Constituents of Crinoidea. 1. Isolation and Structure of Inositolphosphoceramide from the Feather Star *Comanthus japonica*

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An inositolphosphoceramide molecular species, tentatively named CJP1, has been obtained from the feather star *Comanthus japonica*, which belongs to the class of crinoidea of phylum echinodermata of deuterostomia. On the basis of chemical and spectroscopic evidence, the structure of CJP1 was determined as D-*myo*-inositol-1-*O*-phosphoceramide, which contained a $C_{16:1}$ sphingosine and a $C_{22:0}$, $C_{24:0}$ normal fatty acid. The same type of sphingolipid has been obtained from plants and protostomia but not yet from deuterostomia. This is the first report on the isolation and structure elucidation of inositolphosphoceramide from deuterostomia.

Key words echinodermata; crinoidea; feather star; *Comanthus japonica*; sphingolipid; inositolphosphoceramide

In our continuing search for biologically active sphingolipids from echinodermata, the ganglioside molecular species GP-2,[1] which was obtained from the starfish *Asterina pectinifera*, was found to support the survival of cultured neuronal cells and another ganglioside molecular species GAA-7,[2] which was obtained from the starfish *Asterias amurensis versicolor*, showed neuritogenic and growth-inhibitory activity towards the mouse neuroblastoma cell line (Neuro 2a). On the other hand, the ganglioside molecular species CG-1,[3] which was obtained from the sea cucumber *Cucumaria echinata*, exhibited neuritogenic activity towards the rat pheochromocytoma cell line (PC-12). Now we report our investigation of the sphingolipids from the feather star *Comanthus japonica*, belonging to the class of crinoidea.

The water-soluble lipid fraction, obtained from the $CHCl_3$–MeOH extract of the whole bodies of *Comanthus japonica*, was subjected to reversed-phase and subsequently normal-phase column chromatography to give an inositolphosphoceramide molecular species. The phosphosphingolipid, showing a single spot on normal-phase thin-layer chromatography (TLC), is tentatively named CJP1.

CJP1 gave a positive reaction with Dittmer–Lester reagent[4] and modified Hanes and Isherwood reagent,[5] which indicated the presence of phosphate group. In the IR spectrum of CJP1, strong hydroxyl, amide, and phosphate absorptions were observed at 3347, 1643, 1555, and 1229 cm^{-1}. In the negative ion FAB mass spectrum, CJP1 showed the fragment ion peaks at *m*/*z* 79, 97 suggesting the existence of a phosphate group together with molecular ion peaks at *m*/*z* 834, 862. Furthermore the characteristic fragment ion peaks[6] due to inositolphosphoceramide were observed at *m*/*z* 241, 259, 300, 672 and 700 as shown in Fig. 1.

When CJP1 was subjected to mild alkaline hydrolysis with 1 N KOH, ceramides (CJP1Cer) and inositolphosphate were obtained. At first, the inositolphosphate moiety was examined. In the ^{1}H-NMR spectrum, the chemical shift values of the inositolphosphate were in good agreement with the calculated values of *myo*-inositol-1-phosphate (Table 1).[7] As for the absolute configuration, the specific rotation of the inositolphosphate from CJP1 ($[\alpha]_D$ −8.2°) is opposite to that of L-*myo*-inositol-1-phosphate ($[\alpha]_D$ +9.3°),[8] therefore CJP1 contains D-*myo*-inositol-1-phosphate.

Next the ceramide moiety was examined. CJP1 was methanolyzed and extracted with *n*-hexane to give fatty acid methyl ester (FAM). GC-MS analysis of the FAM mixture showed mainly the existence of $C_{22:0}$, $C_{24:0}$ normal fatty acids. On the other hand, the TMS derivatives of the long chain base mixture, which was obtained from CJP1Cer upon methanolysis, was analyzed by GC-MS. The result was that the LCB mixture was mainly composed of a $C_{16:1}$ sphingosine derivative.

The relative stereochemistry of the ceramide was determined as 2,3-*erythro*-4*E*, since the ^{1}H-NMR spectrum of the ceramide was in good agreement with that of the ceramide obtained from the soft coral *Litophyton sp*.[9] possessing 2,3-*erythro*-4*E* configuration (Table 2). Furthermore, signals due to the methyl groups are observed at 14.1 ppm in the ^{13}C-NMR spectrum of CJP1Cer, which indicates LCB also has the normal type of alkylchain.

Accordingly, if LCB is assumed to belong to the most

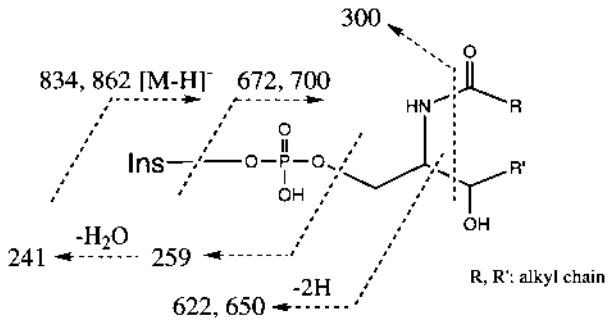

Fig. 1. The Negative Ion FAB-MS Fragmentation of CJP1

Table 1. ^{1}H-NMR Spectral Data of Inositolphosphate (D_2O)

Position	Inositol phosphate from CJP1	*myo*-Inositol	Calculated chemical shifts of *myo*-inositol-1-phosphate[a)]
1	3.99 (ddd, 3.0, 8.6, 9.8 Hz)	3.55	3.99
2	4.29 (t, 3.0 Hz)	4.08	4.26
3	3.59 (dd, 2.6, 9.9 Hz)	3.55	3.57
4	3.68 (t, 10.0 Hz)	3.65	3.65
5	3.36 (t, 9.2 Hz)	3.30	3.32
6	3.78 (t, 9.6 Hz)	3.65	3.83

a) Calculated from the chemical shifts of *myo*-inositol.[7]

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commonly found D-*erythro* (2*S*, 3*R*) type, the structure of the inositolphosphoceramide molecular species CJP1 is characterized as D-*myo*-inositol-1-*O*-phosphoceramide as shown in Fig. 2.

The same type of sphingolipid as CJP1 has been found in plants and protostomia[10] but to our knowledge not yet in deuterostomia. The isolation and characterization of inositolphosphoceramide from deuterostomia is noteworthy.

Experimental

Melting points were determined on a micro melting point apparatus (Yanaco MP-3) without correction. Optical rotations were measured with a Jasco Dip-370 digital polarimeter at 24 °C. IR spectra were obtained on a Jasco FT/IR-410 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded at 270 and 67.8 MHz, respectively, on a JEOL GX-270 spectrometer. Negative-ion FAB-MS spectra were acquired with a JEOL SX/SX102A tandem mass spectrometer (xenon atom beam, 5 kV; ion source accelerating potential, 10 kV; matrix, triethanol amine). GC-MS was taken with a Shimadzu QP-1000 [EI mode; ionization potential, 70 eV; separator and ion-source temperature 250 °C; column, a glass column (1.1 m×2.6 mm) packed with 2% OV-17 on Chromosorb W (mesh 80—100 μm); carrier gas, He (30 ml/min)].

Separation of CJP1 Whole bodies of the feather star *Comanthus japonica* (7.6 kg), collected at Koinoura, Fukuoka Prefecture, Japan, in 1995, were homogenized and extracted with $CHCl_3$–MeOH (1 : 2) (8 l, three times). The $CHCl_3$–MeOH solution was concentrated *in vacuo* to give an aqueous suspension (1 l). The suspension was diluted with H_2O (2 l) and extracted with AcOEt–1-BuOH (2 : 1) for separation of less polar lipids. The aqueous layer was washed with 1-BuOH saturated with H_2O, and concentrated *in vacuo* to 1 l. Then 1 l of MeOH was added to the aqueous layer and the mixture was chromatographed on reversed-phase column chromatography [Cosmosil 140C_{18}-PREP (Merck), solvent 80%MeOH, 100%MeOH and $CHCl_3$–MeOH (3 : 7)]. The 100%MeOH and $CHCl_3$–MeOH (3 : 7) eluate were combined and concentrated *in vacuo* to give a residue (4.1 g). The residue was chromatographed on silica gel [silica gel 60 (Merck), solvent $CHCl_3$–MeOH–H_2O (6.5 : 3.5 : 0.2→6.5 : 3.5 : 0.3→6.5 : 3.5 : 0.5→6.5 : 3.5 : 0.55→6 : 4 : 0.7→6 : 4 : 0.85→6 : 4 : 1→5 : 5 : 1)] to give six fractions. Successive column chromatography of fr. 2, 3 [Iatrobeads (6RS-8060, Iatron Lab.), solvent $CHCl_3$–MeOH–H_2O (6 : 4 : 0.35)] to afford CJP1 (92 mg), which was detected with 5%H_2SO_4–MeOH or Dittmer–Lester reagent[4] on silica gel TLC [solvent $CHCl_3$–MeOH–H_2O (6 : 4 : 0.45)], *Rf*=0.42.

CJP1: Amorphous powder, mp 173—176 °C, $[\alpha]_D$ +3.6° [*c*=0.71, $CHCl_3$–MeOH (9 : 1)]. IR (KBr) cm^{-1}: 3347 (OH), 1643, 1555 (amide), 1229 (phosphate). Negative ion FAB-MS: $(M-H)^-$ *m/z*: 834, 862.

Alkaline Hydrolysis of CJP1 CJP1 was hydrolyzed with 1 N KOH at 35 °C for 48 h. Hydrolysate was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4, evaporated *in vacuo* to give crude ceramide. The crude ceramide was chromatographed on silica gel [solvent $CHCl_3$–MeOH (99 : 1)] to afford ceramide (CJP1Cer). The H_2O layer was neutralized with 2 N HCl, diluted with the same quantity of MeOH, and then passed through Cosmosil 140C_{18}-PREP [solvent 50%MeOH]. The eluate was evaporated *in vacuo* and the residue was applied to a Sephadex LH-20 column [solvent 50%MeOH] to give inositolphosphate, which was detected with modified Hanes and Isherwood reagent[5] on avicel TLC.

Inositolphosphate from CJP1 $[\alpha]_D$ −8.2° (*c*=0.47, H_2O). ^{1}H-NMR (D_2O): see Table 1.

Major Ceramide from CJP1Cer ^{1}H-NMR ($CDCl_3$): see Table 2. ^{13}C-NMR ($CDCl_3$) δ: 173.8 (C-1′), 134.3 (C-5), 129.0 (C-4), 74.7 (C-3), 62.6 (C-1), 54.7 (C-2), 36.9 (C-2′), 14.1 (terminal methyl groups).

GC-MS Analysis of Fatty Acid Methyl Esters from CJP1 CJP1 was methanolyzed with 0.9 N HCl–MeOH aq. at 80 °C for 17 h in a small sealed vial. The reaction mixture was extracted with *n*-hexane, the extract was concentrated *in vacuo* to yield a mixture of FAM, which was subjected to GC-MS [column temperature 180—250 °C (rate of temperature increase 8 °C/min)]. The results were as follows: FAM-1 (methyl octadecanoate), t_R [min] (ratio of peak areas): 7.2 (10), *m/z*: 298 $(M)^+$, 255 $(M-43)^+$; FAM-2 (methyl docosanoate), t_R: 11.4 (29), *m/z*: 354 $(M)^+$, 311 $(M-43)^+$; FAM-3 (methyl tricosanoate), t_R: 12.2 (3), *m/z*: 368 $(M)^+$, 325 $(M-43)^+$; FAM-4 (methyl tetracosanoate), t_R: 13.3 (58), *m/z*: 382 $(M)^+$, 339 $(M-43)^+$.

GC-MS Analysis of TMS Ethers of LCB from CJP1Cer Ceramides (CJP1Cer) (1 mg) were methanolyzed with 5% HCl in MeOH at 70 °C for 2 h. The reaction mixture was extracted with *n*-hexane to remove FAM. The MeOH layer was dried with a N_2 stream to yield long chain bases (LCB). The mixture was heated with 1-(trimethylsilyl)imidazole–pyridine (1 : 1, 20 μl) for 10 min at 70 °C and the reaction mixture was subjected to GC-MS [column temperature 180—250 °C (rate of temperature increase 8 °C/min)].

Table 2. ^{1}H-NMR Spectral Data of Ceramide ($CDCl_3$)

Position	Ceramide from CJP1	Ceramide from *Litophyton sp.*
1	3.70 (br d, 11.2 Hz)	3.70 (br d, 10.2 Hz)
	3.95 (dd, 12.4, 3.6 Hz)	3.95 (dd, 12.7, 3.6 Hz)
2	3.91 (m)	3.90 (m)
3	4.32 (br s)	4.32 (br s)
4	5.53 (dd, 15.5, 6.3 Hz)	5.53 (dd, 15.3, 6.4 Hz)
5	5.78 (dt, 15.5, 6.6 Hz)	5.79 (dt, 15.3, 7.0 Hz)
2′	2.23 (t, 7.6 Hz)	2.23 (t, 7.6 Hz)

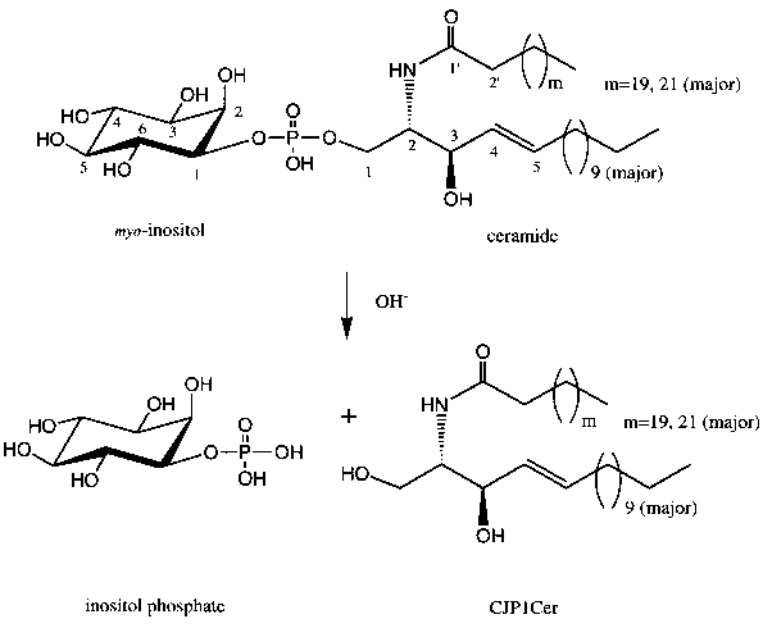

Fig. 2

The results were as follows: LCB-1 (2-amino-1,3-diol-4-hexadecene), t_R [min] (ratio of peak areas): 4.5 (72), *m*/*z*: 312 $(M-103)^+$, 310 $(M-105)^+$, 283 $(M-132)^+$; LCB-2 (2-amino-1,3-diol-4,ξ-octadecadiene), t_R: 6.3 (20), *m*/*z*: 338 $(M-103)^+$, 336 $(M-105)^+$, 309 $(M-132)^+$.

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