SPHINGOSINE-RELATED MARINE ALKALOIDS: CYCLIC AMINO ALCOHOLS†

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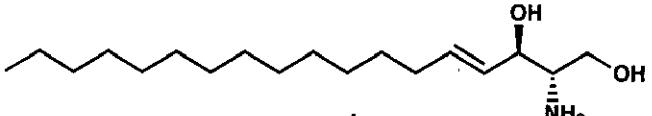
Abstract—Two classes of aliphatic alkaloids, pseudodistomins and penaresidins, were isolated from an Okinawan marine tunicate *Pseudodistoma kanoko* and a sponge *Penares* sp., respectively. They have piperidine and azetidine skeletons, respectively; these cyclic amino alcohols are likely to be biogenetically related to sphingosine derivatives. Other sphingosine-related metabolites from marine microorganisms are also described.

1. INTRODUCTION

Sphingosine (**1**) is a long-chain amino alcohol with generally 18 carbon atoms and one of the basic building blocks of sphingolipids and glycosphingolipids (*e.g.*, ceramides, cerebrosides, or gangliosides); they are constituents of cell walls and membranes and play diverse roles in biological processes such as cell growth, cell differentiation, and the immune response.[1] On



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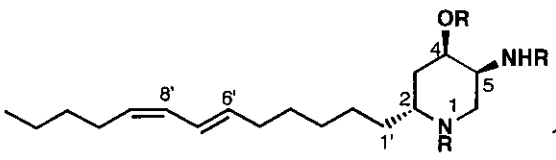
†Dedicated to the memory of Professor Yoshio Ban.

the other hand, sphingolipids and glycosphingolipids are growing class of marine natural products,[2] some of which are reported to have promising antitumor activity.[3] During our continuing studies on search for bioactive substances from marine organisms,[4] we have isolated several sphingosine-related metabolites with various biological activities.[5] This review article overviews the recent results on the study of isolation and structure elucidation of sphingosine-related marine alkaloids, among which two classes of cyclic amino alcohols, pseudodistomins and penaresidins, are described mainly.

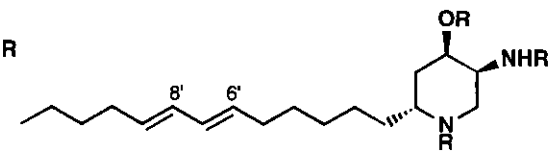
2. PSEUDODISTOMINS

2-1. Isolation of Pseudodistomins A and B[6]

In 1986 we investigated the bioactive substances from an Okinawan tunicate *Pseudodistoma kanoko*, which is an orange-colored compound tunicate and looks like a strawberry (Japanese name, 'ichigo-boya'). The material was collected off Ie Island, Okinawa, by SCUBA (-5 to -10 m). The methanol-toluene (3:1) extract of *P. kanoko* was partitioned between toluene and water. The aqueous layer was successively extracted with chloroform, ethyl acetate, and *n*-butanol. By preliminary screening using mammalian muscle preparations, the chloroform-soluble fraction was found to exhibit marked antispasmodic activity on the isolated guinea-pig ileum; the contractile responses to carbachol and histamine were abolished by this fraction. The chloroform-soluble fraction was therefore subjected to bioassay-guided fractionations using silica gel flash column chromatography eluted with $CHCl_3$/*n*-BuOH/H_2O/AcOH (1.5:6:1:1) followed by the reversed-phase hplc separation (Develosil ODS-5, 50% MeCN with 0.1% TFA) to afford active fraction, which was positive on the ninhydrin-test on tlc plate. This active fraction was revealed to be a mixture of two components [pseudodistomins A (**2**) and B (**3**)], the separation of which was first carried out after converting them into acetates (**4** and **5**, respectively) by ODS-hplc (YMC-Pack, AM)



2 R = H **4** R = Ac



3 R = H **5** R = Ac

with 88% MeOH. The acetates (**4** and **5**) were used for characterizations and structural studies described in the next paragraph. A small amount of **2** and **3** (before acetylation) were obtained by careful hplc (Develosil ODS-5) eluting with 37% MeCN with 0.2% TFA to supply for bioassays. In addition to antispasmodic activity, pseudodistomins A (**2**) and B (**3**) exhibited cytotoxic activity against murine leukemia cells, L1210 and L5178Y, in vitro (IC_{50} values: 2.5 and 0.4 μg/ml against L1210, respectively; 2.4 and 0.7 μg/ml against L5178Y, respectively). Both compounds (**2**) and (**3**) also exhibited calmodulin antagonistic activity; they both inhibited calmodulin-activated brain phosphodiesterase with IC_{50} values of 3×10^{-5} M, being approximately 3 times more potent than W-7, a well-known synthetic calmodulin antagonist.

2-2. Structural Elucidation of Pseudodistomins A and B

Studies for structural elucidation were mostly carried out using pseudodistomin B acetate (**5**). In the ^1H nmr spectrum of the acetate (**5**) (Figure 1), several signals appeared broad and split in an approximately 4:1 ratio; this phenomenon might be ascribed to the presence

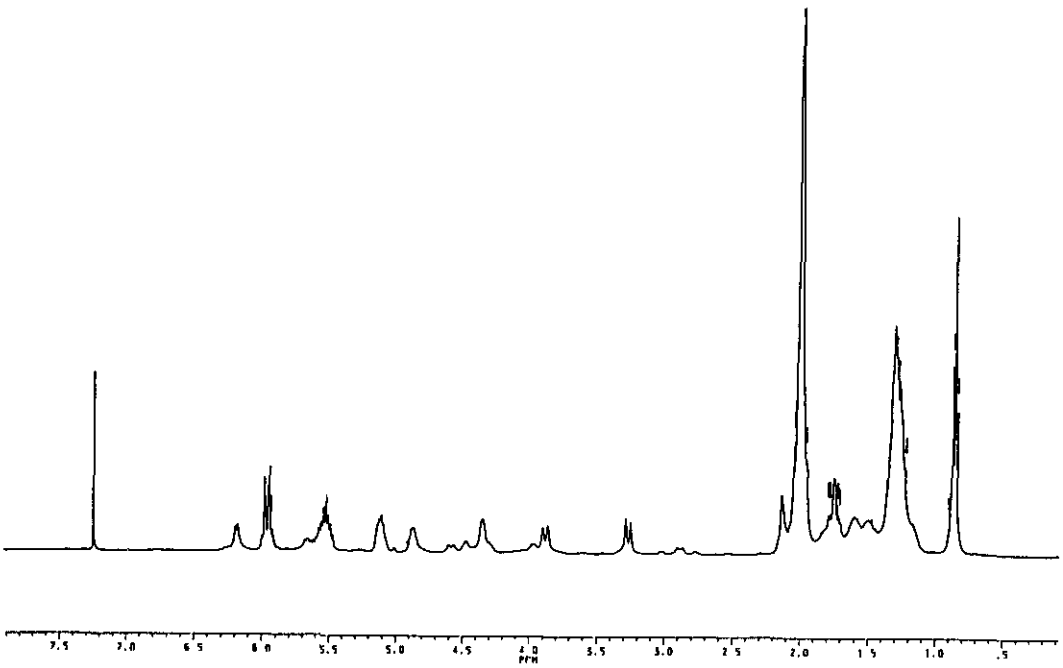
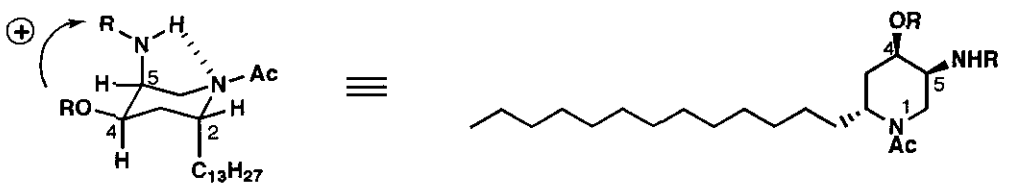


Figure 1. ^1H Nmr Spectrum of Pseudodistomin B Acetate (**5**) in $CDCl_3$

of two slowly interconverting conformations due to the rotation of the secondary amide group. Although the elucidation of the nmr data was disturbed by this phenomenon, we could obtain 2D nmr data of **5** with good quality recorded on a 400 MHz spectrometer by collaboration with Dr. Wälchli, Bruker Japan, and analysis of the ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectral data led to a planar structure of **5** containing a piperidine nucleus and alkyl side chain with two *E*-double bonds. Spectral data of pseudodistomin A acetate (**4**) suggested that pseudodistomin A possesses the same planar structure with different olefin geometry (one *E* and one *Z*). Catalytic hydrogenation of each of the acetate (**4** and **5**) afforded the identical tetrahydro derivative (**6**). The relative stereochemistry of the chiral centers at C-2, C-4, and C-5 was determined by the coupling constants and NOE data using the tetrahydroacetate (**6**, Figure 2). Although it seemed unfavorable that the alkyl side-chain at C-2 is axially oriented, we proposed that the conformation shown in Figure 2 may be stabilized by an intramolecular hydrogen-bond between the N(5)-H and N(1), which was inferred from the FT ir spectrum of a dilute solution of **6**. Knapp and Hales, who achieved the synthesis of the tetrahydroacetate (**6**) in 1993,[7] described that this hydrogen bond would be highly strained,[8] but they also agreed that the conformation in Figure 2 was preferred to the alternative one because of a steric interaction between the N(1)-acetyl group and the alkyl side-chain, which was also supported by the Macromodel calculation.[7]

The absolute stereochemistry of the C-2, C-4, and C-5 positions was deduced on the basis of the exciton chirality method.[9] 1-Acetyl-4,5-bis(*p*-bromobenzoyl) derivative (**7**) was prepared by partial hydrolysis of **6**, followed by *p*-bromobenzoylation. The cd spectrum of **7** showed a positive Cotton effect, implying the 2*R*, 4*R*, and 5*S*-configurations (Figure 2).



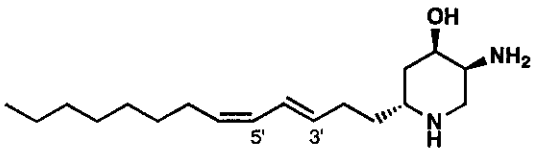
6 R = Ac
7 R = COC_6H_4-*p*-Br

J-values (Hz) for **6**:
$J_{2,3ax} = < 1$, $J_{2,3eq} = < 1$, $J_{3ax,4} = 11$, $J_{3eq,4} = 5\,5$, $J_{4,5} = ca.\ 1$, $J_{5,6ax} = ca.\ 0$, and $J_{5,6eq} = 1.5$ Hz

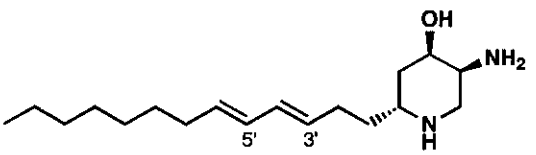
Figure 2. Perspective Drawings of **6** and **7**

2-3. Revision of the Structure of Pseudodistomins A and B

We initially proposed the conjugated diene position in the side chain of pseudodistomins A and B to be at the 3',5'-position (**2a** and **3a**, respectively);[6] this position was, however, later revised to be at the 6',8'-position (**2** and **3**, respectively) by the following studies.



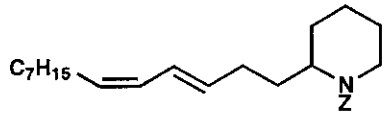
2a (wrong structure)



3a (wrong structure)

2-3-1. Studies on the Synthesis of Pseudodistomins by Other Groups

After we published our work on isolation and structure elucidation of pseudodistomins, these compounds were chosen as a target of organic synthesis by several groups probably because of their unique bioactivities as well as their interesting structure apparently biogenetically related to sphingolipids. In 1990, Nakagawa and coworkers reported the synthesis of compound (**8**) as a model for **2a** (initial structure), and they also prepared an optically active compound (**9**) from L-aspartic acid as a key intermediate.[10]



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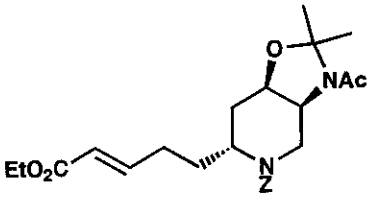

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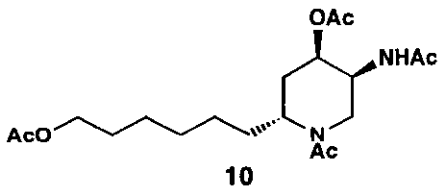
Total synthesis of the tetrahydroacetate (**6**) was achieved by three groups. Natsume and coworkers prepared (±)-**6** from 1,2-dihydropyridine derivative through the singlet oxygen addition reaction.[11] Naito and coworkers described the synthesis of (±)-**6** *via* the route involving the reductive photocyclization of enamide and α-acylamino radical allylation,[12] and they afterward synthesized optically active (+)-**6** through the cycloaddition of a nitrone

to (+)-2-aminobut-3-en-1-ol.[13] Knapp and Hale, *vide supra*, prepared (+)-**6** as optically active form from D-serine,[7] and the optical rotation of the synthetic sample of (+)-**6** corresponded to that of natural one.[14] From these studies the piperidine ring absolute stereochemistry of pseudodistomins A (**2**) and B (**3**) was unambiguously confirmed as those described initially by us.[6]

2-3-2. Revision of the Structure of Pseudodistomin B

Naito and coworkers, however, questioned the side-chain diene position during their synthesis of (±)-**6**; they prepared the triacetates of **3a** (3',5'-diene; our initial structure for pseudodistomin B), and found that the spectral data of the synthetic triacetate were not superimposable to those of natural specimen.[6] We therefore reinvestigated the side-chain diene position of pseudodistomin B by chemical degradation experiments. The triacetate (**5**) prepared from natural specimen of pseudodistomin B (**3**) was treated with ozone followed by reduction with $NaBH_4$ and acetylation with Ac_2O and pyridine afforded the tetraacetate (**10**), the FABms of which clearly afforded the $(M+H)^+$ ion at *m/z* 385, implying the side-chain diene position of pseudodistomin B (**3**) to be at 6',8'-position.[15] After being aware of this result,[16] Naito and coworkers prepared the triacetate with 6',8'-diene (**5**), whose spectral data proved to be identical with those of authentic sample of **5**.[15]



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2-3-3. Revision of the Structure of Pseudodistomin A

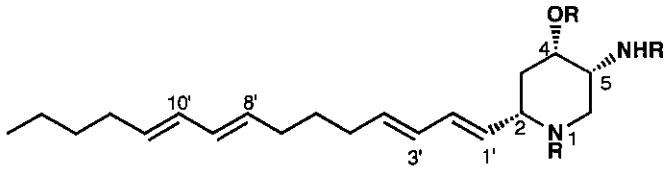
As the result of the above work, the structure of pseudodistomin A had to be also reexamined. Since the natural specimen of pseudodistomin A was unavailable at that time, we had to reinvestigate the extracts of the tunicate *P. kanoko*. Pseudodistomins A and B were first isolated from the chloroform-soluble fraction obtained by partition experiments (see, section 2-1). We reexamined the toluene-soluble fraction, which had been shown to be less active on the antispasmodic activity assay, to detect a ninhydrin-positive spot on tlc. The toluene-soluble fraction was therefore separated by the same procedures as above to succeed

in reisolating pseudodistomin A as its acetate (**4**). Pseudodistomin A acetate (**4**) in hand was subjected to ozonolysis by the same procedures used for pseudodistomin B acetate (**5**) to give the identical product (**10**) on the basis of tlc, ^{1}H nmr, and EIms [*m/z* 325, (M – CH_3CONH_2)$^+$] data. Thus, pseudodistomin A was also revealed to have 6',8'-diene (**2**), and the 6'*E*,8'*Z*-configuration was deduced from the combination of the HOHAHA spectrum and coupling constant data of the acetate (**4**).[17]

2-4. Isolation and Structure of Pseudodistomin C[18]

2-4-1. Isolation of Pseudodistomin C

On the study of reisolation of pseudodistomin A (**2**), we also aimed at isolating new other sphingosine-related alkaloids since sphingolipids are of current interest of many scientists working in a broad range of biological sciences.[1] The ninhydrin-positive fraction was, after acetylation, carefully examined by hplc (Develosil ODS-5) eluting with 85% MeOH to afford a new piperidine alkaloid, named pseudodistomin C (**11**), as its acetate (**12**). Before acetylation, **11** was also obtained by preparative silica gel tlc ($CHCl_3$/MeOH/H_2O, 6:4:0.7), and was revealed to exhibit cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells *in vitro* (IC_{50} values, 2.3 and 2.6 μg/ml, respectively).



11 R = H **12** R = Ac

2-4-2. Planar Structure of Pseudodistomin C

The ^{1}H nmr spectrum of the acetate (**12**) in $CDCl_3$ (Figure 3) showed so broad signals that no signals were able to be assigned, and was quite different from the ^{1}H nmr spectrum of pseudodistomin B acetate (**4**) (Figure 1). The 2D nmr experiments (^{1}H-^{1}H COSY, HSQC, and HMBC) of **12** were carried out in a CD_3OD solution, which showed relatively resolved signals. Since the ^{1}H nmr spectrum of natural compound (**11**) in a C_5D_5N solution appeared

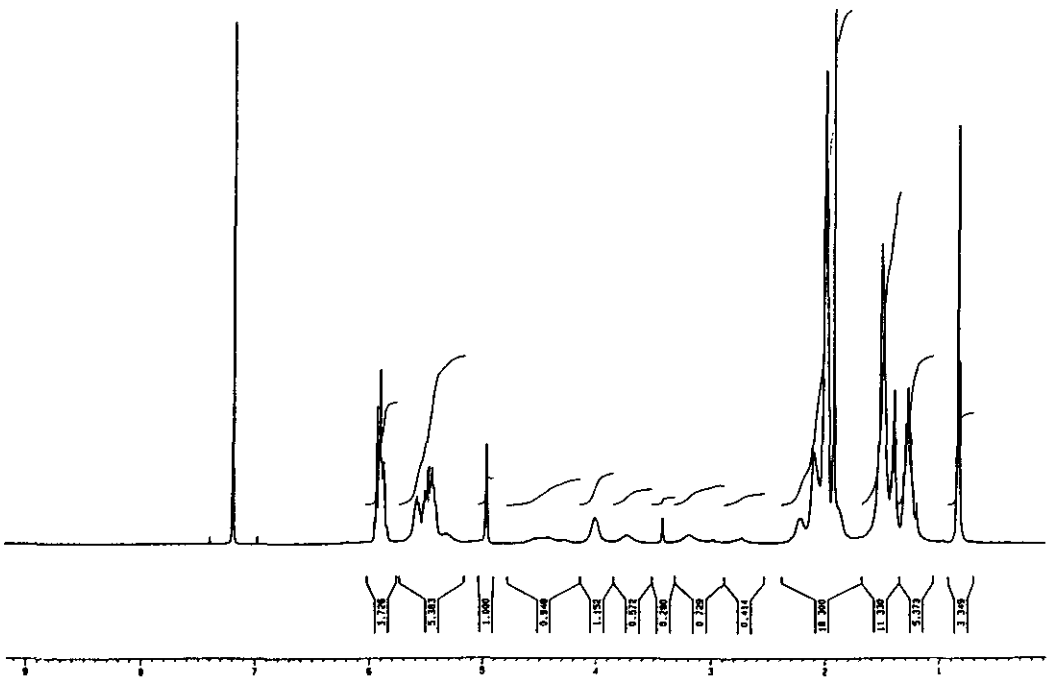
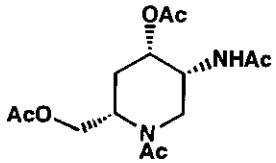


Figure 3. ^{1}H Nmr Spectrum of Pseudodistomin C Acetate (**12**) in $CDCl_3$

better in resolution, the ^{1}H-^{1}H COSY and HSQC spectra of **11** were recorded in this solution. From these spectral data, pseudodistomin C (**11**) was suggested to consist of a piperidine moiety and an unsaturated side-chain; the piperidine ring has the same substituents (4-hydroxyl and 5-amino groups) as those of pseudodistomins A and B (**1** and **2**), and the side-chain attached to C-2 contains two dienes. The positions of two dienes were clarified unambiguously by the following degradations. Pseudodistomin C (**11**) was treated with ozone followed by $NaBH_4$ reduction and acetylation to give a crude product, from which the tetraacetate [**13**, EIms *m/z* 315 $(M+H)^+$ and 255 $(M - CH_3CONH_2)^+$] was obtained by hplc purification, thus revealing one of the two dienes to be located at 1',3'-position. The second diene was deduced to be on 8',10'-position since 1,5-pentanediol diacetate (**14**) was detected



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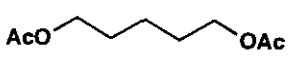
(D-13)



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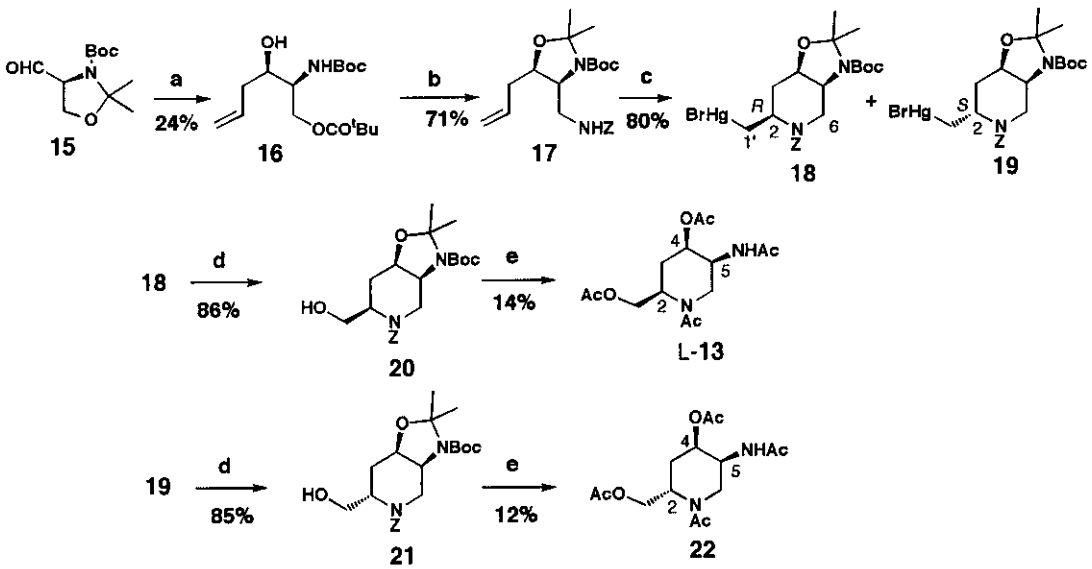
by reversed-phase tlc and hplc analyses from the crude mixture of the ozonolysis products. These olefins were inferred to be all *E* from the coupling constants and the ^{13}C chemical shifts of the allylic methylenes.[19]

2-4-3. Stereochemistry of Pseudodistomin C

Since the ^{1}H nmr spectrum of pseudodistomin C acetate (**12**) (Figure 3) appeared quite different from that of pseudodistomin B acetate (**4**) (Figure 2), a stereochemical evidence of the piperidine ring portion of pseudodistomin C (**11**) was required. Thus, the tetraacetate (**13**), which was obtained by ozonolysis of **11**, was prepared as an optically active form as shown in Scheme 1. Oxazolidine aldehyde (**15**), prepared from L-serine,[20] was treated with allylmagnesium bromide to give a 1:1 diastereomeric mixture of allyl alcohols. After deprotection of acetonide group and conversion into pivaloyl ester, the unnecessary *threo*-isomer was removed by silica gel column chromatography. The *erythro*-monopivaloate (**16**) was transformed *via* 5 steps into a benzyl carbamate (**17**), which was subjected to



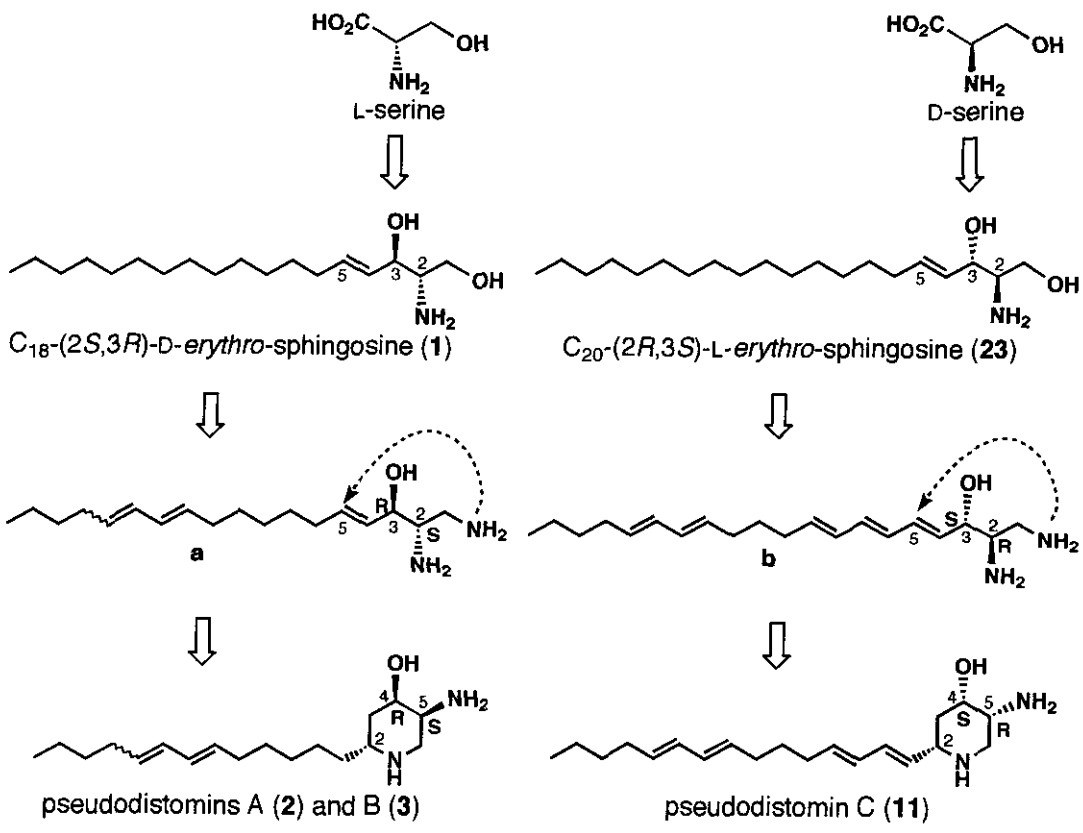
Scheme 1. (a) (1) CH_2=$CHCH_2MgBr$; (2) *p*-TsOH, MeOH; (3) PivCl, pyridine; (4) SiO_2 column, hexane/EtOAc (3:1); (b) (1) DMP, BF_3·OEt; (2) 2.5 N KOH, MeOH; (3) Phthalimide, DIAD, PPh_3; (4) H_2NNH_2·H_2O, EtOH, (5) ZCl, 2N NaOH; (c) (1) $Hg(OCOCF_3)_2$, $CHCl_3$, (2) $NaHCO_3$; (3) NaBr; (d) $NaBH_4$,O_2, DMF, (e) (1) TFA, CH_2Cl_2, (2) Ac_2O, pyridine, (3) H_2, 5% Pd/C, EtOH; (4) Ac_2O, pyridine.

amide mercuration to give 2*R*- and 2*S*-piperidine derivatives (**18** and **19**) in a ratio of 54:46. Oxidative demercuration of **18** and **19** gave primary alcohols (**20** and **21**, respectively), which were deprotected and acetylated to afford tetraacetates (L-**13**) and (**22**), respectively. The ^{1}H nmr spectrum of the tetraacetate (**13**) obtained from natural specimen of pseudodistomin C (**11**) was identical with that of the former [2*R*,4*R*,5*S*-derivative, L-**13**]. Since the latter tetraacetate (**22**) possess the same relative configurations at C-2, 4, and 5 positions on the piperidine ring as those of pseudodistomins A (**2**) and B (**3**), relative configurations of pseudodistomin C (**11**) proved to be different from those of **2** and **3**. The sign of optical rotation of synthetic L-**13** ($[\alpha]_D$ -19°) was opposite to that of the tetraacetate (**13**) ($[\alpha]_D$ +16°) derived from natural specimen of **11**. The absolute configuration of pseudodistomin C (**11**) was therefore revealed as 2*S*, 4*S*, and 5*R*. This result was, however, quite unexpected and remarkable since the piperidine alkaloids isolated from the same tunicate possess different stereochemistries at C-4 and C-5 positions. To obtain further unambiguous confirmation of this conclusion, we prepared the enantiomer (D-**13**) from D-serine by the same procedures as above, and subjected it to chiral hplc analysis (CHIRALPAK AD, Daicel Chemical Ind., Ltd.; 4.6 x 250 mm; flow rate: 0.5 ml/min; uv detection at 215 nm; eluent: hexane/2-propanol, 8:2), which established that the tetraacetate (**13**, t_R 16.5 min) derived from natural specimen (**11**) showed the same retention time as the enantiomer (D-**13**) prepared from D-serine (D-**13**: t_R 16.5 min; L-**13**: t_R 15.1 min), thus firmly establishing the 2*S*, 4*S*, and 5*R*-configurations for pseudodistomin C (**11**).

To supply a series of analogs of pseudodistomin derivatives for the purpose of further biological studies in connection with biological significance of sphingolipids, total synthesis of pseudodistomin C (**11**) is currently in progress with improved procedures by us.

2-5. Biogenetic Consideration of Pseudodistomins

It may be reasonably assumed that pseudodistomins A (**2**) and B (**3**) are biogenetically derived from D-*erythro*-sphingosine (**1**, Scheme 2); the absolute configurations of 4*R*-hydroxyl and 5*S*-amino groups of **2** and **3** are coincident with those of corresponding positions of **1** containing 3*R*-hydroxyl and 2*S*-amino groups. It was, however, quite



Scheme 2. A Hypothesis on the Biosynthesis of Pseudodistomins

surprising that pseudodistomin C (**11**), isolated from the same tunicate as **1** and **2**, possesses 4*S*,5*R*-configurations. Pseudodistomin C (**11**), therefore, has to be derived from unusual L-*erythro*-sphingosine (C_{20}-homologue, **23**; Scheme 2), whose amino alcohol moiety could be assumed to be generated from D-serine. Since all pseudodistomins A - C (**2**, **3**, and **11**) possess the same 2*S*-configuration, it may be presumed that the biosynthetic cyclization process of all these compounds (**2**, **3**, and **11**) affords commonly the same stereochemistry at C-2 of the piperidine ring; in other words, the enzyme which participates in the cyclization, which might be called as 'cyclase' (?), commonly gives the same configuration at C-2 from both hypothetical precursors (**a** and **b**).

Recently leucetamol A (**24**, Chart 1) was isolated from a sponge *Leucetta microraphis*,[21] and this amino alcohol was described as a racemate; *viz.* (2*S*,3*R*)- and (2*R*,3*S*)-isomers (L-**24**

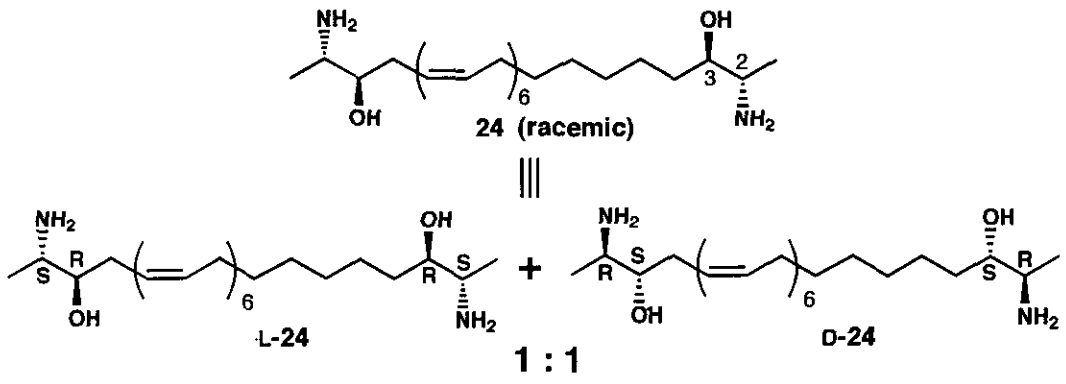
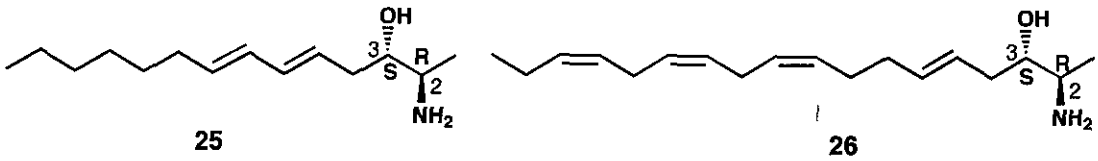


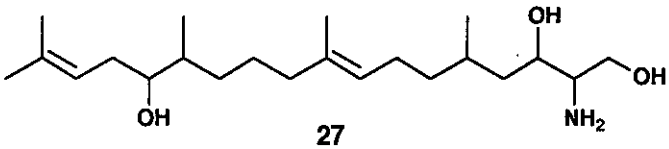
Chart 1. Leucetamol A, a Natural Product Present as a Racemate

and D-**24**, respectively) were concurrently present in the sponge. It may be assumed that L-**24** and D-**24** are generated from L- and D-alanine, respectively. From a sponge *Xestospongia* sp. an amino alcohol (**25**) was isolated[22] and was shown to have 2*R*,3*S*-configurations by synthesis.[23] Crucigasterins (*e.g.*, **26**) were isolated from a tunicate *Pseudodistoma crucigaster*, and were reported to possess 2*R*,3*S*-configurations.[24] Thus, these amino alcohols (**25** and **26**) were proposed to be biosynthetically derived from unusual D-alanine. These facts may justify the unexpected result that pseudodistomins A/B and C with opposite absolute stereochemistries at C-4 and C-5 positions are concurrently contained in the extract of the tunicate *Pseudodistoma kanoko*.



2-6. Other Sphingosine-Related Alkaloids from Tunicates

In 1978 from a tunicate *Aplidium sp.* collected from the Gulf of California, a sphingosine-related lipid, aplidiasphingosine (**27**) was isolated as an antimicrobial and cytotoxic substance, and this compound was regarded as a diterpenoid coupled with serine.[25]



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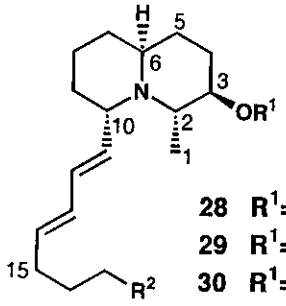
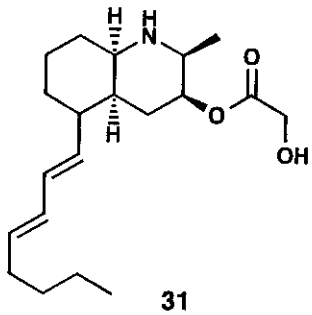
Pseudodistomins A (**2**) and B (**3**), isolated in 1986 and published in 1987 by us,[6] were the first piperidine alkaloids obtained from marine origins, and thereafter several piperidine alkaloids have been isolated from marine organisms, mainly from tunicates. Clavepictines A (**28**) and B (**29**) are cytotoxic quinolizidines from a Bermudian tunicate *Clavelina picta.* Their structures were based on the X-ray diffraction analysis of **29** and their absolute stereochemistry remained undefined.[26] Pictamine (**30**), a bis-nor homologue of clavepictine A (**28**), was isolated from the tunicate *Clavelina picta* collected in Venezuela, methanol



28 R^1=Ac, R^2=$CH_2CH_2CH_3$
29 R^1=H, R^2=$CH_2CH_2CH_3$
30 R^1=Ac, R^2=CH_3

18



31

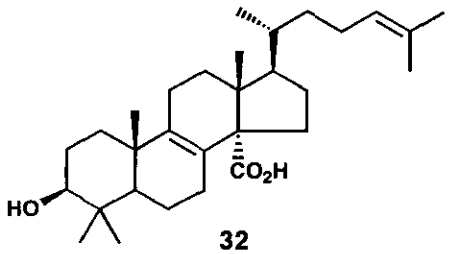
extracts of which were antimicrobial and antifungal; it was not described that these activities were ascribable to pictamine (**30**).[27] It may be noteworthy that much of compound (**30**) was lost by evaporation under high vacuum. From the tunicate *Clavelina lepadiforms* collected in the North Sea, a decahydroquinoline alkaloid, lepadin A (**31**), was isolated and its structure was elucidated by spectral studies.[28]

3. Penaresidins

3-1. Isolation and Structure Elucidation of Penaresidins

During our studies on bioactive substances from marine sponges,[29] we investigated extracts of an Okinawan sponge of the genus *Penares*, which was collected by netting at Unten Bay (-

70 m), Okinawa island, in June 1987. The methanol extract of this sponge was partitioned between toluene and water, and the aqueous phase was subsequently extracted with chloroform, ethyl acetate, and *n*-butanol. From the toluene-soluble fraction a cytotoxic triterpene acid, penasterol (**32**),[30] was isolated, while 6-bromoindole-3-acrylic acid (*penaresin*, **33**) was obtained from the chloroform-soluble fraction and was revealed to exhibit Ca-releasing activity in sarcoplasmic reticulum.[31] Further examination of the



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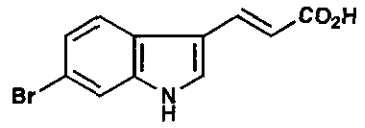
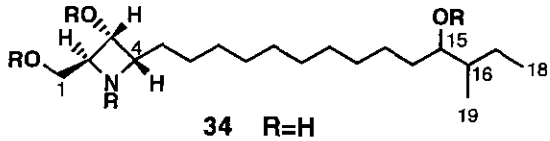
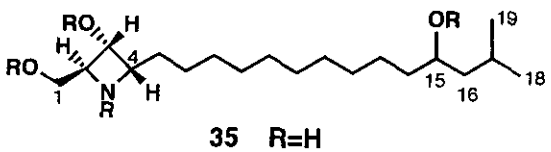


33

EtOAc-soluble fraction of this sponge by column chromatography on Sephadex LH-20 (MeOH/$CHCl_3$, 1:1) and silica gel ($CHCl_3$/*n*-BuOH/H_2O/AcOH, 1.5:6:1:1) resulted in the isolation of a mixture of two azetidine alkaloids, penaresidins A (**34**) and B (**35**),[32] in a ratio of *ca.* 1.5:1. Separation of this mixture using silica gel and ODS reversed-phase hplc was unsuccessful even after conversion of them into tetraacetates (**36** and **37**, respectively). Mixture of penaresidins A and B (**34** and **35**) exhibited potent actomyosin ATPase-activating activity; the mixture elevated the ATPase activity of myofibrils from rabbit skeletal muscle to 181% of the control value at 3 x 10^{-5} mol/l. Very few substances are hitherto known as those modulate the ATPase activities of myosin and actomyosin. Characterization and structural studies of penaresidins were carried out mostly using the mixture of tetraacetate (**36** and **37**). The ^1H nmr spectrum of the mixture of **36** and **37** was shown in Figure 4. Alkaline hydrolysis of the tetraacetate mixture (**36** and **37**) afforded a



34 R=H
36 R=Ac



35 R=H
37 R=Ac

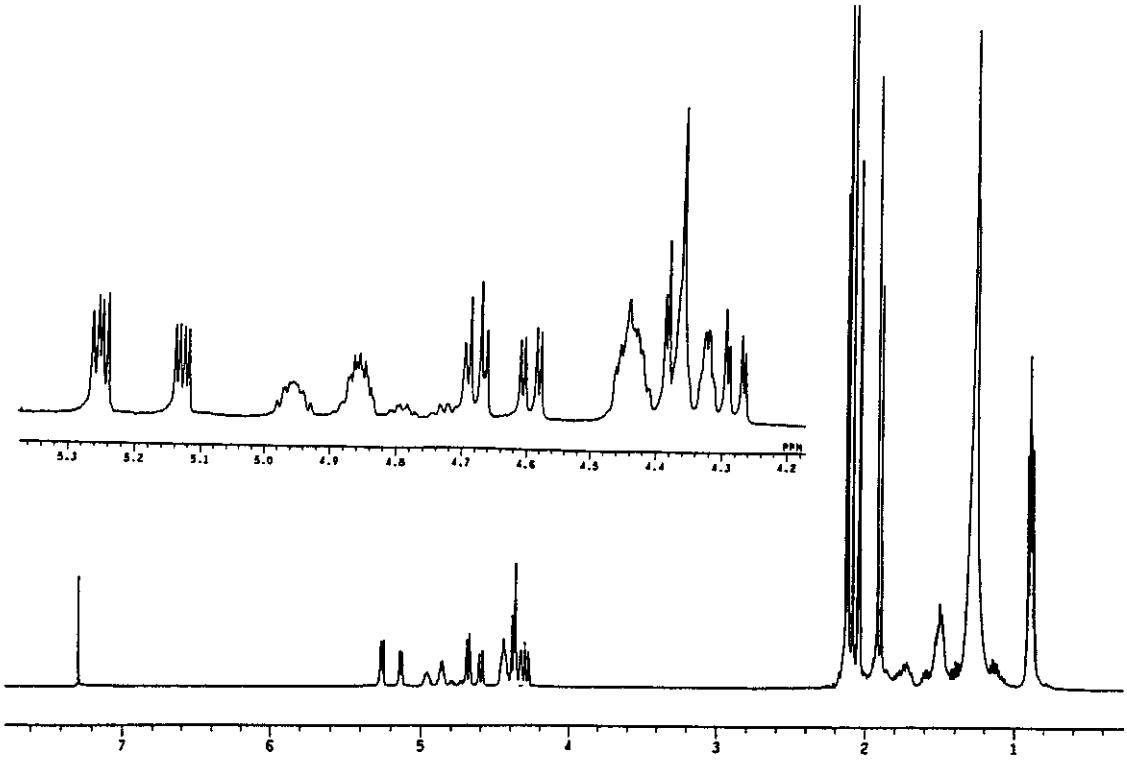
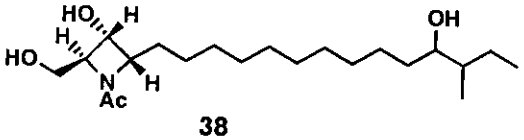


Figure 4. ^1H NMR Spectrum of Mixture of Penaresidins A and B Acetate (**36** and **37**) in $CDCl_3$ (500 MHz)



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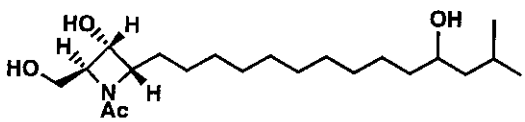
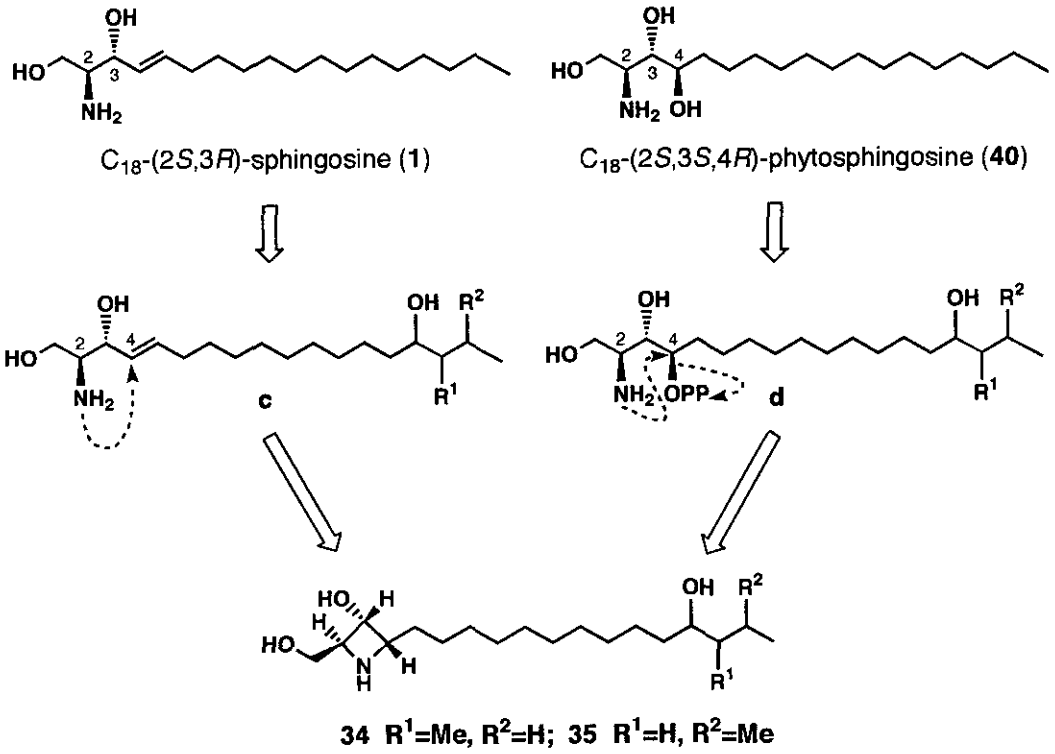


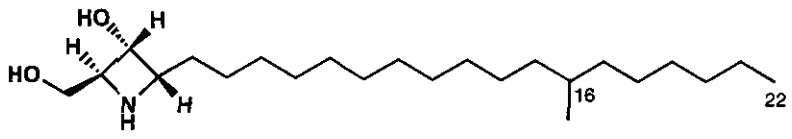
39

mixture of monoacetyl derivatives (**38** and **39**). The presence of the azetidine skeleton was suggested by analyzing the ^1H-^1H COSY and HMBC spectra of the mixture of **36** and **37**, and were also supported by the ^1H nmr chemical shift differences between the tetraacetates (**36/37**) and monoacetates (**38/39**). The relative stereochemistry of the azetidine ring portion was deduced from the coupling constants as 2,3-*trans* and 3,4-*cis*; the *J*-values for the azetidine ring protons were reported to be 5 Hz for *cis* and 9 Hz for *trans*, respectively.[33] The positions of the acetoxyl and secondary methyl groups were also indicated from the COSY and HMBC spectra.



Scheme 3. A hypothetical Biogenetic Path of Penaresidins

Penaresidins A and B (**34** and **35**) seems to be biogenetically derived from sphingosine (**1**) or phytosphingosine (**40**) through cyclization of N-2 to C-4 *via* intermediate **c** or **d**, and the relative stereochemistry at C-2 and C-3 was retained (Scheme 3). Natural products containing this type of sphingosine-related azetidine ring are unprecedented. The third compound belonging to this azetidine alkaloid group, named penazetidine A (**41**),[34] was recently isolated from a potato-shaped Indo-Pacific marine sponge, *Penares sollasi*, and was described to exhibit protein kinase C inhibition activity (IC_{50} 1 μM) and no inhibitory activity against protein tyrosine kinase. The structure of **41** was elucidated on the basis of spectroscopic data including 2D nmr measurements and tandem mass spectrometry of **41**

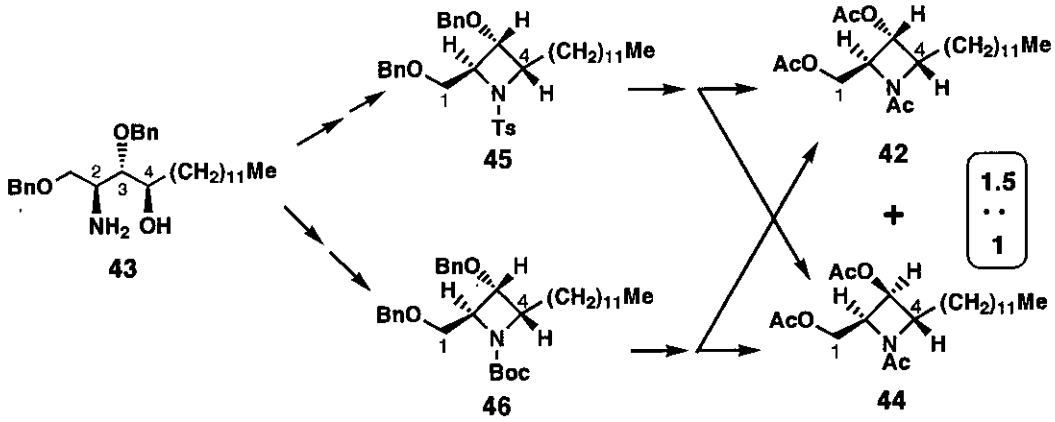


41

itself (not its acetate). It seems interesting because sphingosine (**1**) is known as a protein kinase C inhibitor.[35] Penazetidine A (**41**) was reported to show *in vitro* cytotoxicity against human and murine cell lines as well.

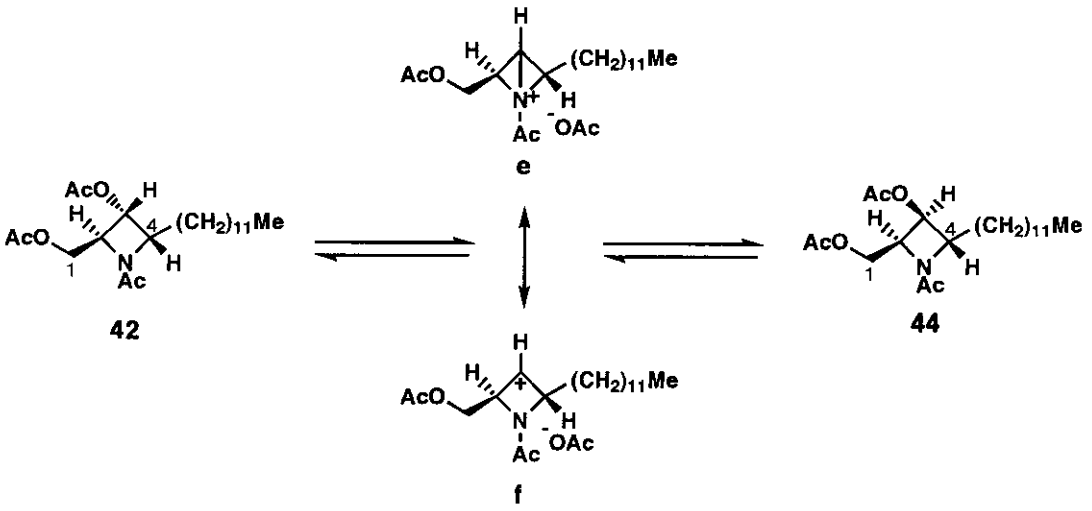
3-2. Studies on the Synthesis of Penaresidins by Other Groups and Revision of the Structure of Penaresidin B

Because of their unprecedented structure and biological activity, synthesis of penaresidins is investigated by plural groups. Kamikawa and coworkers recently achieved the synthesis of a C16 analog (**42**) of penaresidins.[36] They constructed azetidine ring through cyclization of the phytosphingosine derivative (**43**), which was prepared from D-xylose (Scheme 4). They described that the final acetate (**42**) was obtained as a mixture of its diastereomer (**44**) in a



Scheme 4. Synthesis of Penaresidin Analog by Kamikawa and Coworkers

1.5:1 ratio while the azetidine intermediates (**45** and **46**) were both single; this phenomenon was ascribed to the formation of azabicyclobutyl ion-pair (**e**) or an ion-pair of azetidinyl cation and OAc anion (**f**) (Scheme 5), which may be generated during the acetylation reaction through interaction between back-lobe of C(3)-O bonding and the electron on the nitrogen atom. They also pointed out that the ^{1}H nmr spectral data of the mixture of synthetic triacetates (**42** and **44**) (Figure 5) showed good resemblance to those of the natural



Scheme 5. Isomerization of Azetidine Acetates Proposed by Kamikawa and Coworkers

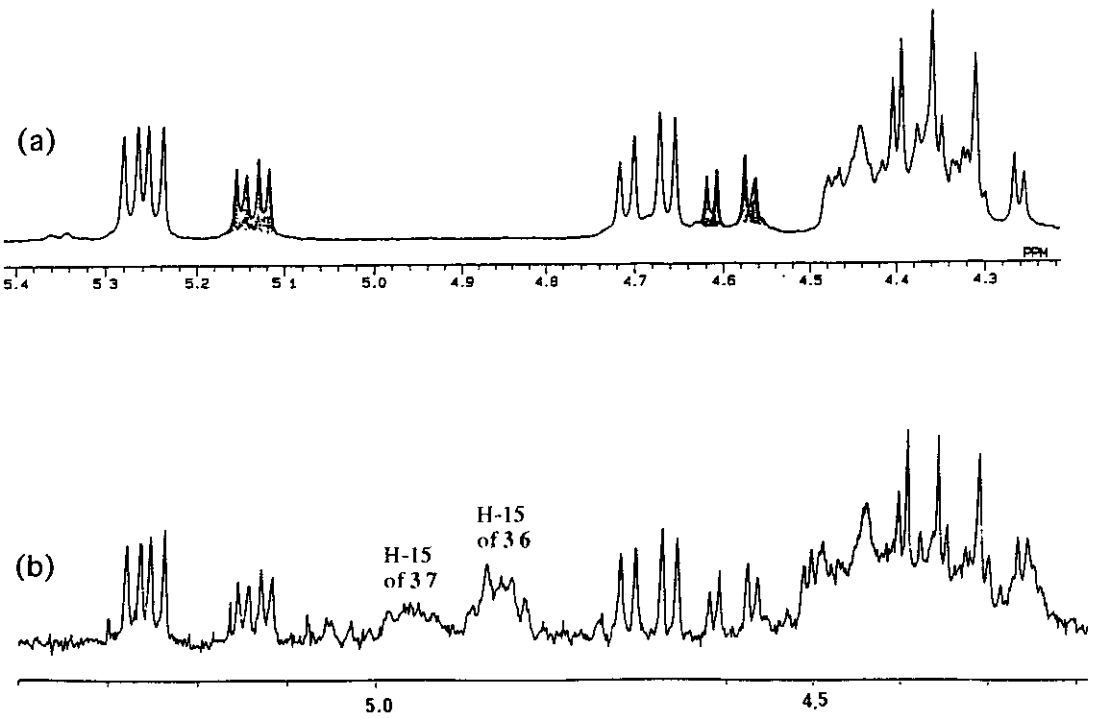
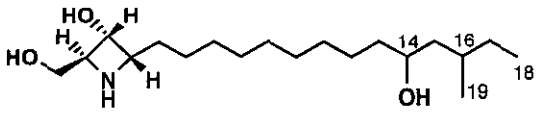


Figure 5. Expanded ^1H nmr Spectra (4.2 - 5.4 ppm region) of (a) Mixture of Synthetic Triacetates (**42** and **44**) Recorded by Kamikawa and Coworkers and (b) Mixture of Natural Penaresidin A and B Acetates (**36** and **37**, a crude sample) Recorded by Us. Both spectra were recorded in $CDCl_3$ at 270 MHz. For the spectrum of the purified sample of the latter, see Figure 4.

penaresidin acetate mixture (**36** and **37**) in their chemical shifts, coupling constants, and the ratio of the isomers.

Quite recently, Mori and coworkers[37] informed us that they also studied the synthesis of penaresidins, and suggested that our initially proposed structure of penaresidin B (**35a**)[32] with 14-hydroxyl and 16-methyl groups should be revised to **35** possessing 15-hydroxyl and 17-methyl groups. We reexamined the ^{13}C nmr data of mixture of penaresidin A and B acetates (**36** and **37**), and found that the ^{13}C nmr chemical shifts of the C-16 ~ C-19 positions of **36** and **37** corresponded well to those of corresponding positions of isoleucine and leucine,[38] respectively (Chart 2). We therefore agreed to the suggestion given by Mori and coworkers.



35a (initially proposed structure)

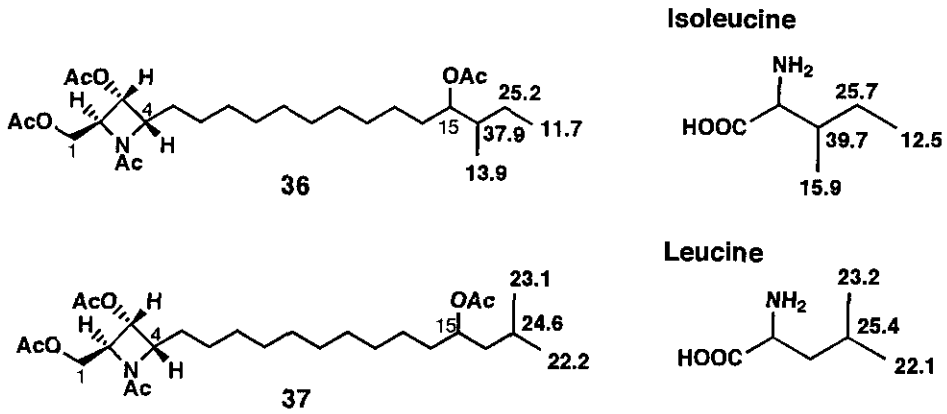
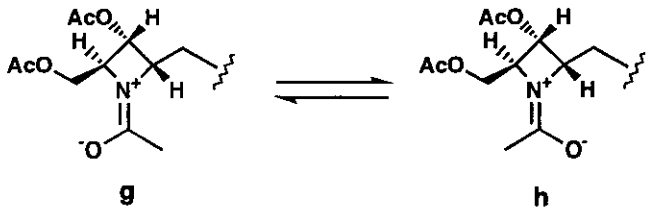


Chart 2. Comparison of the ^{13}C Nmr Chemical Shifts of Penaresidins A and B Acetates (**36** and **37**) with Those of Isoleucine and Leucine

Mori and coworkers also encountered the phenomenon that signals for two isomers were observed in the ^{1}H nmr of the azetidine acetates; they proposed that this phenomenon were attributed to the geometrical isomers due to the amide bond (**g** and **h**, Scheme 6).



g h

Scheme 6. Isomerization of Azetidine Acetates Proposed by Mori and Coworkers

The revised assignments of the nmr data of penaresidins A and B tetraacetates (**36** and **37**)[32] are described in Table 1. The major isomer of penaresidin A tetraacetate (**36**) is represented as **36a** and its minor isomer as **36b**, while isomers of penaresidin B tetraacetate

Table 1. Revised Assignments of the ^{1}H and ^{13}C Nmr Data of Penaresidins A and B Tetraacetates (**36** and **37**)

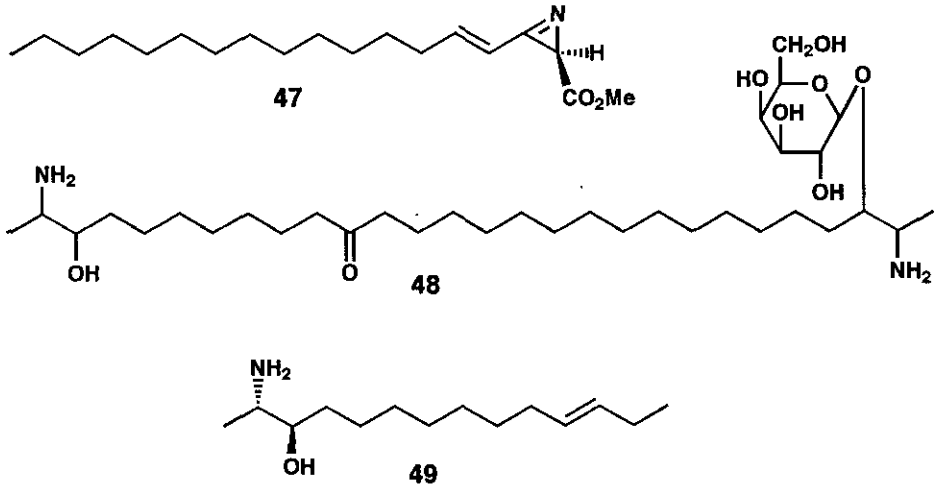
	36a		**36b**		**37a**		**37b**	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1(a)	4.63 dd	60.99 t	4.54 dd	62.27 t	4.63 dd	60.99 t	4.54 dd	62.27 t
(b)	4.33 dd		4.24 dd		4.33 dd		4.24 dd	
2	4.32 m	65.05 d	4.28 ddd	66.62 d	4.32 m	65.05 d	4.28 ddd	66.62 d
3	5.21 dd	66.45 d	5.09 dd	67.41 d	5.21 dd	66.45 d	5.09 dd	67.41 d
4	4.40 m	63.19 d	4.40 m	64.81 d	4.40 m	63.19 d	4.40 m	64.81 d
5(a)	2.10 m	29.04 t	1.92 m	26.86 t	2.10 m	29.04 t	1.92 m	26.86 t
(b)	1.90 m		1.65 m		1.90 m		1.65 m	
1-AcO	2.03 s	20.53 q	2.05 s	20.59 q	2.03 s	20.53 q	2.05 s	20.59 q
		170.08 s		170.25 s		170.08 s		170.25 s
N-Ac	2.07 s	20.62 q	2.08 s	20.64 q	2.07 s	20.62 q	2.08 s	20.64 q
		170.31 s		170.36 q		170.31 s		170.36 q
3-AcO	1.84 s	20.74 q	1.87 s	20.91 q	1.84 s	20.74 q	1.87 s	20.91 q
		169.93 s		169.97 q		169.93 s		169.97 q
14	1.45 m	43.35 t	1.45 m	43.35 t	1.47 m	34.93 t	1.47 m	34.93 t
15	4.81 dt	76.92 d	4.81 dt	76.92 d	4.91 ddt	72.67 d	4.91 ddt	72.67 d
16	1.50 m	37.98 d	1.50 m	37.98 d	1.45 m	25.52 t	1.45 m	25.52 t
17(a)	1.03 m	25.19 t	1.03 m	25.19 t	1.03 m	24.62 d	1.03 m	24.62 d
(b)	1.32 m		1.32 m		1.32 m		1.32 m	
18	0.82 t	11.65 q	0.82 t	11.65 q	0.83 t	22.16 q	0.83 t	22.16 q
19	0.84 d	13.89 q	0.84 d	13.89 q	0.85 d	23.11 q	0.85 d	23.11 q
14-AcO					1.99 s	21.20 q	1.99 s	21.20 q
						170.76 s		170.76 s
15-AcO	1.98 s	21.07 q	1.98 s	21.07 q				
		170.76 s		170.76 s				

J-values (H/H, Hz) for the azetidine part; **36a/37a**: 1a/1b=15.2, 1a/2=5.6, 1b/2=3.3, 2/3=5.2, 3/4=9.0; **36b/37b**: 1a/1b=15.2, 1a/2=4.5, 1b/2=3.7, 2/3=4.2, 3/4=8.7.

(**37**) are also delineated as **37a** (major) and **37b** (minor). The spectrum of a mixture of **36** and **37** (Figure 4), therefore, proved to be a spectrum of a mixture of four components (**36a**, **36b**, **37a**, and **37b**). The ratio of penaresidin A tetraacetate (**36**) and B tetraacetate (**37**) is *ca.* 1.5:1, and the ratios of the isomers (**36a**:**36b** and **37a**:**37b**) are also almost 1.5:1 incidentally. It still remained unestablished at this writing whether these isomers (**36a**/**36b** and **37a**/**37b**) correspond to isomerization shown in Scheme 5 (**42**/**44**) or that in Scheme 6 (**g**/**h**).

3-3. Other Sphingosine-Related Alkaloids from Sponges

Marine sponges are a rich source of sphingosine-related metabolites, among which penaresidins and penazetidin A are few examples possessing a cyclic amino alcohol structure. Another one having a heterocycle is dysidazirine (**47**), which contains an azacyclopropene ring and was isolated from the Fijian sponge *Dysidea fragilis*, and exhibited cytotoxicity as well as inhibition activity against Gram negative bacteria and yeast.[39] Acyclic metabolites such as **24**, **25** (see, section 2-5), and related-metabolites, rhizochalin (**48**)[40] and xestaminol



A (**49**)[41] are likely to be derived through condensation with alanine. Many ceramides and cerebrosides are heretofore isolated from marine sponges,[42] and some of them were reported to have unique bioactivities such as histidine-decarboxylase inhibition,[43]

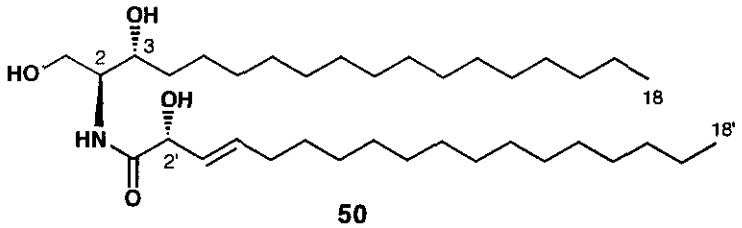
antifungal,[44] antitumor,[45] and immunostimulatroy[45] activities. Therefore, synthesis of the sponge-derived sphingolipids are also studied by several groups.[46]

4. Sphingosine-Related Metabolites from Marine Microorganisms

A number of sphingolipids, *viz.*, ceramides, cerebrosides, and gangliosides, were isolated from various marine organisms such as starfish,[47] sea cucumber,[48] sea anemone,[49] soft coral,[43] sea hare,[50] and algae.[51] This section deals with our recent works on two kinds of ceramides isolated from marine microorganisms.[52]

4-1. Symbioramide

A sphingosine derivative, symbioramide (**50**),[53] was isolated from the extract of a laboratory-cultured dinoflagellate of the genus *Symbiodinium*, which was isolated from the inside of gill cells of the Okinawan bivalve *Fragum* sp. Symbioramide (**50**) was revealed to be a sarcoplasmic reticulum (SR) Ca^{2+}-ATPase activator; compound **50** at 10^{-4} M activated SR Ca^{2+}-ATPase activity by 30%. The Ca^{2+}-ATPase in SR membrane plays an key role in muscle relaxation by energizing Ca^{2+}-pumping from the cytoplasm into the lumen of SR. The structure of **50** was elucidated by spectral and chemical means; the absolute configurations at C-2 and C-3 positions of the sphingosine part were determined by acid hydrolysis of **50** to give 2*S*-amino-3*R*-hydroxyoctadecan-1-ol.



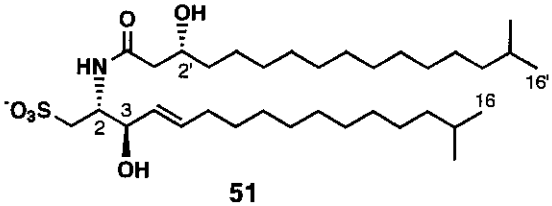
50

Total synthesis of symbioramide (**50**) was accomplished by Nakagawa and coworkers to establish the absolute configuration of the C-2' position of 2-hydroxyoctadec-3*E*-enoic acid moiety as *R*.[54] Mori and coworkers also studied the synthesis of this ceramide (**50**), and they found an interesting phenomenon that the optical rotation of **50** was influenced by the

temperature of the sample solution in a cell for rotation measurements: $[\alpha]_D^{19}$ = +3.6°, $[\alpha]_D^{23}$ = +0.76°, $[\alpha]_D^{28}$ = -1.5°, and $[\alpha]_D^{35}$ = -5.5° (c = 0.31 in $CHCl_3$).[55]

4-2. Flavocristamides

During our studies on bioactive substances from marine bacteria,[56] we recently investigated extracts of cultured marine bacterium *Flavobacterium* sp., which was isolated from bivalve *Cristaria plicata* collected in Ishikari Bay, Hokkaido, and succeeded in isolating two new sphingolipids, flavocristamides A (**51**) and B (**52**),[57] possessing a sulfonic acid group.



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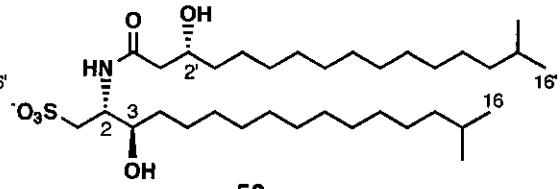

52

Structures of compounds (**51**) and (**52**) along with the stereochemistry of all chiral centers were elucidated on the basis of extensive spectroscopic analyses including FABms/ms measurements as well as degradation experiments such as acid hydrolysis and Lemieux oxidation. Both of **51** and **52** exhibited marked inhibitory activity against DNA polymerase α. Ceramide-1-sulfonic acids were previously obtained from marine diatom *Nitzschia alba*,[58] and studies on the synthesis of ceramide-1-sulfonic acids were also reported.[59]

ACKNOWLEDGMENT

We thank Professor T. Kamikawa, Kinki University, and Professor K. Mori, The University of Tokyo, for informing us of their works on the synthesis of penaresidins before publication and providing us with the spectral data of synthetic compounds as well as valuable discussions. These our works were partly supported by a Grant-in-Aid from the Uehara Memorial Foundation and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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14. It still remains a question that the optical rotation of (+)-**6** reported by Naito *et al.* ($[\alpha]_D$ +70.3° (*c* 0.64, MeOH))[13] was almost twice as large as those described by us ($[\alpha]_D^{23}$ +33° (*c* 1, MeOH))[6] and Knapp and Hale ($[\alpha]_D$ +36.9° (*c* 0.8, MeOH)).[7] Both two

groups except us did not describe the temperature. For a reference of the optical rotation fluctuated by the temperature, see reference 55.

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