Constituents of Ophiuroidea. 1. Isolation and Structure of Three Ganglioside Molecular Species from the Brittle Star *Ophiocoma scolopendrina*

Masanori INAGAKI, a Motohiro SHIBAI, a Ryuichi ISOBE, b and Ryuichi HIGUCHI* a

Faculty of Pharmaceutical Sciences, Kyushu University, a 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan and Department of Industrial Chemistry, Faculty of Engineering, Towa University, b 1–1–1 Chikusigaoka, Minami-ku, Fukuoka 815–0036, Japan. Received June 18, 2001; accepted August 10, 2001

Three ganglioside molecular species, OSG-0 (1), OSG-1 (2), and OSG-2 (3) have been obtained from the polar lipid fraction of the chloroform/methanol extract of the brittle star *Ophiocoma scolopendrina*. The structures of these gangliosides have been determined on the basis of chemical and spectroscopic evidence as 1-O-[(N-glycolyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranosyl]-ceramide (1), 1-O-[8-0-sulfot-(N-acetyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranosyl]-ceramide (2) and 1-O-[(N-glycolyl-α-D-neuraminosyl)-(2→6)-β-D-glucopyranosyl]-ceramide (3). The ceramide moieties were composed of heterogeneous unsubstituted fatty acid, 2-hydroxy fatty acid and phytosphingosine units. Compounds 2 and 3 represent new ganglioside molecular species.

Key words glycosphingolipid; ganglioside; brittle star; *Ophiocoma scolopendrina*

In the course of our continuing research on biologically active glycosphingolipids (GSLs) from echinoderms, a series of studies on the isolation and structure elucidation of the GSLs from starfish, sea cucumber and feather star species have been performed in our laboratory. In continuation of the previous studies, the isolation and characterization of the biologically active GSLs from the brittle star *Ophiocoma scolopendrina* (Udefurikumohitode in Japanese) has now been carried out in order to develop the novel medicinal resources from natural marine products. In this paper, we report the isolation and characterization of three ganglioside molecular species from the whole bodies of *O. scolopendrina*.

The polar lipid fraction, which was obtained from the chloroform/methanol extract of the whole bodies of *O. scolopendrina*, was subjected to repeated column chromatography to give three ganglioside molecular species, OSG-0 (1), OSG-1 (2), and OSG-2 (3), each showing a single spot on silica gel thin-layer chromatography (TLC).

In its 13C-NMR spectrum (Chart 1, Table 1), 1 exhibits the characteristic signals of a phytosphingosine-type ceramide, possessing an unsubstituted fatty acid and a sugar moiety at C-1 [δ: 70.5 (C-1), 53.8 (C-2), 76.0 (C-3), 72.3 (C-4), 176.2 (C-1‘), 36.8 (C-2‘)]. The 13C-NMR spectrum of 1 also features signals due to two anomeric carbons at δ: 105.4 and 100.7, one of which (δ: 100.7) is a quaternary carbon signal, indicating the presence of a sialic acid residue. The negative FAB-MS exhibits a series of quasi-molecular ion peaks [M−H]+ at m/z: 1000—1100. Therefore, 1 is suggested to be a molecular species of a phytosphingosine-type ganglioside, possessing unsubstituted fatty acid groups and two monosaccharide units. Furthermore, 1 is presumed to have mainly normal-type fatty acids and normal and iso-type long-chain bases (LCB), since the carbon signals for the terminal methyl groups are observed at δ: 14.2 (normal form) and δ: 22.9 (iso form) in the 13C-NMR spectrum (Chart 1, Table 1).

The structure of the ceramide moiety was examined first. When 1 was methanolyzed with methanolic hydrochloric acid, a mixture of fatty acid methyl esters (FAM) and LCB was obtained, together with methyl glucopyranoside. The FAM mixture was analyzed by GC-MS, which revealed the presence of five components. These were characterized as methyl octadecanoate (major), methyl eicosanoate, methyl docosanoate, methyl tricosanoate, and methyl tetracosanoate. The LCB mixture was found to be composed of 2-amino-1,3,4-trihydroxy-heptadecane and -octadecane (major), based on GC-MS analysis of its trimethylsilyl (TMS) derivative (Chart 1).

The relative stereochemistry of the phytosphingosine of ceramide moiety is presumed to be (2S,3S,4R), since the aforementioned 13C-NMR signals assignable to C-1, 2, 3, and 4 of 1 are in good agreement with those of the phytosphingosine-type ganglioside molecular species possessing (2S,3S,4R) configurations.

The structure of the disaccharide moiety of 1 was established as follows. The presence of glucose (Glc) was obvious from the results of the methanalysis of 1 (vide supra). A detailed analysis of the 13C-NMR spectrum of 1 revealed the characteristic signals [δ: 175.6 (C-1), 100.7 (C-2), 42.6 (C-3), 53.8 (C-5), 64.4 (C-9), 176.2 (C-10), 62.4 (C-11)] of an N-glycolylneuraminic acid (NeuGc) derivative residue coupled with a β-glucopyranoside derivative residue (Table 1). In the negative FAB-MS of 1, molecular ion and fragment ion peaks arising from cleavage of the glycosidic linkages are observed at m/z: 1000—1100, 720—750, and 550—650, indicating the presence of the disaccharide moiety, NeuGc→Hexose(β-glucopyranose), as shown in Fig. 1.

Methylation of 1, according to Ciucanu–Kerek method,2) afforded the permethylated product 4. Partially methylated undiltol acetate (S-1), prepared from 4, was analyzed by GC-MS and identified as the undiltol derived from 6-linked hexopyranose. On the other hand, upon methanalysis followed by acetylation, the permethylated NeuGc (S-2) derived from the terminal NeuGc was detected by GC-MS analysis. On the basis of the above evidence, the disaccharide moiety of 1 must be NeuGc-(2→6)-β-glucopyranose. The configuration of NeuGc is believed to be α on the basis of its anomeric...
carbon signal ($\delta$: 100.7) in the $^{13}$C-NMR spectrum of 1. In addition, the absolute configuration of the glucose unit was verified as being of D-form by the Hara method. Consequently, if NeuGc and phytosphingosine are assumed to belong to the most commonly found D-series and 2$^S$,3$^S$,4$^R$ type, respectively, then 1 is the (N-glycolyl-a-D-neuraminosyl)-(2$^6$)-b-D-glucopyranoside of a ceramide, composed of heterogeneous (2$^S$,3$^S$,4$^R$)-phytosphingosine and unsubstituted fatty acid units. The major components of the fatty acid and phytosphingosine moiety of 1 are octadecanoic acid and 2-amino-1,3,4-trihydroxyoctadecane, respectively (Chart 1).

Compound 2 exhibits the characteristic signals of a phytosphingosine-type ceramide, possessing a 2-hydroxy fatty acid and a sugar moiety at C-1 ($\delta$: 70.5, 70.3, 70.5) in its $^{13}$C-NMR spectrum (Chart 2, Table 1). The $^{13}$C-NMR spectrum of 2 also shows signals due to two anomeric carbons at $\delta$: 105.0 and 101.0, one of which ($\delta$: 101.0) is a quaternary carbon signal, indicating the presence of a sialic acid residue. The negative FAB-MS exhibits a series of quasi-molecular ion peaks [M-H]$^-$ at $m/z$: 1100—1200, and the fragment ion peaks due to [SO$_4$H]$^-$ and [SO$_3$]$^-$ at $m/z$: 97 and 80.

Therefore, 2 is suggested to be a molecular species of a sulfated phytosphingosine-type ganglioside, possessing 2-hydroxy fatty acid groups and two monosaccharide units. The terminal methyl groups of the ceramide moiety of 2 must be the same as that of 1 from their carbon atom signals (Table 1).

When 2 was methanolyzed with methanolic hydrochloric acid, a mixture of FAM and LCB was obtained, together with

![Chart 1](chart.png)
methyl glucopyranoside. The FAM mixture was analyzed by GC-MS, which revealed the presence of main five components. These were characterized as methyl octadecanoate, methyl 2-hydroxyoctadecanoate, methyl 2-hydroxycosanoate (major), methyl 2-hydroxytricosanoate, and methyl 2-hydroxytetraicosanoate. The LCB mixture was found to be composed of only 2-amino-1,3,4-trihydroxy-octadecane, based on GC-MS analysis of its TMS derivative (Chart 2). Furthermore, the relative stereochemistry of the ceramide moiety is presumed to be \( \text{D-form} \), since the aforementioned \(^{13}\text{C}-\text{NMR} \) signals at \( \delta: 101.2 \) and 101.2, respectively, were found in agreement with those of the phytosphingosine-type ganglioside molecular species possessing \( \text{D,3,4R,2'2''R} \) configurations.\(^{1,3,4}\)

The structure of the disaccharide moiety of 2 was elucidated as outlined below. The presence of Glc was obvious from the results of the methanalysis of this species and the absolute configuration (\( \text{D-form} \)) of the glucose unit was verified as before. In its \(^{13}\text{C}-\text{NMR} \) spectrum (Table 1), 2 shows characteristic signals due to one mol each of \( \text{N-acetylneuraminic acid} \) (NeuAc) derivative and \( \beta\text{-glucopyranose} \) derivative residue. The negative FAB-MS of 2 shows the molecular and fragment ion peaks at \( m/z: 1100—1200, 800—850, \) and \( 650—700 \), corresponding to cleavage of the glycosidic linkages of 2, thus indicating the disaccharide moiety, NeuAc-\( (\text{SO}_3\text{H}) \rightarrow \text{Hexose} (\beta\text{-glucopyranose}) \), as shown in Fig. 1.

Partially methylated alditol acetate prepared from 5, the permethylated 2, was characterized as the alditol derived from 6-linked hexopyranose (S-1) by means of GC-MS. The acetate of partially methylated NeuAc (S-3) derived from 8-linked NeuAc was detected in the acetate of methanolsate prepared from 5. These facts establish the structure of the disaccharide moiety as \( 8\text{-O-sulfo-NeuAc-(2->6)-} \beta\text{-d-Glc} \) (p).

The configuration of C-2 in the sialic acid (NeuAc) is also thought to be \( \alpha \), as in the case of 1, based on its anomeric carbon signals (\( \delta: 101.0 \)) in the \(^{13}\text{C}-\text{NMR} \) spectrum of 2.

Consequently, if NeuAc and LCB are assumed to belong to the \( \alpha \)-series and \( 2\text{S,3S,4R} \) type, 2 is the \( 8\text{-O-sulfo-(N-acetyl-} \alpha\text{-d-neuraminosyl)-(2->6)-} \beta\text{-d-glucopyranoside} \) of a ceramide composed of \( 2\text{S,3S,4R} \)-C\(_{18}\) phytosphingosine and heterogeneous fatty acid units. The major component of the fatty acid is \( (2R)-2\text{-hydroxydocosanoic acid} \) units as shown in Chart 2.

In its \(^{13}\text{C}-\text{NMR} \) spectrum, 3 exhibits characteristic signals attributable to the ceramide moiety, which correspond to those of 2 (Table 1). The \(^{13}\text{C}-\text{NMR} \) spectrum of 3 also features signals due to three anomic carbon atoms at \( \delta: 104.6, 101.2 \) and 101.2, two of which (\( \delta: 104.6 \)) are quaternary carbon atom signals, indicating the presence of two sialic acid residues. The negative FAB-MS exhibits a series of quasi-molecular ion peaks \([\text{M—H}^-] \) at \( m/z: 1400—1500 \). Therefore, 3 is suggested to be a molecular species of ganglioside, like 2, having three monosaccharide units. Since 3 gave the same FAM and LCB mixture as 2, the major fatty acid and LCB of 3 must be \( (2R)-2\text{-hydroxydocosanoic acid} \) and \( (2S,3S,4R)\)-C\(_{18}\) phytosphingosine, respectively.

The methanalysis and acidic hydrolysis of 3, indicating the existence of \( \alpha\text{-Glc} \), together with the signals due to sugar moieties in the \(^{13}\text{C}-\text{NMR} \) spectrum of 3 (Table 1), suggest that the sialosyl trisaccharide moiety of 3 is composed of one mol each of \( \beta\text{-d-glucopyranosyl}, \alpha\text{-NeuAc}, \) and \( \alpha\text{-NeuGc} \). In its negative FAB-MS, 3 shows molecular (\( m/z: 1400—1500 \)) and fragment ion peaks (\( m/z: 1100—1150, 800—850, 650—700 \)) arising from cleavage of the glycosidic linkages of 3, which are indicative of the linear trisaccharide moiety, NeuGc\( \rightarrow \text{Hexose} \rightarrow \alpha\text{-NeuAc} \), as shown in Fig. 1.

The GC-MS analysis of the partially methylated alditol acetates of the neutral sugars and of the acetates of partially methylated sialic acids, which were synthesized from 6, the permethylated 3, indicated the presence of \( 6\text{-linked hexopyranose} \) (S-1), terminal NeuGc (S-2), and 8-linked NeuAc (S-3) together with \( 8\text{-linked NeuGc} \) (S-4) as a minor component in the sugar moiety. On the basis of the above evidence, the sugar moiety of 3 is still heterogeneous for its inner sialic acid residue and therefore, the sialosyl trisaccharide moiety of 3 must be \( \alpha\text{-NeuGc-(2->8)-} \alpha\text{-NeuAc} \) and \( \text{NeuGc-(2->6)-} \beta\text{-d-Glc} \) (p). The major component of inner sialic acid is NeuAc.
Accordingly, if NeuGc and NeuAc are assumed to belong to the d-series, 3 must be (N-glycolyl-d-neuraminosyl)-(2→8)-(N-acetyl- and N-glycolyl-d-neuraminosyl)-(2→6)-β-d-glucopyranosyl of a ceramide composed of the same fatty acid and LCB units as 2.

From the brittle star Ophiura sarsi, 5,6 Ophiocoma echinata 9,10 and Ophiomastrix annulosa, 6,11 five kinds of ganglioside molecular species have been obtained and characterized. However, the ganglioside molecular species isolated in this study, OSG-1 and OSG-2, are, to the best of our knowledge, new ganglioside molecular species. Although ganglioside molecular species possessing the same sugar and core of ceramide moieties as those of OSG-0 have been obtained from Ophiocoma echinata 9,12 and the sea cucumber Stichopus japonicus, 13 OSG-0 slightly differs from them in the fatty acid and LCB components. The biological activity of these gangliosides will be examined.

Experimental

Melting points were determined on a micro melting point apparatus (Yanako MP-3) without correction. IR spectra were obtained on a Jasco FT/IR-410 IR spectrophotometer. NMR spectra were recorded on a Varian Unity-500 spectrometer (500 MHz). Negative-ion FAB-MS spectra were acquired on a JMS-DX300 mass spectrometer (500 MHz). IR spectra were obtained on a Jasco IR-410 IR spectrophotometer. NMR spectra were recorded on a Varian (Y anako MP-3) without correction. IR spectra were obtained on a Jasco OSG-1 (35 mg) was then chromatographed on a silica gel column (2.5 × 25 cm) eluted with 90% MeOH and CHCl3/MeOH (1 : 1) to give four fractions. The crude ganglioside fraction (6.7 g), the CHCl3/MeOH eluate, was chromatographed on silica gel (solvent CHCl3–MeOH–H2O , 7:3:0.3) to give three fractions. Fraction 3 was further chromatographed on silica gel (solvent CHCl3–MeOH–H2O , 7:3:0.2) to afford OSG-0 (1) (38 mg) (Rf = 0.40) and OSG-1 (2) (49 mg) (Rf = 0.33). Fraction 3 was further chromatographed on silica gel (solvent CHCl3–MeOH–H2O , 7:3:0.2 to 7:3:0.5) to afford OSG-2 (3) (57 mg) (Rf = 0.28) [silica gel TLC, solvent CHCl3–MeOH–H2O (6:4:1)].

OSG-0 (1): Amorphous powder, mp 181—183 °C. IR (KBr) cm⁻¹: 3372 (OH), 1647, 1542 (amide). Negative-ion FAB-MS m/z: 1000—1100 [M–H⁻] series (see Fig. 1). 13C-NMR: See Table 1. OSG-1 (2): Amorphous powder, mp 223—228 °C. IR (KBr) cm⁻¹: 3378 (OH), 1648, 1541 (amide), 1230 (sulfate). Negative-ion FAB-MS m/z: 1100—1200 [M–H⁻] series (see Fig. 1). 13C-NMR: See Table 1.

OSG-2: 1) Amorphous powder, mp 210—215 °C. IR (KBr) cm⁻¹: 3367 (OH), 1636, 1547 (amide). Negative-ion FAB-MS m/z: 1400—1500 [M–H⁻] series (see Fig. 1). 13C-NMR: See Table 1.

Methanalysis of 1 Compound 1 (1 mg) was heated with 5% HCl in MeOH (1 ml) at 70 °C for 18 h. The reaction mixture was then extracted with n-hexane, and the extract was concentrated in vacuo to yield a mixture of FAM. The MeOH layer was neutralized with Ag2CO3, filtered, and the filtrate was concentrated in vacuo to give a mixture of LCB and methyl glycoside.

GC-MS Analysis of FAM from 1 A FAM mixture from 1 was subjected to GC-MS [column temp. 150—250 °C (rate of temp. increase 5 °C/min)]. The results were as follows: methyl octadecanoate (major), tR [min] = 16.6, m/z: 298 (M⁺), 255 (M–H⁻)⁺; methyl eicosanoate, tR = 20.0, m/z: 326 (M⁺), 283 (M–H⁻)⁺; methyl docosanoate, tR = 23.4, m/z: 354 (M⁺), 311 (M–H⁻)⁺; methyl tricosanoate, tR = 25.5, m/z: 368 (M⁺), 325 (M–H⁻)⁺; methyl tetracosanoate, tR = 28.0, m/z: 382 (M⁺), 339 (M–H⁻)⁺.

GC-MS Analysis of TMS Ethers of LCB from 1 The mixture of LCB and methyl glycoside from 1 was heated with 1-(trimethylsilyl) imidazole (1:1) for 20 min at 70 °C and the reaction mixture (TMS ethers) was analyzed by GC-MS [column temp. 180—250 °C (rate of temp. increase 5 °C/min)]. The results were as follows: 2-amino-1,3,4-trihydroxy-octadecane (major), tR [min] = 16.4, m/z: 298 (M⁺), 255 (M–H⁻)⁺; 2-amino-1,3,4-trihydroxy-heptadecanec, tR [min] = 16.4, m/z: 298 (M⁺), 255 (M–H⁻)⁺; 2-amino-1,3,4-trihydroxy-octadecanec (major), tR = 18.0, m/z: 340 (M⁺), 299 (M–H⁻)⁺; 2-amino-1,3,4-trihydroxy-heptadecanec (major), tR = 20.0, m/z: 340 (M⁺), 299 (M–H⁻)⁺.

GC-MS Analysis of TMS Ethers of Methyl Glycoside from 1 The mixture of TMS ethers of LCB and methyl glycoside was analyzed by GC-MS [column temp.: 150—200 °C (rate of temp. increase 2.5 °C/min), 200—250 °C (rate of temp. increase 10 °C/min)] 5; tR [min] = 15.5 and 16.1 (methyl glycospyranoside).

Determination of Absolute Configuration of Glucose Moiety of 1 (Hara Method) 6 Compound 1 (1 mg) was heated with 2 N HCl (1 ml) at 90 °C for 24 h. The reaction mixture was then extracted with n-hexane, and the acidic aqueous phase was concentrated. The residue (sugar fraction) was heated with L-cysteine methyl ester hydrochloride (0.3 mg) and pyridine (0.3 ml) at 70 °C for 1 h. Then, 0.1 ml of 1-(trimethylsilyl) imidazole was added and the mixture was heated at 60 °C for a further 0.5 h to yield trimethylsilyl ether of the methyl (4R)-thioldiol-4-carboxylate derivative. The resulting mixture was analyzed by GC-MS [column temp.: 150—200 °C (rate of temp. increase 2.5 °C/min), 200—250 °C (rate of temp. increase 10 °C/min)]; tR [min] = 23.7 (derivative of α-glucose, 23.7; 1-glucose, 24.2).

Methylation of 1 (Ciucanu-Kerek Method) 11 NaOH–dimethylsulfoxide (DMSO) solution, which was prepared from powdered NaOH (40 mg) and DMSO (1 ml), and MeI (0.2 ml) were added to 1 (2 mg), and the mixture was stirred for 30 min. The reaction mixture was then diluted with H2O (15 ml), extracted with CHCl3 (10 ml×3), the CHCl3 phases were washed with H2O, and the solvent was evaporated in vacuo to give permethylated 1, denoted 4 (1.8 mg).

Preparation and GC-MS Analysis of Partially Methyalted Alditol Ac-
FAM and a residue composed of LCB and methyl glycoside. Methanolyzed and the reaction mixture was worked up to give a mixture of trimethylsilylated methyl glucopyranoside were detected. In the same manner as described for 1, 3 was hydrolyzed, the sugar derivatives were analyzed by GC-MS, and d-glucose was detected.

Preparation of Partially Methylated Methylglucosides from 6

The acetates were prepared from 6 (prepared from 3 as above) and analyzed by GC-MS in the same way as for those from 4. S-1 (derived from 6-linked hexopyranosyl) was detected.

Preparation and GC-MS Analysis of Acetates of Partially Methylated Sialic Acids from 5

The acetates were prepared from 5 (0.5 mg) was methanolyzed and then acetylated in the same manner as described for 4. The acetates were subjected to GC-MS under the same conditions as mentioned above, and S-3, \( t_\beta \) [min] = 22.6, \( m/z \) = 129, 201, 254, 318, 376 [methyl N-acetyl-l-8-O-acetyl-N- methyl-2,4,7,9-tetra-O-methylneuramate (derived from 8-linked NeuAc)], was detected.

Analyses of FAM, LCB and Methyl Glycosides from 3

Experiments were conducted in the same manner as in the case of 1, leading to a mixture of FAM and a residue composed of LCB and methyl glycosides derived from 3. The FAM mixture was subjected to GC-MS under the same conditions as described for 1, and methyl octadecanoate, methyl 2-hydroxyoctadecanoate, methyl 2-hydroxycosanoate, and methyl 2-hydroxytricosanoate were detected. The mixture of LCB and methyl glycoside was trimethylsilylated and analyzed by GC-MS as in the case of 1. 2-Amino-1,3,4-trihydroxy-octadecane and methyl glucopyranoside were detected.

Determination of Absolute Configuration of the Glucose Moieties of 3

In the same manner as described for 1, 3 was hydrolyzed, the sugar derivatives were analyzed by GC-MS, and d-glucose was detected.

Preparation of 6 and Partially Methylated Alditol Acetates from 6

The partially methylated alditol acetate was obtained from 6 (prepared from 3 as above) and analyzed by GC-MS in the same way as for those from 4. S-1 (derived from 6-linked hexopyranosyl) was detected.

Preparation and GC-MS Analysis of Acetates of Partially Methylated Sialic Acids from 6

The acetates were prepared from 6 and subjected to GC-MS as described for 4. S-2 (derived from terminal NeuGc), S-3 (derived from 8-linked NeuAc) and a minor component S-4, \( t_\beta \) [min] = 27.2, \( m/z \) = 159, 348, 356, 406 [methyl N-glycolyl-2,4,7,9,11-penta-O-methylneuramate (derived from 8-linked NeuGc)] were detected.

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