Biologically Active Glycosides from Asteroidea, 40[+]

Two New Gangliosides, Acanthagangliosides I and J from the Starfish *Acanthaster planci*

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A new ganglioside molecular species named AG-1 has obtained from the whole body of starfish *Acanthaster planci* (crown of thorns). The structure of this ganglioside have been elucidated by enzymatic hydrolysis with the *endo*-type glycosidase. Enzymatic hydrolysis gave an oligosaccharide and ceramides, quantitatively. The oligosaccharide moiety was determined mainly using 2D-NMR experiments as β-Fuc*f*-(1→4)-α-Gal*p*-(1→4)-α-NeuAc-(2→3)-β-Gal*p*-(1→4)-Glc*p*. Meanwhile, the ceramide moiety was elucidated by NMR and GC-MS analysis as the mixture of (2*S*,2′*S*,3*S*,4*R*)-2-(2′-hydroxydocosanoyl)amino-1,3,4-trihydoxyhexadecane, and (2*S*,2′*S*,3*S*,4*R*)-2-(2′-hydroxytetracosanoyl)amino-1,3,4-trihydoxyhexadecane. Reversed-phase HPLC of AG-1 gave two kinds of gangliosides named acanthagangliosides I (**1**) and J (**2**). Their structures have been identified by negative FAB-MS.

Introduction

As described in a previous paper,[1] we reported the reexamination of the structure of acanthagangliosides isolated from the gangliosides molecular species AG-2 and AG-3,with the aid of 2D-NMR experiments. As a result, the oligosaccharide moieties were revised as β-Gal*f*-(1→3)-α-Gal*p*-(1→4)-α-NeuAc-(2→3)-β-Gal*p*-(1→4)-β-Glc*p* [AG-2] and β-Gal*f*-(1→3)-α-Gal*p*-(1→3)-α-Gal*p*-(1→4)-α-NeuAc-(2→3)-β-Gal*p*-(1→4)-β-Glc*p* [AG-3]. In this report, we isolated a new ganglioside molecular species AG-1 with a modified isolation method. Furthermore, we applied an enzymatic hydrolysis by means of endoglycoceramidase (EGCase)[2] for the structure elucidation of AG-1. The enzyme cleaved the glycoside linkage between the oligosaccharide and ceramide of AG-1 to give the intact oligosaccharide and ceramide moieties. Finally, acanthagangliosides I (**1**), and J (**2**) were purified from the AG-1 by means of reversed-phase HPLC.

Results and Discussion

Modified Isolation Method of Acanthagangliosides

Generally, the starfish gangliosides have been isolated from the water-soluble lipid fraction, which was obtained from the $CHCl_3$/MeOH extract of the whole bodies of starfish. On the other hand, a modified isolation method gave the gangliosides from the *n*-hexane-soluble lipid fraction, which was obtained from the $CHCl_3$/MeOH extract. The *n*-hexane-soluble fraction was extracted with MeOH. This MeOH extract was partitioned between *n*-hexane and aqueous MeOH. The aqueous MeOH soluble fraction was subjected to successive column chromatography on silica gel, Sephadex LH-20, and reversed-phase silica gel (C18) to furnish a new gangliosides molecular species AG-1, together with AG-2 and AG-3 as shown in Scheme 1.

Ganglioside Molecular Species AG-1

The IR spectrum of AG-1 exhibits absorptions due to hydroxy (3410 cm^{-1}) and amide (1645, 1554 cm^{-1}) functionalities. The characteristic ^{13}C-NMR signals of a phytosphingosine-type ceramide possessing an α-hydroxy fatty acid function are quite similar to those of AG-2 (δ_C = 69.5, 51.1, 75.8, and 72.3 derived from phytosphingosine, and δ_C = 175.6, and 72.3 derived from α-hydroxy fatty acid).[1] The negative FAB-MS spectrum shows two pseudo-molecular ion peaks at *m*/*z* = 1577 and 1549 [M − H]$^-$, together with fragment ion peaks, due to the cleavage of the saccharide moiety at 1431, 1403 [M − H − 146; A], 1269, 1241 [A − 162; B], 978, 950 [B − 291; C], 816, 788 [C − 162; D], and 654, 626 [D − 162; ceramide]. These data indicate that AG-1 has a ceramide that consists of an α-hydroxy fatty acid and phytosphingosine, and a tetrasaccharide (deoxyhexose−hexose−NeuAc−hexose−hexose). The ^1H-NMR spectrum of AG-1 in C_5D_5N shows no analyzable spectrum, except for the signal due to four anomeric proton signals [δ_H = 4.74 (d, *J* = 8.4 Hz), 4.88 (d, *J* = 8.0 Hz), 5.64 (br. s), 5.89 (br. s)]. The poorly resolved spectrum, shown in

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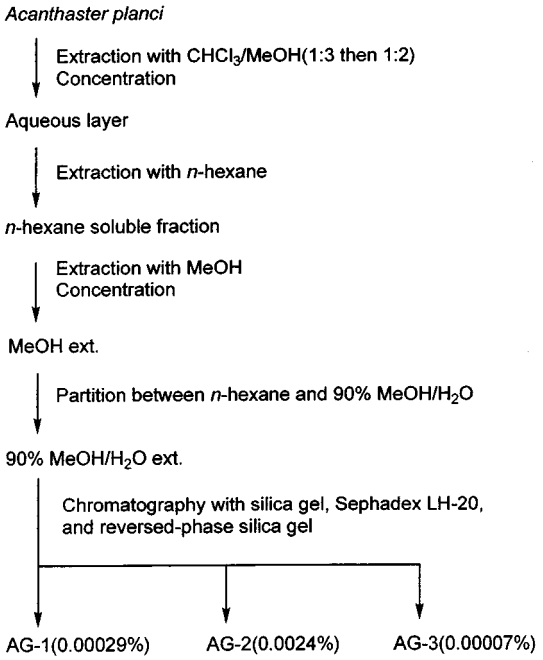


Scheme 1. Modified isolation method of acanthaganglioside molecular species

Figure 1, is due to the molecular species and low solubility of the ceramide in NMR solvents.

Enzymatic Hydrolysis of AG-1 with EGCase

AG-1 was dissolved in 0.02 M sodium acetate buffer, pH = 5.0 with Triton X-100, and incubated at 37 °C for 24 h. The reaction mixture was lyophilized, then extracted with EtOAc. The EtOAc extract was purified by silica-gel column chromatography to give the ceramide of AG-1 (AG-1-Cer). The residue was purified by reversed-phase column chromatography, followed by Sephadex LH-20 to yield oligosaccharide of AG-1 (AG-1-Oligo).

Structure of AG-1-Oligo

The negative FAB-MS of AG-1-Oligo shows a pseudomolecular ion peak at m/z = 940. Acid hydrolysis and methanolysis of AG-1-Oligo reveal the constitutive saccharides as one mol each of glucose (Glc), *N*-acetylneuraminic acid (NeuAc), fucose (Fuc) and two mol of galactose (Gal). The sequence of these monosaccharides was determined using 1D and 2D NMR as follows: The ^{1}H-NMR spectrum of AG-1-Oligo in D_2O shows five anomeric proton signals [δ_H = 4.59 (d, ^3J = 8.6 Hz, 0.5 H), 4.45 (d, ^3J = 8.6 Hz, 1 H), 5.05 (d, ^3J = 4.6 Hz, 1 H), 4.95 (br. s, 1 H), and 5.15 (d, ^3J = 4.6 Hz, 0.5 H)] and characteristic proton signals due to 3-H of NeuAc [δ_H = 2.83 (dd, J = 5.1, 12.7 Hz, 1 H, 3-H_{eq}), 1.69 (t, J = 11.9 Hz, 1 H, 3-H_{ax})] as shown in Figure 2.

Two anomeric proton signals observed at δ = 4.59 and 5.15 predict an α,β-equilibrium mixture of reducing sugar because of their molecular ratio (ca. 1:1). All proton chemical shifts were assigned by the correlation from the above anomeric protons and 3-H of NeuAc. 1D-TOCSY, DQF-COSY,[3] TOCSY,[4] and NOESY[5] spectra, as well as HSQC[6] and HSQC-TOCSY spectra revealed the all-carbon chemical shift assignments as shown in Table 1. These data clarified that the AG-1-Oligo is constructed with ξ−Glc*p*, β-Gal*p*, α-NeuAc, α-Gal*p*, β-Fuc*f*. The linkage of each monosaccharide was examined by an HMBC[8] spectrum. The $^3J_{CH}$ correlations between C-1 of β-Fuc*f* (δ_C = 107.1) and 4-H of α-Gal*p* (δ_H = 4.06), C-4 of α-NeuAc (δ_C = 72.3) and 1-H of α-Gal*p* (δ_H = 5.05), C-2 of α-NeuAc (δ_C = 98.9) and 3-H of β-Gal*p* (δ_H = 4.05), C-1 of β-Gal*p* (δ_C = 101.6) and 4-H of β-Glc*p* (δ_H = 3.60) are clearly apparent. The linkage of each monosaccharide was

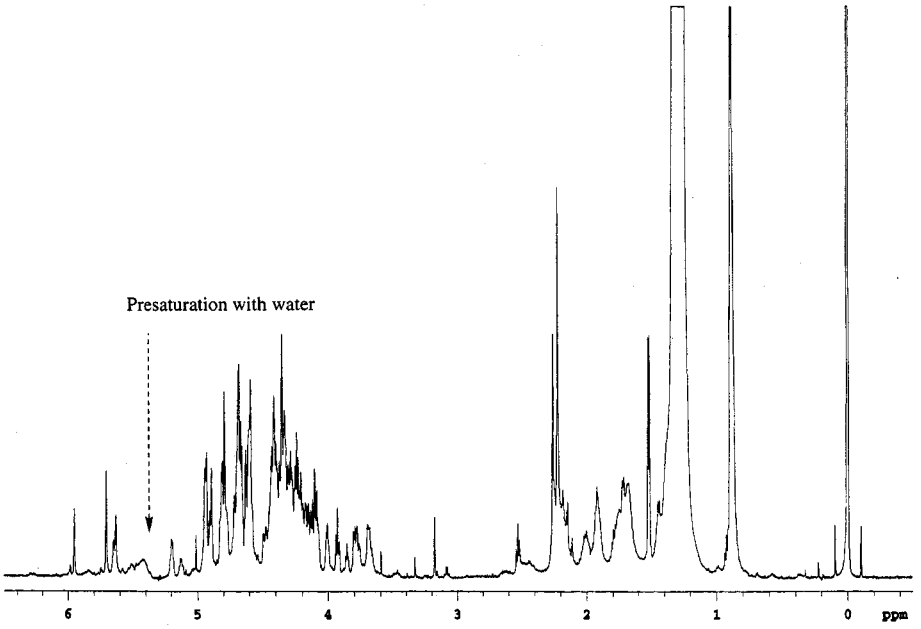


Figure 1. ^{1}H-NMR spectrum of AG-1 (600 MHz, C_5D_5N/D_2O, 95:5, 30 °C)

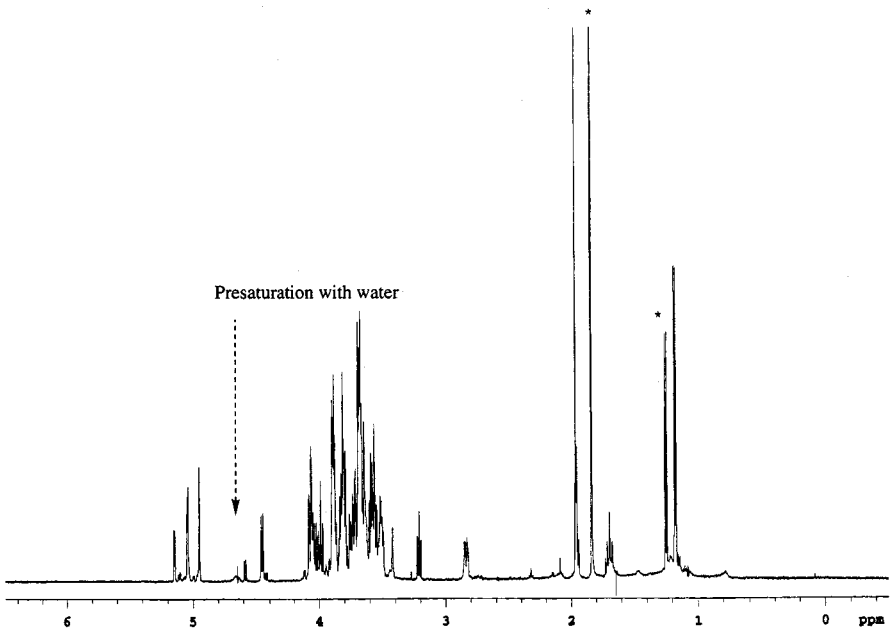


Figure 2. ^{1}H-NMR spectrum of AG-1-Oligo (600 MHz, D_2O, 30 °C; * derived from solubilization agent)

Table 1. ^{1}H- and ^{13}C-NMR chemical shift assignments of AG-1-Oligo in D_2O at 30 °C

		δ_C Ag-1-Oligo	δ_C Ag-2-Oligo	δ_H Ag-1-Oligo
β-Glc*p*	1	94.8	94.7	4.59 (d, 8.6)
	2	72.8	72.8	3.21 (t, 8.9)
	3	73.3	73.3	3.56 (t, 9.2)
	4	77.3	77.2	3.53 (m)
	5	73.8	73.7	3.64[a]
	6	59.1	59.1	3.89[a]
β-Gal*p*	1	101.6	101.6	4.45 (d, 8.6)
	2	68.4	68.3	3.51[a]
	3	74.5	74.5	4.05 (dd, 5.1, 10.1)
	4	66.5	66.5	3.89 (br. s)
	5	74.2	74.1	3.50[a]
	6	60.0	60.0	3.68 (m, 2 H)
α-NeuAc	1	172.6	172.6	
	2	98.9	98.9	
	3	35.2	35.0	2.83 (dd, 5.1, 12.7)
				1.69 (t, 11.9)
	4	72.3	71.5	3.67 (m)
	5	48.4	48.4	3.99 (t, 10.7)
	6	71.5	71.6	3.69[a]
	7	67.0	66.9	3.56[a]
	8	70.8	70.8	3.84[a]
	9	61.6	61.5	3.80[a]
				3.58[a]
	10	173.3	173.2	
	11	21.1	21.1	1.96 (s, 3 H)
α-Gal*p*	1	94.0	93.5	5.05 (d, 4.6)
	2	67.5	65.9	3.72 (dd, 4.6, 10.7)
	3	71.5	75.9	3.68 (dd, 3.6, 10.7)
	4	75.6	75.9	4.06 (br. s)
	5	70.2	69.8	3.63[a]
	6	59.0	59.6	3.68[a]
				3.81[a]
β-Fuc*f*	1	107.1	*108.1*	4.95 (br. s)
β-Galf	2	80.8	*80.4*	4.08 (d, 5.1)
	3	75.7	*75.7*	3.88 (t, 7.6)
	4	86.0	*81.6*	3.87[a]
	5	66.0	*69.6*	3.83 (m)
	6	17.2	*61.7*	1.17 (d, 5.9, 3 H)

[a] Obscured by other signals.

also confirmed by NOESY spectral data. Accordingly, the structure of AG-1-Oligo was determined as shown in Scheme 2. The carbon chemical shifts of AG-1-Oligo are similar to those of AG-2-Oligo, which was prepared by the same manner as AG-1-Oligo, except for the signals due to terminal furanose and α-Gal*p* (Figure 3).

Structure of AG-1-Cer

The ^{1}H- and ^{13}C-NMR spectra of AG-1-Cer are quite similar to those of the (2*S*,3*S*,4*R*,2′*R*)-type ceramide molecular species (AC-1), which was obtained from the same animal (Table 2). The positive FAB-MS of AG-1-Cer shows two pseudomolecular ion peaks at *m*/*z* = 656 and 628. Methanolysis of AG-1-Cer with methanolic hydrochloric acid furnish two fatty acid methyl esters (FAM), and a long-chain base (LCB). The former were identified by means of GC-MS as methyl 2-hydroxydocosanoate and methyl 2-hydroxytetracosanoate, and the latter was identified as 1,3,4-hydroxy-2-aminohexadecane by their trimethyl silyl (TMS) derivatives. These data suggested that AG-1-Cer was a mixture of two kinds of ceramide as shown in Scheme 3.

Structure of AG-1

The β-linkage between the AG-1-Oligo and AG-1-Cer was determined by the ^{1}H *J* value of 1-H of Glc*p* [δ_H = 4.74 (d, ^3J = 8.7 Hz, 1H)], and the chemical shift of C-1 of Glc*p* (δ_C = 104.5), which were assigned by comparison with those of AG-2. Thus, the ganglioside molecular species AG-1 is a mixture of two gangliosides that are slightly different in the length of the acyl chains.

Purification of Acanthagangliosides I (1) and J (2) from AG-1

Isolation of two gangliosides from the molecular species AG-1 was succeeded according to our previous procedure.[1]

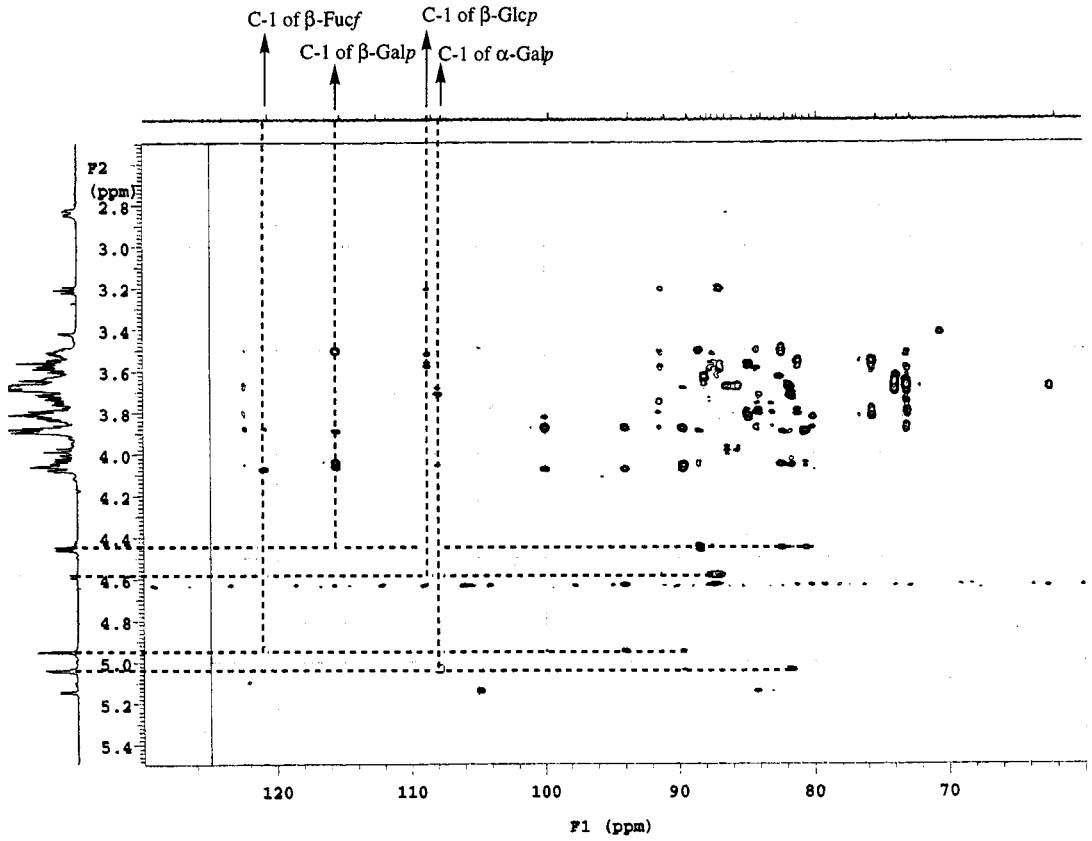
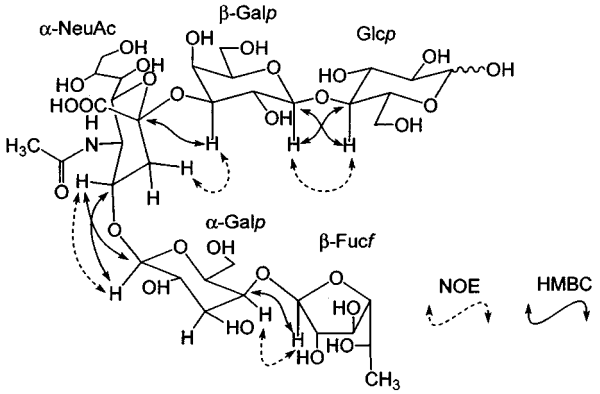


Figure 3. HSQC-TOCSY spectrum of AG-1-Oligo (600 MHz, D_2O, 30 °C, mixing time = 120 ms)



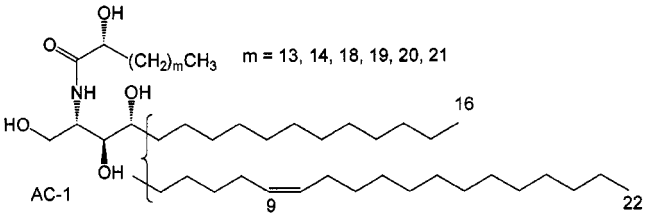
Scheme 2. HMBC and NOESY spectral data of AG-1-Oligo

Reversed-phase HPLC (Cosmosil 5C18 AR-II) of AG-1 using 0.5% TFA/MeOH resulted in two peaks. The solvent from each fraction was removed, leaving the two compounds (**1** and **2**). The negative FAB-MS of **1** shows a molecular ion peak at m/z = 1549 $[M - H]^-$, as well as fragment ion peaks due to the cleavage of the glycosidic linkage at m/z = 1403, 1241, 950, 788, and 626 (ceramide),while the negative FAB-MS of **2** shows a molecular ion peak at m/z = 1577 $[M - H]^-$, and fragment ion peaks at m/z = 1431, 1269, 978, 816, and 654.

These data indicate that acanthaganglioside I (**1**) contains a C-22 fatty acid, and acanthaganglioside J (**2**) contains a C-24 fatty acid (Scheme 4).

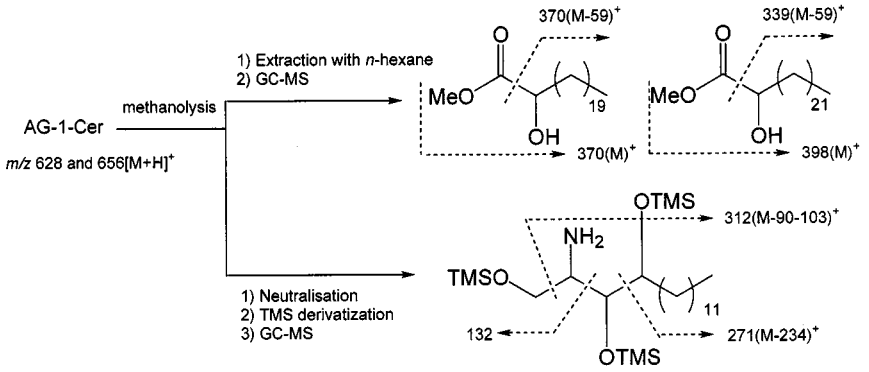
Table 2. ^1H- and ^{13}C-NMR chemical shift assignments of AG-1-Cer and AC-1

	AG-1-Cer		AC-1	
	δ_C	δ_H	δ_C	δ_H
LCB 1a	62.1	4.52 (dd, 4.8, 10.8)	62.1	4.53 (dd, 4.5, 10.7)
1b		4.44 (dd, 4.8,10.8)		4.43 (dd, 4.5, 10.7)
2	53.0	5.12 (m)	52.9	5.12 (m)
3	76.8	4.34 (m)	76.8	4.35 (dd, 4.6,6.5)
4	73.1	4.29 (m)	73.0	4.29 (m)
term.Me	14.3	0.88 (t, 7.0, 3 H)	14.2	0.83 (t, 6.9, 3 H)
NH		8.57 (d, 8.9)		8.55 (d, 8.8)
FAM 1′	175.3		175.2	
2′	72.5	4.63 (m)	72.4	4.63 (dd, 3.7, 7.6)
term. Me	14.3	0.88 (t, 7.0, 3 H)	14.2	0.83 (t, 6.9, 3 H)

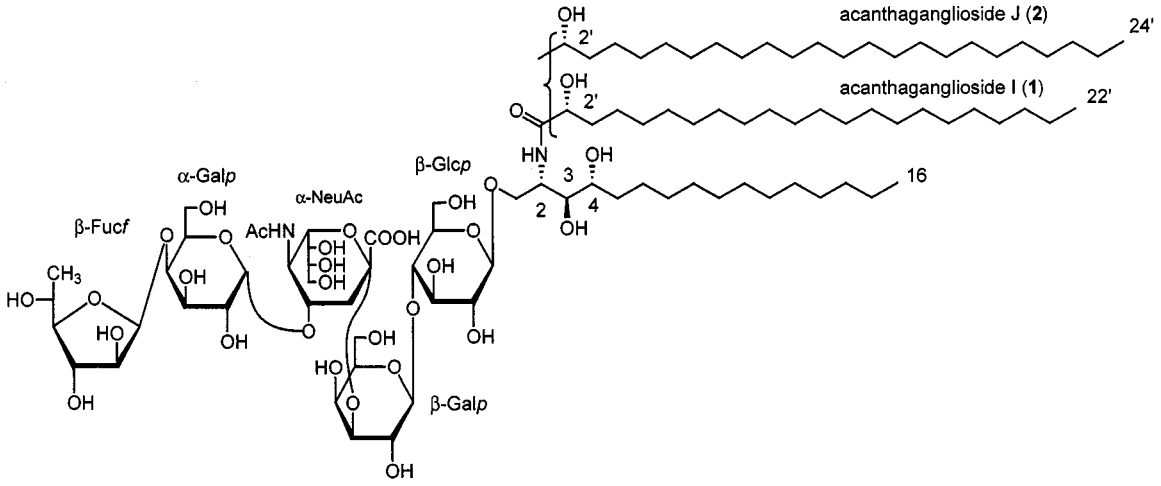


Conclusion

It is easy to prepare the starfish ganglioside molecular species by short steps using the modified isolation method developed here. The oligosaccharide, which was obtained by hydrolysis with EGCase, is more suitable for NMR analysis than the parent ganglioside molecular species. The oli-



Scheme 3. GC-MS analysis of FAM, and LCB from AG-1-Cer



Scheme 4. Structure of acanthaganglioside I (**1**) and J (**2**)

gosaccharide is easily soluble in D_2O, and gives high-resolution spectra. It is clear that the oligosaccharide moiety of AG-1 is different from AG-2 and AG-3 in its terminal monosaccharide moiety. The terminal β-Gal*f* of AG-2 and AG-3 is linked to C-3 of α-Gal*p*, however, the terminal β-Fuc*f* of AG-1 is linked to C-4 of α-Gal*p*. This interesting difference for terminal sugar linkages seemed to be derived from the co-existence of different glycosyltransferases, namely β-1,3-galactofuranosyl transferase and β-1,4-fucofuranosyl transferase. The gangliosides of *A. planci* characteristically have a terminal furanose-type sugar unit. This characteristic oligosaccharide moiety is only found in starfish belonging to the order of Spinulosa, for example *Asterina pectinifera*.[8]

Experimental Section

IR: JASCO FT/IR-410 Fourier transform infrared spectrometer. − FAB-MS: JEOL SX-102 mass spectrometer (xenon atom beam, 5 kV; ion-source accelerating potential, 10 kV; matrix, glycerol for negative ion, *m*-nitrobenzyl alcohol for positive ion. − NMR: Spectra were recorded using UNITY-600 and -500 spectrometers at an operating frequency of 600 MHz and 500 MHz with Varian Instrument equipped with SUN 4-LX computer systems, and a Jeol FX-270 spectrometer. The operating conditions were as follows: ^{1}H: frequency, 600 MHz; sweep width, 8 kHz; sampling point, 44 k; accumulation, 64 pulses; temperature, 30 °C. ^{13}C: frequency, 150 MHz; sweep width, 32 kHz; sampling point, 160 k; accumulation, 10 000 pulses; temperature, 30 °C. Chemical shifts were referenced to the residual solvent signals (δ_H = 7.19, δ_C = 123.5) in D_5D_5N and acetone (δ_H = 2.05, δ_C = 29.8) as an internal standard in D_2O. Conventional pulse sequences were used for DQF-COSY, NOESY, HSQC, and HMBC. The mixing time in the NOESY experiment was set to 500 ms. TOCSY spectra were acquired using the standard MLEV17 spin-locking sequence and 120 ms mixing time. The mixing time in the HSQC-TOCSY spectra was set to 120 ms. All spectra were recorded using the phase-sensitive mode. The size of the acquisition data matrix was 2048 × 256 words in f2 and f1, respectively, and zero filling up to 2k in f1 was made prior to Fourier transformation. Sine-bell or shifted sine-bell window functions, with the corresponding shift optimized for every spectrum, were used for resolution enhancement and baseline correction was applied in both dimensions. Water suppression was carried out by selective pre-saturation placing the carrier on the solvent resonance. − GLC: Shimadzu GC-14B, fused silica capillary column DB-17 (30 m × 0.32 mm); injection temp. 280 °C, column temp. 200 °C, FID detection, carrier: N_2 (1.3 mL/min). − GC-MS: Shimazu QP-1000, EI mode (ionizing potential of 70 eV, separator and ion-source temperature of 250 °C) and a fused silica capillary column TC-1701 (15 m × 0.53 mm, GL Sciences), carrier: He (30 mL/min). − HPLC: JASCO PU-980 HPLC pump and UV/Vis detector 875-UV [column, Cosmosil 5C18-AR-II (Nacalai Tesque)], elution (0.5% TFA/MeOH), flow rate (1.0 mL/min, monitoring at 210 nm).

Isolation of AG-1: Whole bodies of *Acanthaster planci* (12 kg), which were collected at Motobu bay in Okinawa Island, in 1995, were homogenized, and extracted with $CHCl_3$/MeOH (1:3, 30.5 L) then $CHCl_3$/MeOH (1:2, 24 L). The combined extracts were concentrated in vacuo to give an aqueous solution (5 L), which was extracted with *n*-hexane (2.5 L). The *n*-hexane extract was re-extracted with MeOH to give MeOH extracts (207 g). The MeOH extract was partitioned between *n*-hexane and 90%MeOH/H_2O to give 90% MeOH/H_2O extracts (164.6 g). The 90% MeOH/H_2O extracts were purified by chromatography on silica gel using step-gradient solvents [$CHCl_3$, $CHCl_3$/MeOH (20:1, 10:1, 5:1, 3:1), $CHCl_3$/MeOH/H_2O (5:5:1)] to give four fractions [fr.1 (37.5 g), fr.2 (38.0 g), fr.3 (57.3 g), and fr.4 (5.4 g). Fr.4, which included the gangliosides, was further purified by chromatography with silica gel [$CHCl_3$/MeOH/H_2O (65:35:5, 60:40:5)], Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), and reversed-phase silica gel (Cosmosil C18, 90% MeOH/H_2O, 100%MeOH), to give AG-1 (34.6 mg), AG-2 (290.1 mg), and AG-3 (8.9 mg). AG-1, AG-2, and AG-3 each showed a single spot on silica-gel TLC ($CHCl_3$/MeOH/2.5 N aqueousNH_3); R_f = 0.36 (AG-1), 0.29 (AG-2), and 0.22 (AG-3).

AG-1: Amorphous powder. − IR (KBr, cm^{-1}): 3410 (OH), 2924, 2854, 1645, 1554 (amide). − Negative FAB-MS; *m*/*z* (%) = 1577 (33), 1544 (22) $[M - H]^-$, 1431 (48), 1403 (36), 1269 (14), 1241 (8), 978 (18), 950 (12), 816 (32), 788 (23), 654 (100), 626 (67) [ceramide]. − ^1H NMR (C_5D_5N/D_2O = 95:5, 600 MHz); δ = 0.80−0.84 (m, 6 H, terminal methyls), 1.19−1.27 (s, methylenes), 1.46 (d, ^3J = 6.0 Hz, 3 H, 6-H of β-Fuc*f*), 1.6−1.7 (m, 4 H, methylenes), 2.16 (s, 3 H, acetyl methyl), 3.5−5.0 (carbinol protons), 4.74 (d, ^3J = 8.7 Hz, 1 H, 1-H of β-Glc*p*), 4.88 (d, ^3J = 7.2 Hz, 1 H, 1-H of β-Gal*p*), 5.58 (br. d, 1 H, 1-H of α-Gal*p*), 5.64 (br. s, 1 H, 1-H of β-Fuc*f*). − ^{13}C NMR (C_5D_5N/D_2O = 95:5, 150 MHz): 179.6, 175.6, 173.3, 109.1 (C-1 of β-Fuc*f*), 105.0 (C-1 of β-Gal*p*), 104.5 (C-1 of β-Glc*p*), 100.4 (C-2 of α-NeuAc), 97.2 (C-1 of α-Glc*p*), 89.8, 82.4, 81.8, 81.2, 78.6, 77.4, 76.5, 76.1, 75.8, 75.3, 74.2, 74.0, 72.7, 72.3, 72.2, 70.1, 70.0, 69.5, 68.0, 67.4, 64.1, 64.0, 61.8, 61.5, 61.2, 51.1, 51.0, 35.2, 31.9, 30.2, 30.0, 29.8 (methylenes), 29.7, 29.4, 26.4, 25.6, 22.7 (acetyl methyl), 20.0, 14.1 (methyls).

Enzymatic Hydrolysis of AG-1 with EGCase: EGCase was kindly donated by Dr. M. Ito (Kyushu University), and recombinant EGCase was purchased from TAKARA. The reaction mixture contained 7.0 mg (4.5 μmol) of AG-1 and the 400 mU of in EGCase in 0.02M sodium acetate buffer (pH = 5.0) with 0.4% Triton X-100 (600 μL) was incubated at 37 °C for 24 h. After hydrolysis, the reaction mixture was lyophilized, and treated with EtOAc. The EtOAc-insoluble portion was purified by chromatography using Sephadex LH-20 (H_2O) and reversed-phase column chromatography (RP-8) using 50% MeOH/H_2O to give AG-1-Oligo (2.0 mg). The EtOAc-soluble portion was purified by silica-gel column chromatography using $CHCl_3$/MeOH (95:5) to give AG-1-Cer (1.5 mg).

AG-1-Oligo: Negative FAB-MS; *m*/*z* = 940$[M - H]^-$. − ^1H NMR (600 MHz, D_2O) and ^{13}C NMR (150 MHz, D_2O) see Table 1.

GLC Analysis of Alditol Acetates and 1-*O*-Methyl Sugars from AG-1-Oligo: AG-1-Oligo (0.3 mg) was heated with 0.5 N HCl (0.5 mL) at 80 °C for 13 h in a sealed vial, and the solution was concentrated to remove acid. The residue was dissolved in H_2O (0.8 mL) containing 1 drop of NH_4OH solution, and 7 mg of $NaBH_4$ was added. After standing at room temp. for 5 h, the mixture was acidified with AcOH to pH = 3.5 and the solvent evaporated to dryness. H_3PO_3 in the residue was removed by threefold co-distillation with MeOH. The resulting alditol mixture was acetylated with Ac_2O/C_5H_5N (1:1, 1 mL) at 70 °C for 2 h. Excess Ac_2O was removed by distillation, and the residue was dissolved in $CHCl_3$, washed with H_2O, and concentrated to dryness. The mixture was subjected to GLC. The results were as follows: t_R [min] = 7.9 (peak ratio = 1.34; 1,2,3,4,5-penta-*O*-acetyl fucohexitol), t_R [min] = 19.9 (peak ratio = 0.85; 1,2,3,4,5,6-hexa-*O*-acetyl glucohexitol), t_R [min] = 20.7 (peak ratio = 2; 1,2,3,4,5,6-hexa-*O*-acetyl galactohexitol), t_R [min] = 29.6 (peak ratio = 028; alditol acetate derived from NeuAc). − AG-1-Oligo (0.2 mg) was heated with 1.5 N HCl/MeOH (0.5 mL) at 90 °C for 24 h in a sealed vial. The solution was neutralized with Ag_2CO_3, filtered, and concentrated in vacuo to give a residue, which was treated with 1-(trimethylsilyl)imidazole/pyridine (1:1, 0.05 mL) for 20 min at 70 °C to give TMS ether of 1-*O*-methyl sugars. These samples were subjected to GLC. The results were as follows: t_R [min] = 6.7, 8.0, 8.7 (peak ratio = 0.81; methyl 2,3,5-tri-*O*-trimethylsilylfucose), t_R [min] = 21.0, 21.5 (peak ratio = 1.00; methyl 2,3,5,6-tetra-*O*-trimethylsilylglucose), t_R [min] = 14.8, 17.2, 18.6 (peak ratio = 1.91; methyl 2,3,5,6-tetra-*O*-trimethylsilylgalactose), t_R [min] = 102.2 (peak ratio = 0.27; derived from *N*-acetylneuraminic acid). All GLC samples were identified with independently prepared authentic samples.

AG-1-Cer: Positive FAB-MS; *m*/*z* = 628, 656 $[M + H]^+$. − ^1H NMR (500 MHz, C_5D_5N) and ^{13}C NMR (125 MHz, C_5D_5N) see Table 2.

GC-MS Analysis of FAM, and LCB from AG-1-Cer: AG-1-Cer (1.5 mg) was heated with 5% HCl in MeOH (1 mL) at 70 °C for 3 h in a screw-cap vial. The reaction mixture was extracted with *n*-hexane, the extract concentrated in vacuo to give fatty acid methyl esters (FAMs). The MeOH layer was neutralized with Ag_2CO_3, filtered, and the filtrate was concentrated in vacuo to give a long-chain base (LCB). The LCB was heated with 1-(trimethylsilyl)imidazole/pyridine (1:1, 0.2 mL) for 10 min at 70 °C to give the TMS ether of LCB. These samples were subjected to GC-MS. The results are as follows: methyl-2-hydroxydocosanoate, [min] = 14.5, ratio of peak area = 1.0, *m*/*z* = 370 $[M^+]$, 311 $[M^+ - 59]$, 57, 43 [base peak]; methyl-2-hydroxyhtetracosanoate, [min] = 16.1, ratio of peak area = 1.1, *m*/*z* = 398 $[M^+]$, 57, 43 [base peak]; 1,3,4-tri-*O*-trimethylsilyl-2-amino-hexadecane, [min] = 7.4, *m*/*z* = 312 $[M^+ - 193]$, 271 $[M^+ - 234]$, 204, 132, 73 [base peak].

HPLC Separation of AG-1: AG-1 (3 mg) was dissolved in 200 μL of pyridine. Aliquots of 50 μL of this solution were each subjected to HPLC to give **1** (acanthaganglioside I, 0.29 mg), [min] = 12.5, and **2** (acanthaganglioside J, 0.18 mg), [min] = 16.5.

Acanthaganglioside I (1): Amorphous powder, m.p. 159−161 °C (decomp.). − $[\alpha]_D^{24}$ = +33.0 (*c* = 0.024 in $CHCl_3$/MeOH, 1:1). − Negative FAB-MS; *m*/*z* = 1549 $[M - H]^-$, 1403, 1241, 950, 788, 626.

Acanthaganglioside J (2): Amorphous powder, m.p. 170−172 °C (decomp.). − $[\alpha]_D^{24}$ = +40.8 (*c* = 0.015 in $CHCl_3$/MeOH, 1:1). − Negative FAB-MS; *m*/*z* = 1577 $[M - H]^-$, 1431, 1269, 978, 816, 654.

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