Isolation and Structure of Three New Ceramides from the Starfish *Acanthaster planci*

Masanori Inagaki[a], Ryuichi Isobe[b], Yasuhiro Kawano[a], Tomofumi Miyamoto[a], Tetsuya Komori[a], and Ryuichi Higuchi*[a]

Faculty of Pharmaceutical Sciences, Kyushu University[a],
3-1-1 Maidashi, Higashi-ku, Fukuoka 812-82, Japan

Department of Industrial Chemistry, Faculty of Engineering, Towa University[b],
1-1-1 Chikushigaoka, Minami-ku, Fukuoka 815, Japan

Received June 30, 1997

Keywords: Sphingolipids / Ceramides / Starfish / *Acanthaster planci* / FABMS/MS

Three new phytosphingosine-type ceramides, AC-1-6, AC-1-10 and AC-1-11, were isolated from the ceramide molecular species AC-1, obtained from the less polar fraction of the $CHCl_3$/MeOH extract of the starfish *Acanthaster planci*. The structures of these ceramides were determined on the basis of chemical and spectroscopic evidence as (2*S*,2′*R*,3*S*,4*R*,9*Z*)-2-(2-hydroxyhexadecanoylamino)-9-docosene-1,3,4-triol (AC-1-6), (2*S*,2′*R*,3*S*,4*R*,)-2-(2-hydroxytricosanoylamino)hexadecane-1,3,4-triol (AC-1-10) and (2*S*,2′*R*,3*S*,4*R*,)-2(2-hydroxytetracosanoylamino)hexadecane-1,3,4-triol (AC-1-11). Positive ion FABMS/MS of each ceramide gave important information for their structure elucidation. Mass spectrometry of the dimethyl disulfide derivatives of ceramide was also useful for the determination of the double bond position in the long-chain base.

A series of studies on the isolation and structure elucidation of the glycosphingolipids from several starfish species have been performed in our laboratory. From *Acanthaster planci*, we have isolated and characterized six cerebrosides[1], two ceramide lactosides[2] and five gangliosides[3]. Continuing our previous studies, the isolation and characterization of the ceramide, which is regarded as the aglycone part of the glycosphingolipids, were carried out. There is considerable interest and importance attached to the determination of the composition of the sphingolipids mixture and it is hoped that biologically active compounds from marine natural products may be discovered.

In this paper we report on the isolation and structure determination of three new ceramides, AC-1-6, AC-1-10, and AC-1-11, from the starfish *A. planci*.

The acetone soluble part, obtained from the less polar fraction of the $CHCl_3$/MeOH extract of the whole bodies of *A. planci*, was separated by normal-phase silica-gel column chromatography to give a compound AC-1, which appears as a single spot on normal-phase TLC.

Structure of Ceramide Molecular Species AC-1

In the IR and positive ion fast-atom-bombardment mass spectrometry (FABMS) of AC-1, strong hydroxyl and amide absorptions and a series of molecular ion peaks were observed. The ^{13}C-NMR spectra of AC-1 exhibited the characteristic signals of phytosphingosine-type ceramides possessing 2-hydroxy fatty acids (Table 1). Therefore, AC-1 must be a molecular species of a phytosphingosine-type ceramide containing 2-hydroxy fatty acids. Furthermore, AC-1 was thought to possess *normal*[4] types of side chains, since the carbon atom signals due to terminal methyl groups were observed at $\delta = 14.2$ (*normal* form) in the ^{13}C-NMR spectrum of AC-1 (Table 1). The ^{1}H-NMR spectrum of AC-1 corresponded to that of the synthetic ceramide[5], (2*S*,2′*R*,3*S*,4*R*)-2-(2-hydroxytetracosanoylamino)hexadecane-1,3,4-triol, with regard to the signals due to 1-H_2, 2-H, 3-H, 4-H and 2'-H (Table 2 and Figure 1). The above fact and the optical rotations of the AC-1 series, AC-1-6, AC-1-10, AC-1-11 (+8.4 to +11.5) (vide infra) and the synthetic ceramide (+9.1)[5] suggested that AC-1 has the same absolute configuration as that of the synthetic one for the core structure, the 2,3,4,2' part. Therefore, the structure of the ceramide molecular species AC-1 was determined to be that shown in Figure 1.

Table 1. ^{13}C-NMR chemical shifts (δ values) of AC-1, AC-1-6, AC-1-10, and AC-1-11 in C_5D_5N

C	AC-1	AC-1-6	AC1-10	AC-1-11
1 (t)	62.1	62.1	62.0	62.0
2 (d)	52.9	53.0	53.0	52.9
3 (d)	76.8	76.9	76.8	76.7
4 (d)	73.0	73.0	73.0	73.0
=CH (d)		130.2		
=CH (d)		130.3		
1′ (s)	175.2	175.3	175.2	175.3
2′ (d)	72.4	72.5	72.4	72.4
CH_3 (q)	14.2	14.3	14.2	14.2

Isolation and Structure of Ceramides from AC-1

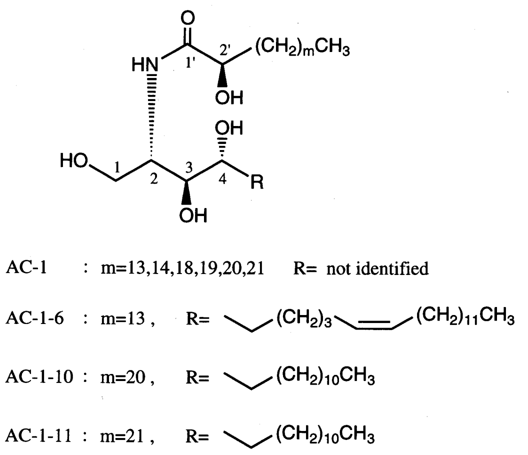
AC-1 was separated by reversed-phase HPLC into thirteen peaks, which were recovered to give the thirteen compounds AC-1-1 to AC-1-13. Of the thirteen compounds, eight of them, AC-1-6 to AC-1-13, had $[M + Na]^+$ as the

Table 2. ^{1}H-NMR spectral data (δ and J values) of AC-1 and synthetic ceramide in C_5D_5N

H	AC-1	synthetic ceramide[a]
1-H_a	4.53 (dd, J = 10.7, 4.5)	4.52 (dd, J = 10.7, 4.5)
1-H_b	4.43 (dd, J = 10.7, 4.5)	4.43 (dd, J = 10.6, 5.0)
2-H	5.12 (m)	5.12 (m)
3-H	4.35 (dd, J = 6.5, 4.6)	4.36 (dd, J = 6.6, 4.6)
4-H	4.29 (m)	4.29 (m)
2′-H	4.63 (dd, J = 7.6, 3.7)	4.63 (dd, J = 7.6, 4.0)

[a] Data from ref.[5].

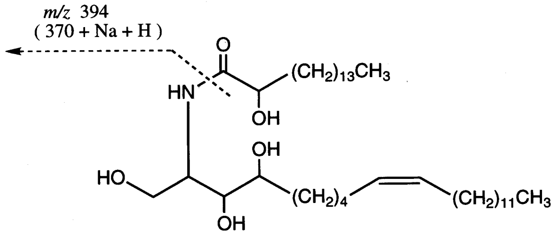
Figure 1



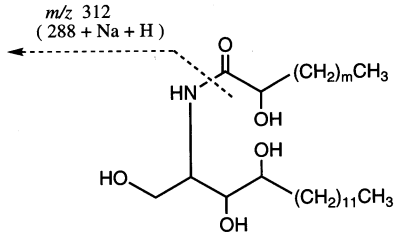
single molecular ion peak and [M + H]$^+$ in the positive ion FAB mass spectra, but the other five exhibited diverse molecular ion peaks. Because we have found[6] that positive ion FAB tandem mass spectrometry (FABMS/MS) of [M + Na]$^+$ ions, obtained from positive ion FABMS of sphingolipids possessing 2-hydroxy fatty acid, afforded characteristic high intensity fragment ions due to fission of the amide bond, indicating the structure of the ceramide residue as shown in Figure 2, the positive ion FABMS/MS of the above-mentioned eight compounds were also measured. Three of the eight compounds (AC-1-6, AC-1-10, AC-1-11) contained a single fragment ion at m/z 394, 312 and 312, respectively, due to fission of the amide bond as shown in Figure 2. However, another five compounds showed diverse fragment ion peaks. Accordingly, only three compounds, AC-1-6, AC-1-10 and AC-1-11, can be pure ceramides.

AC-1-6 was verified as the ceramide component of AC-1, since the ^{13}C-NMR spectrum of AC-1-6 is essentially identical to that of AC-1, and showed the signals ascribable to monounsaturated *normal* phytosphingosine-type ceramides possessing a *normal* 2-hydroxy fatty acid. Taking the molecular mass of AC-1-6 (m/z 625) and characteristic fragment ion (m/z 394) obtained from the positive ion FABMS/MS into account, we may regard the long chain base of AC-1-6 as a docosene derivative and the fatty acid as 2-hydroxyhexadecanoic acid. The location and geometry of the double bond in the long chain base were determined as follows. Positive ion FABMS of the dimethyl disulfide (DMDS) derivative of AC-1-6 showed a remarkable fragment ion peak at m/z 229 due to cleavage of the double bond between the carbon atoms bearing a methylthio group (Figure 3). This fragment ion was verified by high resolution positive FABMS to belong to $C_{14}H_{29}S$. These data indicated that the double bond in the long chain alkene was located at C-9 in AC-1-6. On the other hand, it is known[7] that the geometry of the double bond in a long chain alkene can be determined from the ^{13}C-NMR chemical shift of the methylene carbon atom next to the olefinic carbon atom: The carbon signal is observed at δ ≈ 27 in the (Z) type and δ ≈ 32 in the (E) type. When the C-H long-range COSY spectrum of AC-1-6 was measured, remarkable correlations were observed between the signals of the olefinic proton at δ = 5.47 and the signals for the methylene carbon atoms at δ = 27.6 and 27.8. Accordingly, these methylene carbon atoms must be those next to the double bonds. Thus, the olefinic group in the long-chain part of AC-1-6 was characterized to be of the (Z) type.

Figure 2. Positive ion FABMS/MS fragmentation of AC-1-6, AC-1-10, and AC-1-11 ([M + Na]$^+$ ion was selected as a precursor ion)

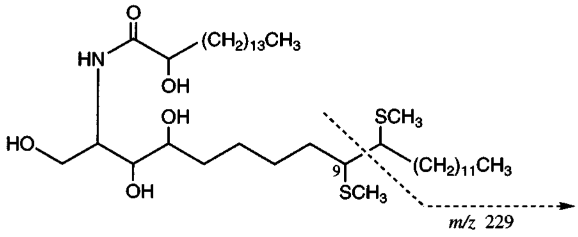


AC-1-6



AC-1-10 (m=20)
AC-1-11 (m=21)

Figure 3. Positive ion FABMS fragmentation of DMDS derivative of AC-1-6



AC-1-10 and AC-1-11 were also verified to be the ceramide components of AC-1 from ^{13}C-NMR spectra. Both of them showed the signals ascribable to saturated *normal* phytosphingosine-type ceramides possessing a normal 2-hydroxy fatty acid in their ^{13}C-NMR spectra (Table 1). Taking the molecular mass of AC-1-10 (*m/z* 641) and AC-1-11 (*m/z* 655) and the above-mentioned positive ion FABMS/MS fragmentation (*m/z* 312) of AC-1-10 and AC-1-11 into account, the long-chain base and fatty acid of AC-1-10 and AC-1-11 must be a hexadecane derivative (base) and 2-hydroxytricosanoic- and 2-hydroxytetracosanoic acid, respectively.

Since the absolute configuration of the basic skeleton of the molecular species AC-1 has already been determined (vide supra), the structures of the homogeneous components of AC-1, AC-1-6, AC-1-10 and AC-1-11, are (2*S*,2′*R*,3*S*,4*R*,9*Z*)-2-(2-hydroxyhexadecanoylamino)-9-docosene-1,3,4-triol, (2*S*,2′*R*,3*S*,4*R*,)-2-(2-hydroxytricosanoylamino)hexadecane-1,3,4-triol and (2*S*,2′*R*,3*S*,4*R*,)-2(2-hydroxytetracosanoylamino)hexadecane-1,3,4-triol, respectively, as shown in Figure 1.

To our knowledge, this has been the first time that ceramides from starfish have been isolated and characterized. AC-1-6, AC-1-10 and AC-1-11 are new ceramides, although AC-1-11 has been obtained as a synthetic product[5] before.

This work was supported in part by Grants-in-Aid for Scientific Research (No.07680632 and 09470486) from the *Ministry of Education, Science and Culture, Japan*, which is gratefully acknowledged. We also thank Mr. *Y. Tanaka* and Ms. *Y. Soeda* of the Faculty of Pharmaceutical Sciences, Kyushu University, for providing NMR data.

Experimental Section

Melting points: Micro melting point apparatus (Yanaco MP-3); uncorrected values. – Optical rotations: Jasco Dip-370 digital polarimeter at 25 °C. – IR spectra: Jasco IR-700 infrared spectrophotometer. – ^{1}H- and ^{13}C-NMR spectra: 270 MHz and 67.8 MHz, respectively, with a Jeol GX-270 spectrometer. – C-H long-range COSY spectrum: Varian Unity Plus 500 MHz NMR spectrometer. – Positive-ion FABMS and FABMS/MS: Jeol SX/SX102A tandem mass spectrometer (xenon atom beam, 5 kV; ion-source accelerating potential, 10 kV; matrix: *m*-nitrobenzyl alcohol or *m*-nitrobenzyl alcohol/satd. NaCl solution). – HPLC: Jasco 880-PU, RI detector.

Separation of AC-1: Whole bodies of the starfish *Acanthaster planci* (4 kg), collected at Naha bay in Okinawa, Japan, in 1988, were homogenized and extracted with $CHCl_3$/MeOH (1:3) (16 l), followed by further extraction with $CHCl_3$/MeOH (1:2) (9 l), and the combined $CHCl_3$/MeOH solutions were concentrated in vacuo to give a residue of 337 g. This residue was partitioned between H_2O (2 l) and AcOEt/1-BuOH (2:1) (1.8 l), and the organic layer was concentrated in vacuo to give the less polar fraction (67 g), which was washed with cold acetone (400 ml). The acetone-soluble part (45 g) was chromatographed on silica gel [solvent *n*-hexane/AcOEt/MeOH (6:4:0.7) for the first chromatography and $CHCl_3$/MeOH (97:3) for the second chromatography] to afford AC-1 (198 mg). AC-1 showed a single spot on silica-gel TLC [solvent $CHCl_3$/MeOH (19:1), $R_f = 0.21$].

AC-1: Amorphous powder. – IR (KBr): $\tilde{\nu} = 3350\ cm^{-1}$ (OH), 1640, 1540 (amide). – Positive FABMS; *m/z*: 626, 640, 642, 654, 656 $[M + H]^+$ series. – ^{13}C and ^{1}H NMR: See Tables 1 and 2.

Isolation of the Ceramides AC-1-6, AC-1-10 and AC-1-11: HPLC of AC-1 showed thirteen peaks [column WAKOSIL 5C18 (C-18, 5μ, 10 mm ϕ × 30 mm); solvent, MeOH; flow rate, 3.0 ml/min]: t_R [min] (ratio of peak areas) = 21.5 (1.3), 22.5 (1.9), 24.2 (8.0), 25.5 (1.9), 27.5 (56.9), 29.0 (19.9), 32.5 (4.8), 35.0 (1.0), 38.0 (9.6), 44.5 (4.5), 52.0 (9.3), 62.0 (1.4), 73.0 (1.8). 180 mg of AC-1 was separated by HPLC into the above-mentioned thirteen fractions by using the conditions described above: Fraction 1 (AC-1-1, 0.4 mg), fraction 2 (AC-1-2, 1.2 mg), fraction 3 (AC-1-3, 4.8 mg), fraction 4 (AC-1-4, 1.4 mg), fraction 5 (AC-1-5, 63.9 mg), fraction 6 (AC-1-6, 22.8 mg), fraction 7 (AC-1-7, 3.3 mg), fraction 8 (AC-1-8, 0.6 mg), fraction 9 (AC-1-9, 9.1 mg), fraction 10 (AC-1-10, 4.0 mg), fraction 11 (AC-1-11, 9.9 mg), fraction 12 (AC-1-12, 1.7 mg), fraction 13 (AC-1-13, 2.8 mg). – Positive FABMS of AC-1-1 to AC-1-13: AC-1-1 to AC-1-5 showed diverse molecular ion peaks, AC-1-6 to AC-1-13 each revealed a single molecular ion peak as $[M + H]^+$ and $[M + Na]^+$ at *m/z*: 626, 648 (AC-1-6), 640, 662 (AC-1-7), 654, 676 (AC-1-8), 628, 650 (AC-1-9), 642, 664 (AC-1-10), 656, 678 (AC-1-11), 670, 692 (AC-1-12), 684, 706 (AC-1-13). – Positive-ion FABMS/MS of AC-1-6 to AC-1-13 ($[M + Na]^+$ ion was selected as a precursor ion): AC-1-6, AC-1-10, AC-1-11 gave rise to a single fragment ion peak due to fission of the amide bond at *m/z* 312 (AC-1-10, -11) and 394 (AC-1-6), respectively. On the other hand, the other five compounds revealed diverse fragment ion peaks.

AC-1-6: Amorphous powder, m.p. 135–137 °C. – $[\alpha]_D = +11.5$ ($c = 1.0$ in pyridine). – ^{13}C NMR: See Table 1. – C-H long-range COSY (C_5D_5N, $J = 10$ Hz): Correlations were observed between $\delta = 27.6$ and 27.8 (methylene carbon atoms) and 5.47 (olefinic protons). – $C_{38}H_{76}NO_5$ $[M + H]^+$: calcd. 626.5724, found 626.5727 (high-resolution positive-ion FABMS).

DMDS Derivative of AC-1-6: 2 mg of AC-1-6 was dissolved in carbon disulfide (0.2 ml) and dimethyl disulfide (DMDS) (0.2 ml), and iodine (1 mg) was added to the solution. The obtained mixture was kept at 60 °C for 40 h in a small sealed vial. The reaction was quenched with aqueous $Na_2S_2O_3$ (5%), and the reaction mixture was extracted with *n*-hexane (0.3 ml). The extract was concentrated and the residue (DMDS derivative) was purified by silica-gel column chromatography [solvent $CHCl_3$/acetone (8.5:1.5)] to give the DMDS derivative as amorphous powder. – High-resolution positive-ion FABMS of AC-1-6 DMDS derivative ($C_{14}H_{29}S$): calcd. 229.1990, found 229.1990.

AC-1-10: Amorphous powder, m.p. 143–145 °C. – $[\alpha]_D = +10.0$ ($c = 0.43$ in pyridine). – ^{13}C NMR: See Table 1. – $C_{39}H_{80}NO_5$ $[M + H]^+$: calcd. 642.6037, found 642.6037 (high-resolution positive-ion FABMS).

AC-1-11: Amorphous powder, m.p. 142–143 °C. – $[\alpha]_D = +11.5$ ($c = 0.96$ in pyridine). – ^{13}C NMR: See Table 1. – $C_{40}H_{82}NO_5$ $[M + H]^+$: calcd. 656.6193, found 656.6197 (high-resolution positive-ion FABMS).

---

[1] Y. Kawano, R. Higuchi, R. Isobe, T. Komori, *Liebigs Ann.* **1988**, 19–24.

[2] Y. Kawano, R. Higuchi, R. Isobe, T. Komori, *Liebigs Ann.* **1988**, 1181–1183.

[3] Y. Kawano, R. Higuchi, T. Komori, *Liebigs Ann.* **1990**, 43–50.

[4] *normal* means straight chain (...$CH_2CH_2CH_2CH_3$).

[5] S. Sugiyama, M. Honda, R. Higuchi, T. Komori, *Liebigs Ann.* **1991**, 349–356.

[6] R. Isobe, M. Inagaki, Y. Harano, H. Sakiyama, R. Higuchi, *Chem. Pharm. Bull.* **1997**, *45*, 1611–1614.

[7] N. Fusetani, K. Yasumoto, S. Matsunaga, H. Hirota, *Tetrahedron Lett.* **1989**, *30*, 6891–6894.

[97196]