Pergamon

Tetrahedron 57 (2001) 1229–1234

TETRAHEDRON

Ancorinosides B–D, inhibitors of membrane type 1 matrix metalloproteinase (MT1-MMP), from the marine sponge *Penares sollasi* Thiele

Masaki Fujita,[a] Yoichi Nakao,[a] Shigeki Matsunaga,[a] Motoharu Seiki,[b] Yoshifumi Itoh,[b] Rob W. M. van Soest[c] and Nobuhiro Fusetani[a,*]

[a]*Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*

[b]*Department of Cancer Cell Research, Institute of Medical Science, The University of Tokyo, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108-0071, Japan*

[c]*Institute for Systematics and Ecology, University of Amsterdam, 1090 GT Amsterdam, The Netherlands*

Received 16 October 2000; accepted 30 November 2000

Abstract—Ancorinosides B–D were isolated from the marine sponge *Penares sollasi* Thiele as inhibitors of membrane type 1 matrix metalloproteinase (MT1-MMP).[1] Their structures were elucidated to be tetramic acid glycosides related to ancorinoside A by spectroscopic and chemical methods. Ancorinosides B–D inhibited MT1-MMP with IC_{50} values of 180–500 μg/mL.

1. Introduction

The matrix metalloproteinases (MMPs) are members of a large subfamily of proteases which contain a catalytic zinc-binding domain.[2] They play important roles in the extracellular matrix (ECM) remodeling and degradation and are linked to a range of physiological and pathological processes, including wound healing, bone remodeling, angiogenesis, inflammation, tumor progression, and metastasis. Gelatinase A (MMP-2) is particularly implicated in tumor progression and now known to be derived from progelatinase A, which is processed by membrane-type MMPs (MT-MMPs).[3]

During the screening of Japanese marine invertebrates for inhibitors of the recombinant MT1-MMP,[4] we found considerable activity in the aqueous extract of the marine sponge *Penares sollasi* Thiele collected in southern Japan. Bioassay-guided fractionation resulted in isolation of three new active metabolites related to ancorinoside A.[5] This paper describes isolation, structure elucidation, and activity of these compounds.

2. Results and discussion

The combined organic extracts were concentrated, suspended in H_2O, and washed successively with $CHCl_3$ and *n*-BuOH. The aqueous phase was fractionated by ODS flash chromatography followed by repeated reverse-phase HPLC to afford ancorinosides B (**1**), C (**2**), and D (**3**) together with the known ancorinoside A (**4**) (Chart 1).[†]

Ancorinoside B (**1**) had a molecular formula of $C_{41}H_{69}NO_{17}$ as established by HR–FABMS. The ^{1}H NMR spectrum exhibited a methylene envelope (δ 1.3 ppm), two methylenes (1.55 and 1.60), two non-equivalent methylenes (2.37/2.78 and 2.73/2.78), an *N*-methyl (2.88), two oxygenated methylenes (3.51/3.88 and 3.68/3.76), nine oxygenated methines (between 3.3 and 3.9), and two anomeric protons (4.29 and 4.35). In addition to these functionalities, six non-protonated carbon signals were observed at δ 102.3 (C3), 175.7 (C2), 176.2 (C6′), 177.1 (C7), 195.7 (C4), and 197.0 (C8) in the ^{13}C NMR spectrum (Tables 1 and 2).

Interpretation of COSY and HMQC[6] data led to substructures **a**–**f**. Starting from the anomeric proton at δ 4.29 (H1′), the connectivities of five contiguous oxymethine protons were established (substructure **a**). Another anomeric proton

Keywords: biologically active compounds; enzyme inhibitors; marine metabolites; sponges.

* Corresponding author. Tel.: +813-5841-5299; fax: +813-5841-8166; e-mail: anobu@mail.ecc.u-tokyo.ac.jp

† Although **1**–**4** were isolated as the tris(diethylammonium) salts due to diethylammonium acetate buffer used for the final HPLC purification step, their structures are drawn as the free acid for the sake of convenience.

0040–4020/01/$ - see front matter
PII: S0040-4020(00)01128-5

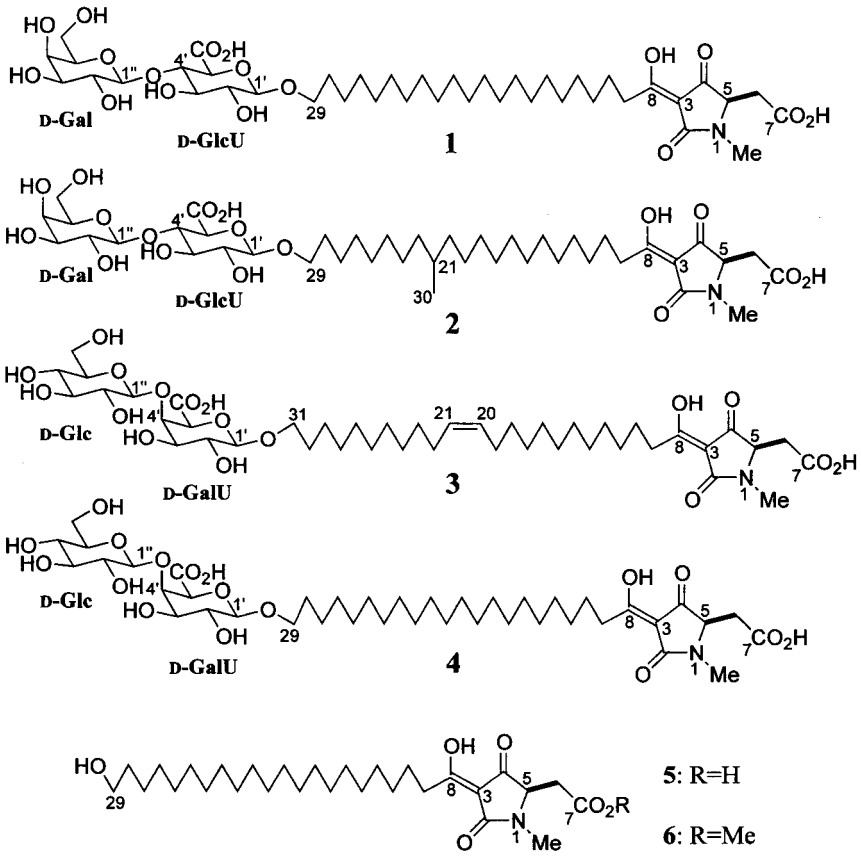


Chart 1.

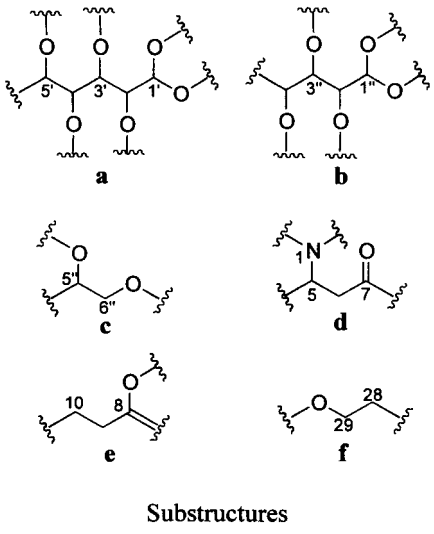


Chart 2.

at δ 4.35 (H1″) was placed at the end of four contiguous oxymethines (substructure **b**). There were two CH–CH_2 substructures; both carbons were oxygenated in substructure **c**, while substructure **d** contained a nitrogenous methine (C5) and a methylene adjacent to a carbonyl carbon. Two methylenes were left unassigned; obviously one (C9) was adjacent to a carbonyl carbon, and the other (C29) was oxygenated judging from their ^{1}H and ^{13}C chemical shifts. These methylenes were coupled to the distinct methylenes at δ 1.55 and 1.60, respectively, both of which were in turn correlated to signals in the methylene envelope (substructures **e** and **f**) (Chart 2).

Further structural analysis was carried out by HMBC[7] experiments (Fig. 1). Substructure **e** was expanded to a hexopyranosyluronic acid unit on the basis of the cross peaks between H4′/C6′, H5′/C6′, and H5′/C1′. Cross peaks between H5″/C4″, H4″/C5″, and H5″/C1″ could connect substructures **b** and **c**, thus completing a hexopyranose unit. The two hexose units were connected through a glycoside linkage between C1″ and C4′ which was implied from cross peaks H1″/C4′ and H4′/C1″. Substructure **d** was connected to a carboxyl group (C7) on the basis of the cross peaks between H5/C7 and H6$\alpha\beta$/C7. The chemical shifts of C2, C3, C4, and C8 were reminiscent of a tetramic acid moiety,[8] which was in agreement with UV absorption at 282 nm. The HMBC correlation network constructed a tetramic acid functionality; connectivity between C5 and C4 was evident from cross peaks between H5/C4 and H6$\alpha\beta$/C4, while connectivity from C5 to C2 through a methylimino group was implied from the cross peaks between H5/C2, *N*-CH_3/C2 and *N*-CH_3/C5. The other end of the tetramic acid moiety (C8) was connected to substructure **e** through C9 on the basis of the cross peak between H9$\alpha\beta$/C8. Substructure **e** was linked to a long methylene chain whose terminus, substructure **f**, was in turn connected to the uronic acid unit (Fig. 1). To fulfill the molecular formula, a C_{21} methylene unit could be accommodated between these two substructures obtained, thus completing the gross structure of **1**.

Table 1. ^{1}H NMR data for **1**–**3** in CD_3OD

#	**1** δ_H mult. (*J* (Hz))	**2** δ_H mult. (*J* (Hz))	**3** δ_H mult. (*J* (Hz))
5	3.88 dd (8.1, 4.2)	3.88 m	3.88 dd (6.5, 4.7)
6	2.78 dd (16.2, 4.2)	2.78 dd (15.8, 4.8)	2.79 dd (15.8, 4.7)
	2.37 dd (16.2, 8.1)	2.37 dd (15.8, 7.9)	2.48 dd (15.8, 6.5)
9	2.73 m	2.74 m	2.75 m
	2.78 m	2.78 m	2.79 m
10	1.55 quint. (7.3)	1.55 quint. (7.3)	1.59 m
11	1.32 m	1.32 m	1.32 m
12	1.27 m	1.28 m	1.28 m
13	1.27 m	1.28 m	1.28 m
14	1.27 m	1.28 m	1.28 m
15	1.27 m	1.28 m	1.28 m
16	1.27 m	1.28 m	1.28 m
17	1.27 m	1.28 m	1.28 m
18	1.27 m	1.28 m	1.32 m
19	1.27 m	1.28 m	2.02 q (6.5)
20	1.27 m	1.25 m	5.33 t (4.6)
21	1.27 m	1.35 m	5.33 t (4.6)
22	1.27 m	1.25 m	2.02 q (6.5)
23	1.27 m	1.28 m	1.32 m
24	1.27 m	1.28 m	1.28 m
25	1.27 m	1.28 m	1.28 m
26	1.27 m	1.28 m	1.28 m
27	1.36 m	1.37m	1.28 m
28	1.60 quint. (7.3)	1.61 quint. (6.9)	1.28 m
29	3.88 m	3.88 m	1.37 m
	3.51 m	3.51 m	–
30	–	0.85 d (6.5)	1.61 m
31	–	–	3.97 dt (9.2, 7.1)
	–	–	3.49 m
*N*Me	2.88 s	2.88 s	2.88 s
1′	4.29 d (7.7)	4.29 d (8.1)	4.21 d (7.7)
2′	3.28 t (8.1)	3.28 t (8.5)	3.54 dd (10.0, 7.7)
3′	3.50 t (8.9)	3.50 t (8.6)	3.59 dd (10.0, 3.5)
4′	3.59 t (9.2)	3.59 t (9.6)	4.33 d (3.5)
5′	3.72 d (9.6)	3.73 d (10.0)	3.91 s
1″	4.35 d (7.7)	4.34 d (7.7)	4.47 d (8.1)
2″	3.53 dd (10.0, 7.7)	3.54 dd (9.8, 7.7)	3.20 m
3″	3.47 dd (10.0, 3.8)	3.47 dd (9.8, 3.2)	3.35 m
4″	3.78 d (3.8)	3.78 d (3.2)	3.20 m
5″	3.56 m	3.56 m	3.20 m
6″	3.76 dd (11.5, 7.3)	3.77 dd (11.4, 6.9)	3.81 dd (11.9, 1.9)
	3.68 d (11.5, 4.6)	3.69 dd (11.4, 4.6)	3.62 m

Table 2. ^{13}C NMR data for **1**–**3** in CD_3OD

#	**1** δ_C	**2** δ_C	**3** δ_C
2	175.7	175.7	175.6
3	102.3	102.3	102.4
4	195.7	195.7	195.5[a]
5	63.6	63.5	63.2
6	39.1	39.0	37.9
7	177.1	177.0	175.9
8	197.0	197.0	196.3[a]
9	40.5	40.5	40.6
10	27.3	27.2	27.2
11	30.8	30.7	30.8
12	30.8	30.7	30.8
13	30.8	30.7	30.8
14	30.8	30.7	30.8
15	30.8	30.7	30.8
16	30.8	30.7	30.8
17	30.8	30.7	30.8
18	30.8	30.7	30.8
19	30.8	28.1	28.2
20	30.8	38.2	130.9
21	30.8	33.9	130.9
22	30.8	38.2	28.2
23	30.8	28.1	30.8
24	30.8	30.7	30.8
25	30.8	30.7	30.8
26	30.8	30.7	30.8
27	27.2	27.1	30.8
28	30.8	31.0	30.8
29	71.0	71.0	27.2
30	–	11.6	30.8
31	–	–	71.2
*N*Me	27.4	27.4	27.2
1′	104.2	104.1	104.7
2′	74.6	74.5	72.9
3′	76.7	76.6	75.6
4′	83.5	83.4	81.2
5′	77.2	77.0	76.5
6′	176.2	176.1	174.9
1″	105.5	105.4	105.8
2″	72.7	72.6	75.7[b]
3″	75.0	74.9	78.1
4″	70.3	70.2	78.1[b]
5″	77.2	77.2	71.6[b]
6″	62.5	62.5	62.8

[a] Assigned from HMBC cross peaks.
[b] Interchangeable.

Although the gross structure of **1** obtained was identical with that of ancorinoside A (**4**), the ^{1}H NMR spectrum of our compound was significantly different from that reported for **4**.[5] Thus, determination of the relative stereochemistry of the disaccharide portion of **1** was carried out on the basis of the ^{1}H–^{1}H coupling constants, which disclosed the identification of glucuronic acid and galactose. Therefore, ancorinoside B (**1**) was different from ancorinoside A (**4**) in two sugar components; the latter consists of galacturonic acid and glucose. Two anomeric protons at δ 4.29 (d, J=7.7 Hz) and δ 4.35 (d, J=7.7 Hz) implied β-glycosidic linkage of sugar units.

The absolute stereochemistry of **1** was determined as follows. Ancorinoside B (**1**) was subjected to methanolysis to afford the methyl ester of the aglycon **6** and the methyl glycosides. Compound **6** was oxidized with RuO_4 followed by acid hydrolysis to furnish MeAsp, which was determined to be in the D-form by Marfey analysis.[9] The D-configuration of two monosaccharides resulted from GC analysis using a chiral stationary phase.[10]

Ancorinoside C (**2**) had a molecular formula of $C_{42}H_{71}NO_{17}$, which was larger than that of **1** by CH_2. The ^{1}H NMR spectrum readily revealed the presence of the disaccharide and tetramic acid units found in **1**. The most significant difference was the presence of a methyl doublet signal in **2**. Since the methyl group was present in the middle of the long alkyl chain, we decided to determine the position of the methyl group by FAB–MS/MS analysis of the aglycon methyl ester (**7**), in which a negative charge was located in the tetramic acid moiety to effect the charge remote fragmentation. In fact, intense fragment ions at *m/z* 378 and 406 allowed us to place the methyl group at C21 (Fig. 2A). The chemical degradation of **2** resulted in D-MeAsp, D-Gal, and D-GlcU. The stereochemistry at C21 remained to be determined.

The molecular formula of ancorinoside D (**3**) was determined to be $C_{43}H_{71}NO_{17}$, which was larger than that of **1** by C_2H_2. Interpretation of 2D NMR data indicated that **3** had a disubstituted olefin, the tetramic acid core as found in **1**, a disaccharide unit, and a methylene chain. 2D NMR data indicated that the disaccharide unit was identical with that in ancorinoside A (**4**). Therefore, **3** was a vinylogous homolog of **4**.

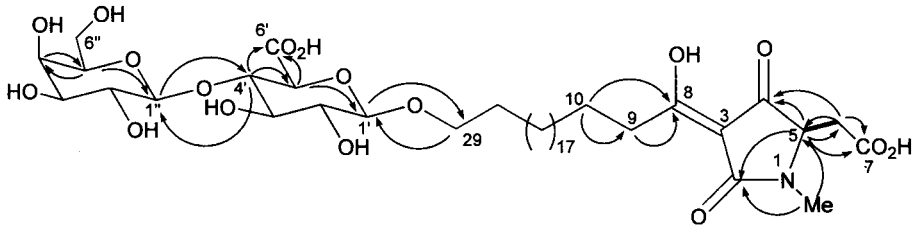


Figure 1. Key HMBC correlations.

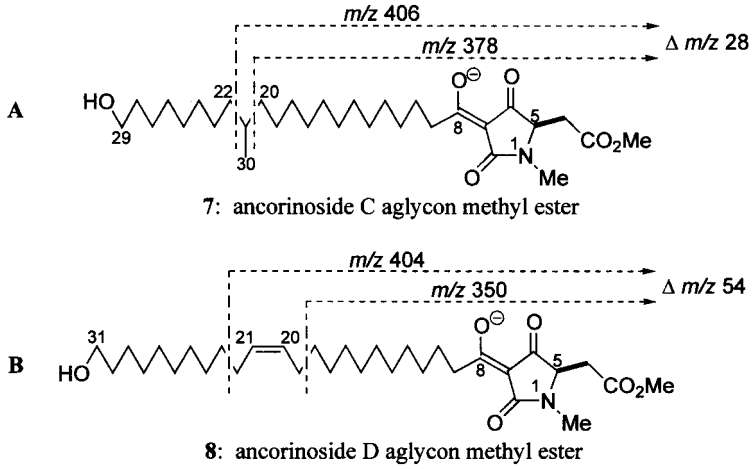


Figure 2. FAB–MS/MS fragmentation of **7** and **8**.

Table 3. Biological activity of ancorinosides

#	Compound	MT1-MMP	MMP2	Cytotoxicity[a]
		IC_{50} (μg/mL)	IC_{50} (μg/mL)	IC_{50} (μg/mL)
1	ancorinoside B	500	33	>22
2	ancorinoside C	370	NT	NT
3	ancorinoside D	180	NT	NT
4	ancorinoside A	440	NT	NT
5	aglycon of ancorinoside B	160	100	22
6	aglycon methyl ester	130	48	22
9	FN-439	25	1.5	>22
10	tenuazonic acid	22	1.1	4.5

[a] Against P-388 murine leukemia cells. NT: not tested.

The position of the double bond was determined again by negative mode FAB–MS/MS analysis of the aglycon methyl ester (**8**), which exhibited prominent ions at *m/z* 350 and 404 corresponding to those arising from cleavage at allylic positions (Fig. 2B), thereby indicating that the olefin was located between C20 and C21. Its geometry was assigned as *Z* on the basis of the ^{13}C chemical shift values of C19 and C22. The absolute stereochemistry of **3** was similarly determined as D-MeAsp, D-Glc, and D-GalU.

Ancorinosides B–D (**1**–**3**) inhibited MT1-MMP with IC_{50} values of 500, 370, and 180 μg/mL, respectively. As shown in Table 3, ancorinosides are 10 times less potent than the known inhibitor FN-439 (**9**),[11] which encompasses a hydroxamate group. The MMP inhibitors known to date contain either hydroxamate or carboxylate groups.

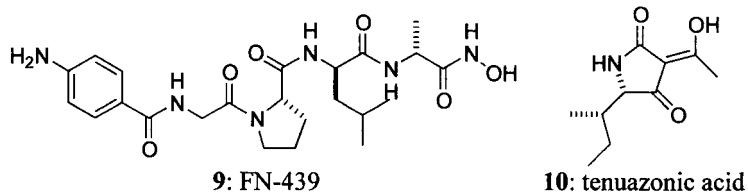
Ancorinosides contain two carboxylic acids and a tetramic acid group. In order to disclose which group was essential for the activity, we examined activity of the aglycon of ancorinoside B (**5**), its methyl ester **6**, and tenuazonic acid (**10**).[12] Compounds **5** and **6** were slightly more potent than ancorinoside B (**3**), indicating the irrelevance of the two carboxylic acid groups in the activity. The importance of the tetramic acid group was evident from the potent activity of tenuazonic acid, which has no functional group other than tetramic acid (Chart 3).

Ancorinoside A (**4**) was originally isolated from a marine sponge *Ancorina* sp. as an inhibitor of blastulation of starfish embryos. At present it is unknown if this activity is due to inhibition of MT1-MMP.

3. Experimental

3.1. General procedures

NMR spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts were



9: FN-439 **10**: tenuazonic acid

Chart 3.

referenced to the solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD and δ_H 7.24 for $CDCl_3$. FAB mass spectra were measured using triethanolamine as a matrix. Negative mode HR–FABMS were obtained at resolution of 5000 using PEG 600 sulfate as a marker. FAB–MS/MS experiments were performed on a four-sector tandem mass spectrometer with EBEB geometry. The accelerating voltage was 10 or 6 kV, and a collision cell located in the third field free region was not floated. Helium was introduced to cause the dissociation at a pressure that reduced the intensity of precursor ions to 10, 30, or 50%. Optical rotations were determined on a digital polarimeter in CH_3OH or $CHCl_3$. UV spectra were recorded in CH_3OH.

3.2. Animal material

The sponge was collected using SCUBA at depths of 15–20 m off Uke-jima Island near Amami-Oshima Island (28°02′80″N, 129°13′40″E), immediately frozen and preserved at −20°C until extraction. The sponge is a black lobate mass, approximately 7×6×4 cm, with smooth glistening black surface. The interior is cream. Ectosomal regions consist of an outer thickly felted mass of microxeas regularly arranged around porefields of 50–80 μm diameter, carried by the cladomes of triaenes and by the ends of radiating bundles of large oxeas. Microscleres are abundant in the interior. Spicules consist of (1) orthotriaenes with cladi of 70–120×7–10 μm and rhabds of 600–800 μm, (2) large oxeas 650–900×10–12 μm, (3) microxeas in two distinct size categories, 90–150×1–2 μm and 25–30×1 μm, and (4) asters in two distinct categories, large ones 25–42 μm with up to eight rays, and smaller multirayed ones 8–20 μm. These features conform quite closely to *Penares sollasi* Thiele, 1903, originally described from the Moluccas, the only difference being the presence of dichotraenes instead of orthotriaenes. That is considered as a minor difference. A voucher specimen was incorporated in the Zoological Museum of Amsterdam under ZMA POR. 15733.

3.3. MT1-MMP inhibition assay

The recombinant MT1-MMP[4] which was truncated the transmembrane domain and fluorescent substrate MOCAc-Pro-Leu-Gly-Leu-A_2pr(Dnp)-Ala-Arg-NH_2 purchased from Peptide Institute Inc., Osaka, were used. Inhibition assay of MT1-MMP was carried out by the modified procedure of Knight et al.[13] Test samples (2 μL) were added to a well of 96-well microtiter plates containing 100 μL of TNC buffer (50 mM Tris–HCl pH 7.5+150 mM NaCl+10 mM $CaCl_2$+0.02% NaN_3+0.05% Brij-35). Fifty microliters of enzyme solution (40 ng/mL) were added to the sample solution, and pre-incubated at 37°C for 10 min. After pre-incubation, 50 μL of substrate solution (8 μM) was added to the mixture to begin the reaction. The fluorescence values were measured at an excitation of 328 nm and an emission of 393 nm after incubation at 37°C for 1 h.

3.4. Extraction and isolation

The frozen sample (400 g, wet wt) was homogenized and exhaustively extracted with 50% aqueous MeOH, MeOH and $CHCl_3$/MeOH (1:1) (500 mL×2, each). The combined extracts were concentrated and partitioned between $CHCl_3$ and H_2O, and the aqueous layer was further extracted with *n*-BuOH. The active aqueous layer was fractionated by ODS flash chromatography with stepwise elution of aqueous MeOH and $CHCl_3$/MeOH/H_2O (7:3:0.5). The inhibitory activity was found in the fractions eluted with 50%, 70%, and 100% MeOH; the 100% MeOH fraction gave white precipitates during evaporation. The precipitates were separated by centrifugation, and both supernatant and the precipitates were subjected to enzyme inhibition assay. As the white precipitates were more active than the supernatant, the precipitates were dissolved in aqueous *n*-PrOH containing 0.1% TFA and subjected to ODS HPLC (COSMOSIL $5C_{18}$ AR-II; solvent 40% *n*-PrOH containing 0.1% TFA). The active fractions were further fractionated on ODS HPLC (Inertsil ODS-3) with the same solvent system to obtain four partially purified compounds. Other active side fractions were also separated in the same manner, and the peaks which gave identical retention time were combined. These fractions were finally purified by ODS HPLC [CAPCELL PAK UG-80; solvent *n*-PrOH/MeCN/H_2O (25:15:60) with 0.125 M diethylamine and 0.05 M AcOH] to yield ancorinoside B (**1**: 14.3 mg, 3.58×10^{-3}% yield based on wet weight), ancorinoside C (**2**: 13.6 mg, 3.40×10^{-3}%), ancorinoside D (**3**: 4.6 mg, 1.15×10^{-3}%), and ancorinoside A (**4**: 2.3 mg, 5.75×10^{-4}%).

3.4.1. Ancorinoside B (1). White powder; $[\alpha]_D^{24}=+1.5°$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} 282 nm (ϵ 8540); HR–FABMS (triethanolamine) *m/z* 846.4483 $(M-H)^-$ ($C_{41}H_{69}NO_{17}$; calcd for 846.4488); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.4.2. Ancorinoside C (2). White powder; $[\alpha]_D^{24}=+2.8°$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} 282 nm (ϵ 8430); HR–FABMS (triethanolamine) *m/z* 860.4689 $(M-H)^-$ ($C_{42}H_{71}NO_{17}$; calcd for 860.4645); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.4.3. Ancorinoside D (3). White powder; $[\alpha]_D^{24}=-5.2°$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} 282 nm (ϵ 9630); HR–FABMS (triethanolamine) *m/z* 872.4680 $(M-H)^-$

($C_{43}H_{71}NO_{17}$; calcd for 872.4645); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.5. Methanolysis and chiral GC analysis

Each compound (0.5 mg) was dissolved in 0.5 mL of 10% HCl–MeOH and heated at 100°C for 2 h. The reaction mixture was dried in a stream of N_2 gas, and the residue was partitioned between H_2O and *n*-BuOH. The H_2O layer was dried, and dissolved in a mixture of CH_2Cl_2 (0.2 mL) and trifluoroacetic anhydride (0.2 mL), and the mixture was heated at 100°C for 5 min. After removal of the solvent, the residue was dissolved in CH_2Cl_2 (0.1 mL), and a 5 μL portion was subjected to the chiral GC analysis on Chirasil-L-Val capillary column (25 m×0.25 mm, i.d.): detection, FID; initial temperature 50°C for 6 min, final temperature 160°C for 1 min, temperature raised at 4°C min^{-1}. Retention times: standard D-GlcU (28.147, 32.142 min), L-GlcU (28.078, 32.613 min), D-Gal (27.620, 33.017 min), L-Gal (28.043, 33.492 min), D-GalU (24.843, 29.863 min), L-GalU (24.923, 29.917 min), D-Glc (26.888, 32.145 min), L-Glc (26.700, 31.878 min); products from ancorinoside B (**1**), 27.872, 28.382, 32.330, and 33.255 min; products from ancorinoside C (**2**), 27.842, 28.360, 32.337, and 33.257 min; products from ancorinoside D (**3**), 24.750, 26.993, 29.857, and 32.183 min. Because the retention times fluctuated, identity of the peaks was examined by co-injection with the standards. Absolute configuration of GlcU, GalU, Glc, and Gal in the methanolysates were all assigned as D.

3.6. RuO_4/$NaIO_4$ oxidation, acid hydrolysis, and Marfey analysis

The *n*-BuOH layer described above was dried in a stream of N_2 gas, and the residue was dissolved in a mixture of MeCN, aqueous $NaIO_4$ (10 mg/mL), and CCl_4 (1:1:1, total 4.5 mL). To the mixture was added 1 mg of $RuCl_3$–nH_2O (n=1–3), and the mixture was stirred for 2 h at room temperature. Solvents were removed in a stream of N_2 gas. The residue was dissolved in 0.5 mL of 6 N HCl, and the solution was heated at 105°C for 2 h. The hydrolysate was passed through an ODS column with H_2O, and the eluate was dried and derivatized with 50 μL of 0.1% 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (L-FDAA) in acetone and 100 μL of 0.1 M $NaHCO_3$ at 80°C for 10 min. To the reaction mixture were added 50 μL of 0.2 M HCl and 100 μL of 50% aqueous MeCN containing 0.05% TFA. A 20 μL portion was subjected to HPLC analysis [COSMOSIL $5C_{18}$ AR-II (ϕ 4.6×250 mm); mobile phase, aqueous MeCN containing 0.05% TFA].

3.7. Preparation of aglycon of ancorinoside B 5 and the aglycon methyl ester 6

Ancorinoside B (2 mg) was methanolyzed with 10% HCl–MeOH (500 μL) by stirring at 100°C for 2 h. Solvent was removed and the residue was partitioned between *n*-BuOH and H_2O to yield the aglycon methyl ester **6** in the *n*-BuOH layer. A half portion of **6** was hydrolyzed with 0.5 N KOH (500 μL) at room temperature overnight. The reaction mixture was extracted with *n*-BuOH to yield aglycon **5**.

3.7.1. Aglycon 5. White powder; $[\alpha]_D^{24}$=+3.5° (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} 282 nm (ϵ 10200); HR–FABMS (triethanolamine) *m/z* 508.3654 $(M-H)^-$ (calcd for 508.3638); ^1H NMR ($CDCl_3$) δ 4.05 (1H, brs, H-5), δ 3.59 (2H, t, J=6.5 Hz, H-29), δ 2.90 (1H, dd, 16.5, 3.5, H-6), δ 2.69 (3H, s, *N*-Me), δ 2.79 (2H, m, H-9), δ 2.68 (1H, dd, 16.5, 6.2, H-6), δ 1.61 (2H, quint., 6.9, H-10), δ 1.51 (2H, quint., 7.6, H-28), δ 1.31 (2H, m, H-11), δ 1.26 (2H, m, H-27), δ 1.21 (30H, m, H-12-26).

3.7.2. Aglycon methyl ester 6. White powder; $[\alpha]_D^{24}$=+3.5° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} 282 nm (ϵ 9740); HR–FABMS (triethanolamine) *m/z* 522.3785 $(M-H)^-$ (calcd for 522.3794); ^1H NMR ($CDCl_3$) δ 4.00 (1H, brs, H-5), δ 3.66 (3H, s, *O*-Me), δ 3.59 (2H, t, J=6.3 Hz, H-29), δ 2.92 (3H, s, *N*-Me), δ 2.86 (1H, dd, 16.7, 4.6, H-6), δ 2.79 (2H, m, H-9), δ 2.70 (1H, dd, 16.7, 6.5, H-6), δ 1.62 (2H, quint., 7.6, H-10), δ 1.53 (2H, quint., 7.1, H-28), δ 1.32 (2H, quint., 7.2, H-11), δ 1.27 (2H, m, H-27), δ 1.21 (30H, m, H-12-26).

Acknowledgements

We are indebted to the crew of R/V *Toyoshio-maru* of Hiroshima University for assistance in collection of the sponge samples. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan and Japan Society for the Promotion of Science, 'Research for the Future Program' (JSPS-RFTF 96I00301).

References

1. Part 105 of the Bioactive Marine Metabolites Series. Part 104: Nakao, Y.; Fujita, M.; Warabi, K.; Matsunaga, S.; Fusetani, N. *J. Am. Chem. Soc.* **2000**, *122*, 10462–10463.
2. Woessner Jr, J. F. *Ann. N. Y. Acad. Sci.* **1999**, *732*, 11–21.
3. (a) Seiki, M. *APMIS* **1999**, *107*, 137–143; (b) Polette, M.; Birembaut, P. *Int. J. Biochem. Cell. Biol.* **1998**, *30*, 1195–1202.
4. Kinoshita, T.; Sato, H.; Takino, T.; Itoh, M.; Akizawa, T.; Seiki, M. *Cancer Res.* **1996**, *56*, 2535–2538.
5. Ohta, S.; Ohta, M.; Ikegami, S. *J. Org. Chem.* **1997**, *62*, 6452–6453.
6. Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285–4294.
7. Bax, A.; Azolos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056–8063.
8. Sakuda, S.; Ono, M.; Furihata, K.; Nakayama, J.; Suzuki, A.; Isogai, A. *J. Am. Chem. Soc.* **1996**, *118*, 7855–7856.
9. Marfey, P. *Carlesberg Res. Commun.* **1984**, *49*, 591–596.
10. Ushio-Sata, N.; Wada;, S.; Matsunaga, S.; Watabe, S.; Soest, R. W. M.; Fusetani, N. *J. Org. Chem.* **1999**, *64*, 2331–2339.
11. Odake, S.; Morita, Y.; Morikawa, T.; Yoshida, N.; Hori, H.; Nagai, Y. *Biochem. Biophys. Res. Commun.* **1994**, *199* (3), 1442–1446.
12. Rosset, T.; Sankhala, R. H.; Stickings, C. E.; Taylor, M. E. U.; Thomas, R. *Biochem J.* **1957**, *67*, 390.
13. Knight, C. G.; Willenbrock, F.; Murphy, G. *FEBS Lett.* **1992**, *296*, 263–266.