Constituents of Crinoidea. 2. Isolation and Structure of the Novel Type Gangliosides from the Feather Star *Comanthus japonica*

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Two novel type gangliosides CJP2 and CJP3 have been obtained from the feather star *Comanthus japonica*. On the basis of methylation linkage analysis combined with ammonolysis and other chemical and spectroscopic evidence, the chemical structures of CJP2 and CJP3 were determined to be α-9-O-Me-NeuGc-(2→3)-inositolphosphoceramide and α-9-O-Me-NeuGc-(2→11)-α-9-O-Me-NeuGc-(2→3)-inositolphosphoceramide, respectively. These gangliosides are unique in that they are inositolphosphoceramide derivatives possessing sialic acid; such gangliosides have not previously been identified. The presence of 9-O-methyl-N-glycoly neuraminosyl residues is also unique in naturally occurring gangliosides.

Key words echinodermata; feather star; ganglioside; glycosphingolipid; inositolphosphoceramide; 9-O-methyl-N-glycoly neuraminic acid

A series of studies on the isolation and structure elucidation of biologically active glycosphingolipids (GSLs) from the echinodermata has been performed in our laboratory. In the study of the GSLs of the feather star, we reported on the isolation and structure of a ω-my o-inositol-1-O-phosphoceramide, CJP1, from *Comanthus japonica*. Continuing this work, the more polar GSLs from *C. japonica* were isolated and characterized. In this paper, we report on the isolation and structure of the novel type gangliosides, tentatively called CJP2 and CJP3, from the whole bodies of *C. japonica*.

The water-soluble lipid fraction, obtained from the CHCl₃/MeOH extract of the whole bodies of *C. japonica*, was subjected to reversed-phase and subsequently normal-phase column chromatography to give CJP2 and CJP3. They showed a single spot on normal-phase TLC and exhibited a positive reaction with Dittmer-Lester reagent, which indicated the presence of a phosphate group. In their IR spectra, strong hydroxyl, amide and phosphate absorption was observed. When CJP2 and CJP3 were hydrolyzed with 5% acetic acid, CJP1, ω-my o-inositol-1-O-phosphoceramide, was obtained from both compounds. Therefore, CJP2 and CJP3 were suggested to be the derivatives of CJP1.

**Structure of CJP2** In the negative ion FAB-MS spectrum, CJP2 showed quasi-molecular ion peaks due to (M−H)⁻ at m/z 1155 and 1183 together with fragment ion peaks similar to those of CJP1 as shown in Fig. 1. The loss of 321 mass units from the molecular ion suggested the existence of a monomethylated N-glycoly neuraminic acid residue.

The structure of the sialic acid residue in CJP2 was analyzed as follows. CJP2 was methylated with CD₃I according to the Ciucanu and Kerek method and afforded the perdeutermethylated product. The product was methanolyzed, and the methanolysate was acetylated to give the sialic acid derivative. GC-MS analysis of the sialic acid derivative revealed the presence of terminal 9-O-methylated N-glycoly neuraminic acid (9-O-Me-NeuGc) residue as shown in Fig. 2A.

The position of the 9-O-Me-NeuGc linkage site to the myo-inositol part was elucidated by methylation linkage analysis combined with ammonolysis. The permethylated CJP2 was hydrolyzed with NH₄OH to give the partially methylated inositol derivative. GC-MS analysis of its TMS ether revealed that it was tetramethylated. In the ¹H-NMR spectrum of the tetramethylated inositol, four kinds of oxymethin proton signals were observed and could be assigned as shown in Table 1 on the basis of J values, which indicated the symmetrical structure of the tetramethylated inositol. Furthermore, the NOE correlations of methoxyl and oxy methyl proton signals (Fig. 3) revealed that the inositol derivative was 2,4,5,6-tetramethylated myo-inositol. Consequently, 9-O-Me-NeuGc residue must be linked at the C3-OH group of the inositol part as shown in Fig. 1.

The anomeric configuration of 9-O-Me-NeuGc residue was determined as below. When CJP2 was subjected to mild alkaline hydrolysis with 1N KOH, the sugar part and ceramide (CJP2Cer) were obtained (Fig. 1). In the ¹H-NMR spectrum of this sugar part, the signal due to H-3eq of 9-O-Me-NeuGc was observed at 2.73 ppm (1H, dd, J=12.5, 4.7 Hz), which suggests the α-configuration of 9-O-Me-NeuGc. This part of CJP2 was therefore characterized as α-9-O-Me-NeuGc-(2→3)-inositolphosphate.

Next, the detailed structure of the ceramide moiety was examined using CJP2Cer obtained as above. When CJP2Cer was methanolyzed, fatty acid methyl ester (FAM) and the

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long chain base (LCB) mixture were obtained. GC-MS analysis of the FAM mixture indicated the presence of C$_{22}$:0 and C$_{24}$:0 normal fatty acids. On the other hand, the LCB mixture was converted to their TMS ether and analyzed by GC-MS. The result showed that the LCB mixture was composed of C$_{16}$:1 sphingosine as major component, and a small amount of C$_{16}$:0 and C$_{18}$:0 phytosphingosines.

The relative stereochemistry of CJP2Cer could be characterized as 2,3-erythro-4E, because the $^1$H-NMR spectrum was in good agreement with that of the ceramide obtained from the gorgonian *Acabaria undulata* possessing 2,3-erythro-4E configuration. Furthermore, in the $^{13}$C-NMR spectrum of CJP2, the signals of the terminal methyl group are observed at 13.7 ppm, which indicates both long alkyl chains are straight chains.

Consequently, if LCB and NeuGc are assumed to belong to the most commonly found D-erythro (2'S,3'R) and D series, and myo-inositol-1-O-phosphate is also assumed to be $\alpha$-series, as true of the co-existing CJP1, then CJP2 is $\alpha$-9-O-Me-NeuGc-(2→11)-$\alpha$-9-O-Me-NeuGc-(2→3)-inositolphosphoceramide as shown in Fig. 1.

**Structure of CJP3** In the negative ion FAB-MS spectrum, CJP3 revealed the quasi-molecular ion peaks due to (M+K−2H)$^-$ at m/z 1514, 1540, 1542 (Fig. 4), which indicate CJP3 contains one additional mole of monomethylated N-glycolyl-neuraminic acid compared with CJP2. The structure of these sialic acids and the linkage sites of the sialic acid and inositol residues were analyzed in the same manner as CJP2, as follows.

GC-MS analysis of sialic acid derivatives, prepared from perdeuteromethylated CJP3, revealed the existence of terminal 9-O-Me-NeuGc and 11-linked 9-O-Me-NeuGc as shown in Fig. 2A and 2B. Therefore, 9-O-Me-NeuGc must be linked at C11-OH of another 9-O-Me-NeuGc residue (Fig. 4). The inositol derivative prepared from permethylated CJP3 was identified as 2,4,5,6-tetramethylated myo-inositol by the $^1$H-NMR and NOESY spectra, which suggested that 9-O-Me-NeuGc residue was linked at C3-OH of the inositol part as true of CJP2. In the $^{13}$C-NMR spectrum of CJP3, two anomic carbon signals due to two sialic acid residues were observed at $\delta$ 99.8 and 100.3, indicating their $\alpha$-configurations. Consequently, the sugar part of CJP3 was determined to be $\alpha$-9-O-Me-NeuGc-(2→11)-$\alpha$-9-O-Me-NeuGc-(2→3)-inositolphosphoceramide.

For the analysis of the ceramide moiety, CJP3Cer was obtained by means of alkaline hydrolysis of CJP3. CJP3Cer was methanolyzed and FAM and TMS ether of LCB were analyzed by GC-MS. The results indicated the presence of C$_{22}$:0, C$_{24}$:0, and C$_{24}$:1 normal fatty acid and C$_{16}$:1 sphingosine.
as major components. The relative stereochimistry of ceramide was determined to have 2,3-erythro-4E configuration with the aid of the ^1H-NMR spectrum of CJP3Cer.

Consequently, if LCB, NeuGc and myo-inositol-1-O-phosphate are assumed to belong to the \(\alpha\)-erythro \((2S,3R)\) and \(\beta\) series as CJP2, CJP3 is characterized to be \(\alpha\)-9-O-Me-NeuGc(2→11)-\(\alpha\)-9-O-Me-NeuGc(2→3)-inositolphosphoceramide as described in Fig. 4.

Many kinds of gangliosides of echinodermata have been obtained from species of starfish, sea cucumber, sea urchin and brittle star. This is the first report of isolation and characterization of gangliosides from the feather star. The gangliosides obtained this time, CJP2 and CJP3, are specially novel in the respect that they are inositolphosphoceramide derivatives containing a sialic acid residue. To the best of our knowledge, such unique gangliosides have not previously been reported. Furthermore, the presence of the 9-O-methyl-N-glycolylnuraminic acid residues is unique in the naturally occurring gangliosides. The biological activity of these gangliosides will be examined.

Experimental

Melting points were determined on a micromelting point apparatus (Yanaco MP-3) without correction. IR spectra were obtained on a Jasco FT/IR-410 infrared spectrophotometer. \(^1H\)- and \(^13C\)-NMR spectra were recorded on a Jeol JNM-GX-270 spectrometer (270 and 67.8 MHz) or a Varian XL-270 spectrometer (270 MHz; 10.2 cm). 

Preparation and GC-MS Analysis of Acetate of Partially Trideuteromethylated Sialic Acid from Perdeuteromethylated CJP2

Perdeuteromethylated CJP2 was methanolysed with 5% HCl in MeOH (1 ml) at 85 °C for 12 h in a small-volume sealed vial, and dried with a N₂ stream. The reaction mixture was heated with Ac₂O/pyridine (1:1, 0.5 ml) at 85 °C for 2 h, and dried with a N₂ stream. The residue was subjected to GC-MS (QP-1000, capillary column) (column temperature: 180—250 °C (rate of temperature increase 4 °C/min)); \(t_f\) min=5.7, \(m/z\): 168, 204, 293, 313, 393 [myo-inositol-2,4,5,6-tetramethyl-7,8,11-tetra-O-trideuteromethyl-sialic acid (from terminal 9-O-NeuGc)].

Methylation (Trideuteromethylation) of CJP2 (Ciucanu and Kerek Method)

NaOH/DMSO suspension (0.5 ml) and CH₂Cl₂ (0.1 ml) was added to CJP2 (7.9 mg), and the mixture was sonicated for 30 min. The reaction mixture was diluted with H₂O and extracted with CHCl₃, three times to give perdeuteromethylated CJP2.

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LCB-1 (2-amino-hexadec-4-ene-1,3-diol), \( t_{R} \) [min] (ratio of peak areas): 4.5 (65), \( m/z: 312 \) (M—103), 310 (M—105), 283 (M—132), 132; LCB-2 (2-amino-hexadecane-1,3,4-triol), \( t_{R}: 5.6 \) (17), \( m/z: 312 \) (M—193), 271 (M—234), 204, 132; LCB-3 (2-amino-octadecane-1,3,4-triol), \( t_{R}: 7.3 \) (17), \( m/z: 340 \) (M—193), 299 (M—234), 204, 132.

Preparation and GC-MS Analysis of Acetate of Partially Trideuteromethylated Sialic Acid Derived from CJP3 CJP3 was trideuteromethylated according to the Ciucanu and Kerek method, then methanolyzed and acetylated in the same manner as for CJP2. The residue was subjected to GC-MS (QP-5050A) [column temperature 200°C (2.0 min)—250°C (rate of temperature increase 2.5°C/min)]; \( t_{R}: 25.3 \) (37), \( m/z: 193 \) (M1—105), 283 (M1—132). In the mixture of fatty acid methyl esters (FAM), \( t_{R}: 38.2 \) (24), \( m/z: 382 \) (M1). The reaction mixture was evaporated and dried to give a residue.

Methylation Linkage Analysis Combined with Ammonolysis of CJP3 CJP3 (10.0 mg) was permethylated and ammonolyzed in the same manner as for CJP2. The reaction mixture was evaporated and dried to give a residue containing a partially methylated inositol derivative. The residue was chromatographed on silica gel to give an inositol derivative (2,4,5,6-tetramethyl-inositol derivative).

Alkaline Hydrolysis of CJP3 CJP3 was hydrolyzed with 1 N KOH. The hydrolysate was neutralized with 2 N HCl, and extracted with CHCl3 three times, and evaporated in vacuo.

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