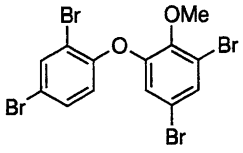


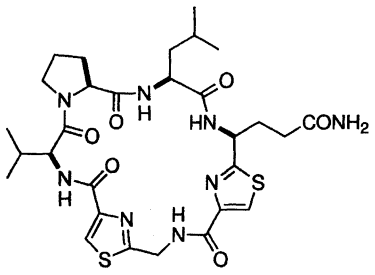
49 R = β-OH
50 R = β-OAc
51 R = O



52

Chart 10.

We have carried out the bioassay-directed investigation of the cytotoxic constituents of the Japanese specimens of *D. auricularia* and obtained cytotoxic peptides and depsipeptides as minute constituents (Chart 12). Dolastatin C (**60**) was shown to be a linear depsipeptide; its structure was determined by chemical and spectral methods and was confirmed by its synthesis.[51] Dolastatin C (**60**) exhibited weak cytotoxicity. Dolastatin D (**61**) is a cyclodepsipeptide, the structure of which was elucidated by chemical and spectral means and was confirmed by its synthesis.[52] Dolastatin D (**61**) contains a new β-amino acid and exhibited moderate cytotoxicity (HeLa S_3, IC_{50} 2.2 $\mu g\,mL^{-1}$). Dolastatin E



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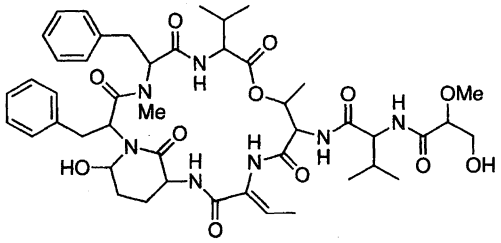

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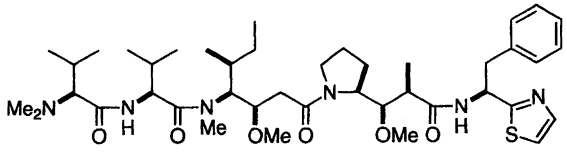

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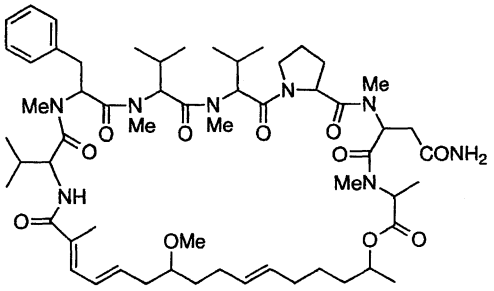

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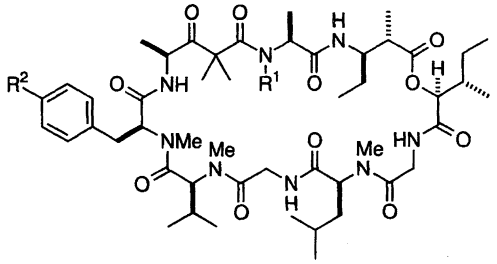
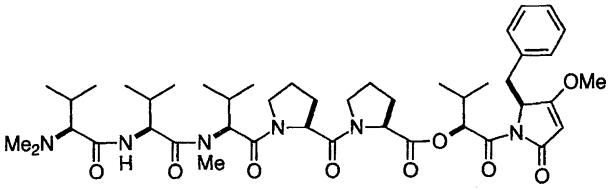


55 R^1 = H, R^2 = MeO
56 R^1 = Me, R^2 = H



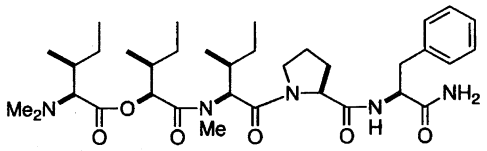
59

Chart 11.

(**62**) is a cyclic hexapeptide that contains three kinds of five-membered heterocycles (oxazole, thiazole, and thiazoline); the gross structure was elucidated on the basis of the spectral data.[53] Since it is known that the chiral center of a thiazoline ring is liable to be racemized, it was not reliable to determine the stereochemistry of the thiazoline ring in dolastatin E (**62**) by the standard method of analyzing the chirality of the amino acid obtained by acid hydrolysis of dolastatin E (**62**). Thus, the stereostructure of dolastatin E (**62**) was determined by its enantioselective synthesis.[54] Dolastatin E (**62**) exhibited weak cytotoxicity. Dolastatin G (**63**) is a 35-membered depsipeptide that contains a new dihydroxylated fatty acid and a new β-methoxy $\alpha,\beta,\gamma,\delta$-unsaturated fatty acid.[55] Its gross structure was established by spectroscopic analysis. The stereostructure of **63** was determined by chemical methods and was confirmed by its enantioselective synthesis.[55,56] Nordolastatin G (**64**) is a congener of dolastatin G (**63**), the structure of which was determined by chemical correlation with dolastatin G (**63**) and by its synthesis.[55,56] Both compounds, **63** and **64**, were shown to be moderately cytotoxic [HeLa S_3, IC_{50} ($\mu g\,mL^{-1}$): 1.0 for **63** and 5.3 for **64**]. Dolastatin H (**65**) and isodolastatin H (**66**) were isolated in trace amounts (0.3 mg each from 33 kg of *D. auricularia*).[57] On the basis of spectroscopic analysis, these compounds were shown to be peptides that were closely related to dolastatin 10 (**54**). The absolute stereostructures of both compounds, **65** and **66**, were unambiguously determined by their enantioselective synthesis. Owing to the scarcity of the natural samples, cytotoxicity of dolastatin H (**65**) and isodolastatin H (**66**) was evaluated by using synthetic samples. Dolastatin H (**65**) and isodolastatin H (**66**) showed strong cytotoxicity against HeLa S_3 cells with IC_{50} values of 2.2 and 1.6 $ng\,mL^{-1}$, respectively. A cytotoxicity test for synthetic dolastatin H (**65**), isodolastatin H (**66**), and their analogs revealed that the stereochemistry of the 3-phenylpropane-1,2-diol moiety on the C-terminus plays an important role in their cytotoxicity. It is interesting to note that isodolastatin H (**66**) exhibited in vivo antitumor activity comparable to that of dolastatin 10 (**54**), whereas dolastatin H (**65**) did not.

2.1.2 Doliculide. Doliculide (**67**) is a 16-membered depsipeptide that contains a new dihydroxylated fatty acid (Chart 13).[58] The gross structure of **67** was elucidated on the basis of spectral data and the absolute stereostructure was determined by the detailed NMR spectral analysis coupled with the chiral HPLC analysis of the amino acid obtained by acid hydrolysis of **67**. The enantioselective synthesis of doliculide (**67**) unambiguously confirmed its absolute stereostructure.[59,60] Doliculide (**67**) exhibited strong cytotoxicity against HeLa S_3 cells with an IC_{50} of 1 $ng\,mL^{-1}$.

2.1.3 Aurilide. As a trace cytotoxic constituent, au-



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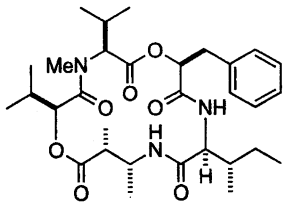

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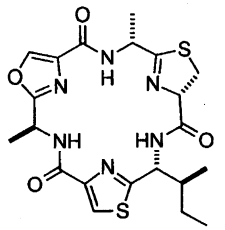

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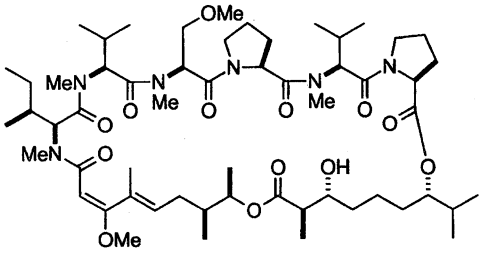

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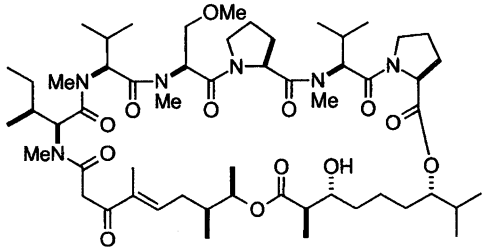

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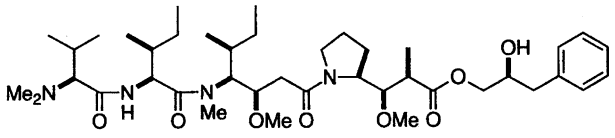

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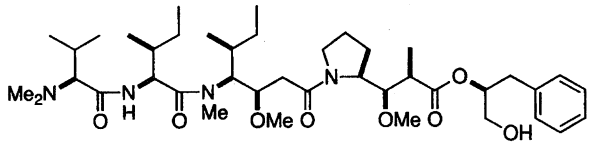
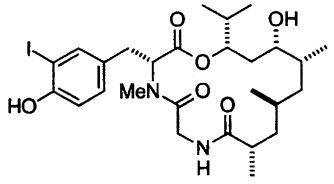
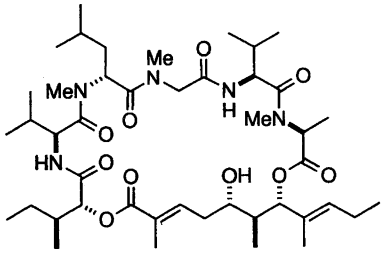


66

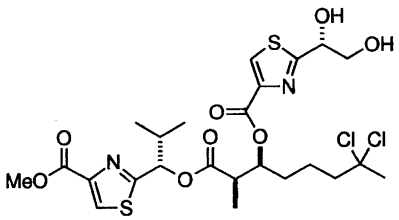
Chart 12.



67



68



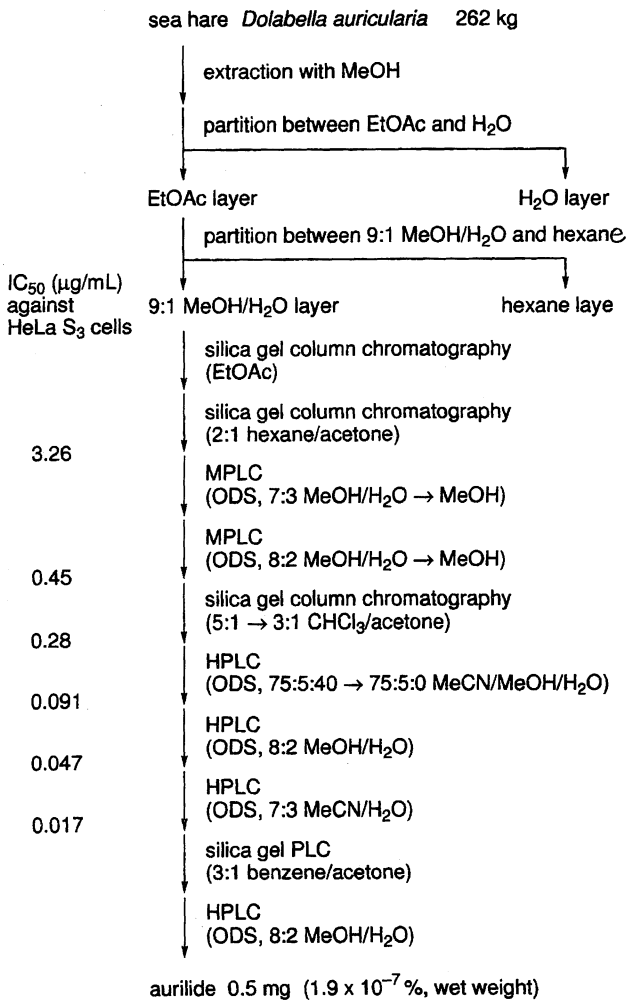
70

Chart 13.

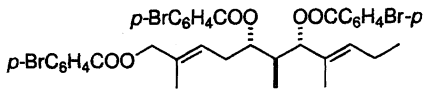
rilide (**68**) was isolated (0.5 mg from 265 kg of the sea hare) (Chart 13).[61] The isolation procedure for aurilide (**68**) is shown in Scheme 4. The gross structure of aurilide (**68**) was determined by interpretation of NMR spectral data (DQF-COSY, HSQC, HMBC) and the absolute stereochemistry of the peptide moiety was inferred by chiral HPLC analysis of the component amino acids and hydroxy acid obtained by acid hydrolysis of aurilide (**68**). The absolute stereochemistry of a new dihydroxylated fatty acid part in aurilide (**68**) was determined by the enantioselective synthesis of four possible diastereomers of the fragment **69** derived from aurilide (**68**) and comparison of their spectral (^{1}H NMR and CD) data with those of the natural fragment **69** (Chart 14). Thus, the absolute stereostructure of aurilide (**68**) was determined. The efficient enantioselective synthesis of aurilide (**68**) was achieved to unambiguously confirm the stereostructure and to supply the sufficient amount for the evaluation of the bioactivities.[62] Using the synthetic sample, aurilide (**68**) was found to exhibit strong cytotoxicity against HeLa S_3 cells with an IC_{50} of 11 ng mL^{-1}.

2.1.4 Dolabellin. Dolabellin (**70**) is a bisthiazole metabolite, the gross structure of which was elucidated on the basis of the spectral data (Chart 13).[63] The stereostructure was determined by an organic synthetic method. Enantioselective synthesis of dolabellin (**70**) was performed, which confirmed the absolute stereostructure. Dolabellin (**70**) was shown to be moderately cytotoxic (HeLa S_3, IC_{50} 6.1 μg mL^{-1}).

2.2 Polyketides. Aurisides A (**71**) and B (**72**) were separated in sub-milligram amounts (0.8 mg of **71** and 0.7 mg of **72** from 278 kg of the sea hare) (Chart 15).[64] Their gross structures were elucidated by spectroscopic analyses, including 2D NMR techniques. On the basis of the NOESY spectral analysis and the degradation experiments, their absolute stereostructures were determined to be 14-membered



Scheme 4. Isolation procudure for aurilide (**68**).



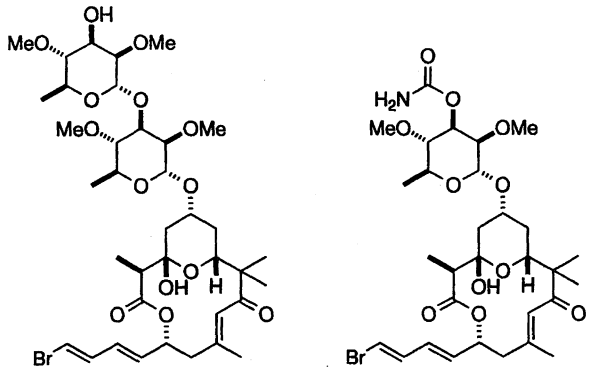
69

Chart 14.

macrolide glycosides. Aurisides A (**71**) and B (**72**) showed cytotoxicity against HeLa S_3 cells with IC_{50} values of 0.17 and 1.2 μg mL^{-1}, respectively. Two related polypropionates, auripyrones A (**73**) and B (**74**) were isolated as trace constituents; their structures were determined by spectroscopic analysis.[65] Relative stereochemistry except for the ester part of auripyrone B (**74**) was elucidated on the basis of the NOESY data. Auripyrones A (**73**) and B (**74**) exhibited cytotoxicity against HeLa S_3 cells with IC_{50} values of 0.26 and 0.48 μg mL^{-1}, respectively. Dolabelides A (**75**), B (**76**), C (**77**), and D (**78**) are macrolides with moderate cytotoxicity (HeLa S_3, IC_{50} 1.3—6.1 μg mL^{-1}) and their absolute stereostructures were determined by spectral and chemical means.[66,67]

2.3 Terpenes. Pettit investigated the cytotoxic constituents of the Indian Ocean sea hare *D. auricularia* and isolated dolatriol (**79**) and dolatriol acetate (**80**), which were shown to be weakly cytotoxic (Chart 15). Their structures were determined by X-ray crystallography.[68]

In summary, the sea hares of two genera *Aplysia* and *Dolabella* have proven to be a rich source of new compounds possessing novel structures and strong bioactivities, although these compounds are contained in trace amounts in the animals, many of which are probably of dietary origin and/or originate from symbiotic microorganisms such as cyanobacteria. In the future, some of these bioactive com-



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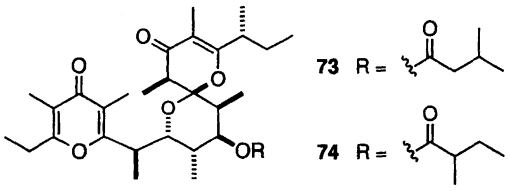
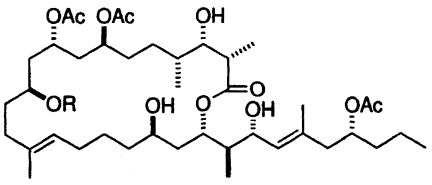
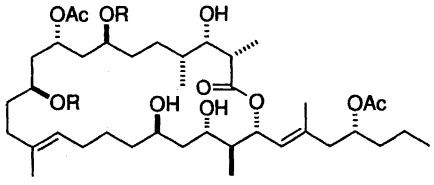
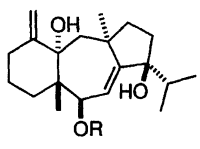




75 R = Ac
76 R = H



77 R = Ac
78 R = H



79 R = H
80 R = Ac

Chart 15.

pounds may serve as useful reagents in life science fields, and be supplied in sufficient quantities by synthesis.

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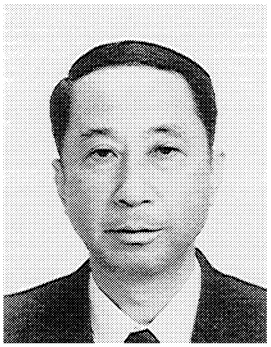

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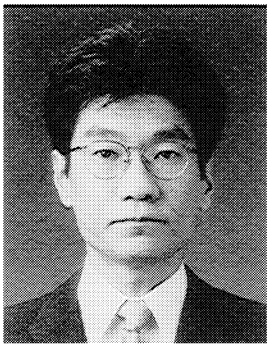

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