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Analysis and Comparison of Glucocerebroside Species from Three Edible Sea Cucumbers Using Liquid Chromatography−Ion Trap−Time-of-Flight Mass Spectrometry

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ABSTRACT: Sphingolipids constitute a highly diverse and complex class of molecules and exhibit important physiological functions. Glucocerebrosides are anticipated to play a positive role in human nutrition. In this study, complicated glucocerebrosides from three specimens of edible sea cucumbers, specifically, *Acaudina molpadioides*, *Cucumaria frondosa*, and *Apostichopus japonicus*, were rapidly identified using liquid chromatography−ion trap−time-of-flight mass spectrometry (LCMS-IT-TOF), which is a powerful analysis tool. $[M + H]^+$, $[M + Na]^+$, and $[M + H - H_2O]^+$ in positive electrospray ionization (ESI) mode were used for MS/MS analysis to obtain product ion spectra. Various long-chain bases of glucocerebrosides were found in these sea cucumbers. Two of the most common long-chain bases were 2-amino-1,3-dihydroxy-4-heptadecene (d17:1) and 4,8-sphingadienine (d18:2), which were acylated to form saturated and monounsaturated nonhydroxy and monohydroxy fatty acids with 18−25 carbon atoms. The glucocerebroside molecular species were the most complicated in the sea cucumber *C. frondosa* and were the simplest in the sea cucumber *A. molpadioides*.

KEYWORDS: LCMS-IT-TOF, sphingolipids, glucocerebrosides, sea cucumbers, long-chain bases

INTRODUCTION

Sphingolipids, for example, cerebrosides, are a complex and essential class of lipids that are found in plants, mammals, fungi, and marine invertebrates, which are ubiquitous constituents of food. The sphingolipids comprise a complex range of lipids in which fatty acids are linked via amide bonds to a long-chain base or a sphingoid base. Sphingolipids are highly bioactive compounds that not only serve as components of cellular membranes but also participate in diverse cellular processes.[1,2] Furthermore, some dietary sphingolipids have been reported to exhibit antiulcerogenic, antihepatotoxic, antitumor, and immunostimulatory activities.[3,4]

Cerebrosides, including glucocerebrosides (glucosylceramides) and galactocerebrosides (galactosylceramides), are usually considered the principal glycosphingolipids in plants[5] and are found at low levels in animal tissues.[6] Variations in chain length and the degree of saturation and/or hydroxylation of the long-chain base and the fatty acid lead to the extensive variation of cerebrosides. Recently, dietary glucocerebrosides have gained attention because of their potential to protect the intestine from inflammation and cancer[7,8] and to inhibit the growth of cancer cells.[9,10] A long-chain base, such as sphingosine (d18:1), is the simplest possible functional sphingolipid.[11] Our previous findings indicated that long-chain bases prepared from dietary sources can induce apoptosis in colon cancer cell lines[12−14] and that dietary glucocerebrosides from plants and yeast could prevent the formation of aberrant crypt foci in 1,2-dimethylhydrazine-treated mice.[15] Understanding the nature and composition of the sphingoid bases and fatty acids that constitute dietary glucocerebrosides is important for evaluation of the possible biological effects of these compounds. Therefore, structural elucidation of the intact, not hydrolyzed, glucocerebroside species is necessary.

There are fragmented studies concerning the structural analysis and characterization of sphingolipids in plant tissues. The structures of the long-chain bases and fatty acids of sphingolipid molecules in those studies were mainly determined by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), or gas chromatography-tandem mass spectrometry (GC-MS) following hydrolysis and derivatization.[16,17] Liquid chromatography−mass spectrometry (LC-MS) with electrospray ionization is a powerful tool for the identification and detection of the chemical structures of sphingolipids, including known and unknown molecular species.[18−20] However, recent mass spectrometric investigations of sphingolipids have mainly been used to analyze cell samples and rarely to analyze complex food samples.

Sea cucumbers have been used as a traditional tonic food in China and other Asian countries for thousands of years. In the oceans, there are more than 1000 varieties of sea cucumbers out of which only approximately 40 kinds of sea cucumber are edible. The major edible parts of sea cucumbers are the body walls, which are rich in many kinds of bioactive substances, such as collagen,[21] polysaccharides,[22] triterpene glycosides,[23] glucocerebrosides,[24,25] and gangliosides.[26] We previously demonstrated[27] that glucocerebrosides

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from the sea cucumber *Acaudina molpadioides* have activity against nonalcoholic fatty liver disease. Glucocerebrosides are important components of lipids derived from sea cucumbers.

However, analysis of the structure−activity relationships of glucocerebrosides and relative long-chain bases is restricted by the complexity of their structures. Generally, the structures of bioactive substances in marine organisms are distinct from those of terrestrial plants and animals. Therefore, it is necessary to research the structural characteristics of sea cucumber glucocerebrosides. Numerous studies have demonstrated that different species of sea cucumbers contain components with different structures with the different components being related to the bioactivities of ingested sea cucumber material.[22] In the present study, we selected three species of sea cucumbers with different commercial values and total lipid contents and isolated glucocerebrosides from them. *A. molpadioides* (Molpadiida, Caudinidae) is from the Zhejiang coast of southern China, which is located at 28° N (north latitude). *Cucumaria frondosa* (Dendrochirotida, Cucumariidae) is from the North Atlantic coast of Norway, situated above 60° N. *Apostichopus japonicus* (Aspidochirotida, Stichopodidae) is from the Shandong coast of northern China, which is located at 38° N and has become the most important cultured aquatic species in recent years. The glucocerebroside components of these three sea cucumbers have not been investigated previously. Determination of their diverse structures, including the type of sphingoid backbone present, is critical for understanding the functional and nutritional significance of dietary glucocerebrosides from sea cucumbers. Thus, the objective of this study was to identify the structures of glucocerebrosides from three different species of sea cucumbers by liquid chromatography−ion trap−time-of-flight mass spectrometry (LCMS-IT-TOF).

MATERIALS AND METHODS

Materials. Dried edible sea cucumber material (*A. molpadioides*, *C. frondosa*, and *A. japonicus*) was purchased from the Zhou-Shan Fishery Company (Zhejiang Province, China). The specimens were identified by Professor Liao Yulin from the Chinese Academy of Sciences Institute of Oceanography (Qingdao, China). All solvents used for sample extraction were of reagent grade, and those for chromatography and MS experiments were purchased from Merck (Darmstadt, Germany) and were gradient grade. Silica gel (0.061−0.100 mm) was obtained from Merck. For use as cerebroside standards, β-glucocerebroside was purchased from Avanti Polar Lipids (Alabaster, AL), and β-galactocerebroside from bovine brain was purchased from Sigma Chemical (St. Louis, MO) with purity greater than 99%. Water for HPLC analysis was purified by a Milli-Q Elix-5 system (Millipore, Bedford, MA).

Sample Preparation. Briefly, the sea cucumber body walls were minced and homogenized. The total lipid from the dried sea cucumber powder (approximately 50 g) was extracted four times with 150 mL of $CHCl_3$/MeOH (2:1, v/v). The combined extracts were concentrated in vacuo to give an aqueous solution (50 mL) that was subsequently extracted three times with 150 mL of EtOAc/*n*-BuOH (2/1, v/v). The EtOAc/*n*-BuOH phase was concentrated in vacuo to yield a residue, which was washed with cold acetone to produce an acetone-insoluble fraction (less polar lipid fraction). The less polar lipid fraction was chromatographically separated on silica gel (solvent $CHCl_3$/MeOH/H_2O, 95/5/0 to 5/5/1, v/v/v) to give five fractions. Successive column chromatography of fractions 2 and 3 (silica gel, solvent $CHCl_3$/MeOH, 25/1 to 10/1, v/v) yielded the glucocerebroside-containing fractions. Next, the glucocerebroside-containing fractions were evaporated to dryness using a rotary evaporator. The dried crude glucocerebrosides were stored in the dark at −20 °C until analysis.

GC-MS Analysis of Glycosides. Cerebroside (2.0 mg) was heated with 10% HCl in MeOH (1 mL) at 70 °C for 18 h. The reaction mixture was subsequently extracted with *n*-hexane, and the MeOH layer was neutralized with Ag_2CO_3 followed by filtration. The filtrate was concentrated in vacuo to give a mixture of long-chain bases and methyl glycoside. The mixture was heated with 1-(trimethylsilyl) imidazole-pyridine (1:1) for 15 min at 70 °C, and the reaction mixture (TMS ethers) was subjected to GC-MS (EI mode; ionizing potential, 70 eV; carrier gas, He) using a 5973i-6890N instrument (Agilent, Santa Clara CA) equipped with an HP-5MS fused silica column (30 m × 0.25 mm i.d., 0.25 μm, J&W, Agilent Technologies) (column temperature, 180−250 °C; rate of temperature increase, 5 °C/min).

LCMS-IT-TOF Analyses. A Prominence HPLC system coupled with a LCMS-IT-TOF system equipped with an electrospray ionization (ESI) interface (Shimadzu, Kyoto, Japan) was used. One milligram of the dried crude glucocerebroside from a sea cucumber specimen was resuspended in 2 mL of methanol. Following centrifugation (at 10000 rpm, 10 min and 4 °C), an aliquot of 5 μL was injected into the LCMS-IT-TOF system. The HPLC system consisted of an LC-20AB binary pump, an SIL-20AC autosampler, a CTO-20AC column oven and an SPD-M20A PDA. Chromatographic separation of the analytes was achieved using a 50 mm × 2.0 mm i.d., 3 μm, TSK gel ODS-100Z column (Tosoh, Tokyo, Japan), and the compounds were eluted with acetonitrile/water (95/5, v/v) at a flow rate of 0.2 mL/min. The column temperature was set at 25 °C. Mass detection was carried out using a hybrid Shimadzu ion trap-time-of-flight mass spectrometer (IT-TOF). The IT-TOF mass operation parameters were set as follows: positive ion electrospray, nebulizing gas (N_2) flow rate of 1.5 L/min, drying gas (N_2) pressure of 0.1 MPa, applied probe voltage of 4.50 kV, curved desolvation line (CDL) voltage set at constant mode (optimized by autotuning), CDL temperature of 200 °C, and a block heater temperature of 200 °C. Mass spectrometry was conducted in the full scan and automatic multiple stage fragmentation scan modes over a range of m/z 650−900 for MS and m/z 200−300 for the MS^2 scan. The ion accumulation time was set at 100 ms. The collision-induced dissociation (CID) parameters were set as follows: The energy was 100%, and the collision gas (Ar) was 100%. Data acquisition and analysis were performed with LC solution 3.0 (Shimadzu, Kyoto, Japan).

For structural analysis of glucocerebrosides, $[M + H]^+$, $[M + Na]^+$, and $[M + H - 18]^+$ (loss of water) in ESI positive scan mode were used for MS/MS analysis to obtain the product ions. The typical signals that are characteristic of sphingoid base moieties were observed by auto MS/MS detection mode in this system. The structures and characteristic product ions of diverse sphingoid bases contained in glucocerebrosides are given in Figure 1 [d16:1, d16:2, 2-amino-1,3-dihydroxy-4-heptadecene (d17:1), t17:1, d19:1, d20:1, and d20:2] and in our previous study [d18:1, 4,8-sphingadienine (d18:2), 2-amino-1,3-dihydroxy-4,8, 10-octatriene (d18:3), 9-methyl-4,8-sphingadienine (d19:2), and 2-amino-1,3-dihydroxy-9-methyl-4,8,10- octatriene (d19:3)].[28] Paired ions of these structurally specific sphingoid bases and their precursor ions were used for the identification of glucocerebroside molecular species.

RESULTS AND DISCUSSION

Molecular Species Investigation of Cerebrosides in Sea Cucumbers. For LCMS-IT-TOF analysis, cerebrosides should be purified from the crude lipid extract to remove glycerolipids using silica gel column chromatography. The results of the GC-MS analysis of the methyl glycoside from β-galactocerebroside were t_R[min] = 5.16 and 5.56 (methyl α- and β-galactocerebroside), and the results of the analysis of β-glucocerebroside and the cerebrosides from the three sea cucumbers were t_R[min] = 5.84

and 6.06 (methyl α- and β-glucopyranoside). These results showed that the cerebrosides in the sea cucumbers *A. molpadioides*, *C. frondosa*, and *A. japonicus* were glucocerebrosides. The ratios of methyl α-glycoside to β-glycoside all were approximately 7:3. It is well-known that the sugar moiety of glucocerebrosides in sea cucumbers is predominantly β-glucose.[24,25] We speculated that isomerization occurred during the heating hydrolysis process of GC-MS analysis, resulting in the transformation of some β-type glycosidic bonds into α-type bonds. The patterns of chromatograms obtained using the present HPLC conditions were similar among glucocerebrosides from different sea cucumber species. In the positive full scan mode, $[M + Na]^+$, $[M + H]^+$, and $[M + H - H_2O]^+$ were the predominant signals in each peak. Glucocerebrosides containing various long-chain bases were found in these sea cucumber specimens. Two long-chain bases, specifically, d17:1 and d18:2, were the most common and were acylated to from saturated and monounsaturated nonhydroxy and monohydroxy fatty acids with 18−25 carbon atoms.

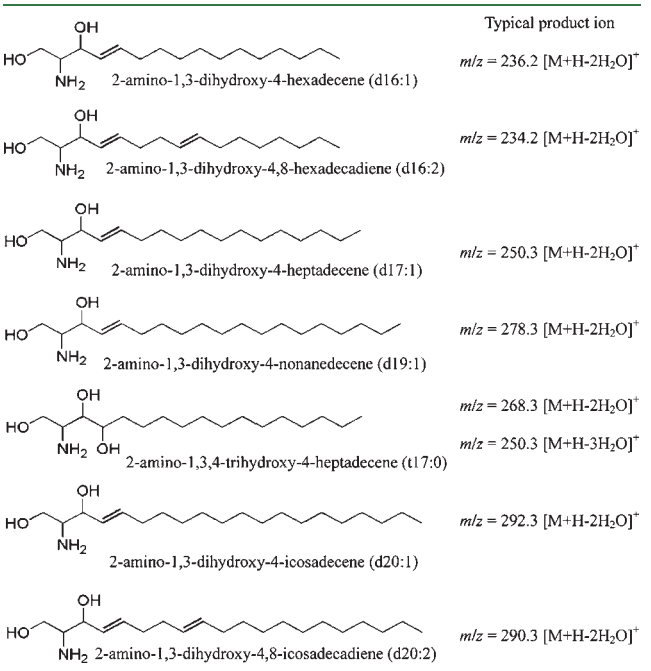

Figure 1. Partial structures and characteristic product ions of diverse sphingoid bases contained in sea cucumber glucocerebrosides.

Figure 2 shows the total ion and selected ion chromatograms of glucocerebrosides prepared from the sea cucumber *A. molpadioides*. The transition of the precursor ions $[M + Na]^+$, $[M + H]^+$, and $[M + H - H_2O]^+$ to the product ions of the sphingoid bases was used for the identification of glucocerebroside molecular species (Figure 3). Mass measurements of 12 molecules that were identified using pairs of specific product ions of sphingoid bases and their precursor ions are depicted in Table 1. The molecular species profile of *A. molpadioides* glucocerebrosides is the simplest among these three sea cucumbers, and the molecular mass region was from m/z 714.6 to m/z 814.6 ($[M + H]^+$). The most predominant long-chain base of *A. molpadioides* glucocerebroside was d17:1. Glucocerebroside molecules containing d19:2 were identified. Nonhydroxy and monohydroxy saturated fatty acids containing 18−24 carbon atoms, including odd-numbered fatty acids (C19:0h, C23:0h, C23:0, and C25:0), were detected in *A. molpadioides* glucocerebrosides. According to the long-chain base subunit structure, these 12 molecules were suspected to be tentative new compounds based on a SciFinder search.

The molecular species profile of *C. frondosa* glucocerebrosides is more complicated than those of *A. molpadioides* and *A. japonicus*. As described in Table 2, a number of glucocerebroside species (52 molecular species) were clearly identified. For *C. frondosa*, their molecular mass distribution was from m/z 714.6 to m/z 854.7 ($[M + H]^+$). The total ions chromatograms of glucocerebrosides from *C. frondosa* are shown in Figure 4. The major long-chain bases of glucocerebrosides in *C. frondosa* were d17:1, d18:2, d18:1, and d19:1. Glucocerebrosides molecules consisting of d20:1 and d20:2 were also detected in this sample. Glucocerebrosides consisting of t17:0 acylated to C20:1h (peak no. 25 in Table 2) was identified. Glucocerebroside molecules containing different monounsaturated fatty acids were identified (C18:1, C22:1 C23:1, C24:1, C20:1h, C21:1h, C23:1h, and C24:1h). In addition, using paired ions of product fragments, some glucocerebroside isomers that could not be separated were identified in *C. frondosa* as shown in Table 2. For example, glucocerebroside isomers were identified as d19:1-C22:0, d18:1-C23:0, and d17:1-C24:0 using product ions with m/z values of 278.3, 264.3, and 250.3

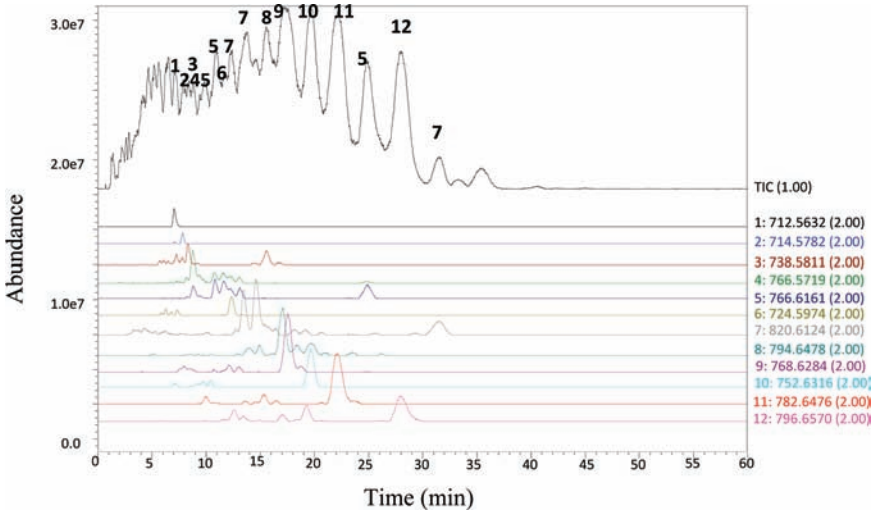

Figure 2. Total ion and selected ion chromatograms of glucocerebrosides from the sea cucumber *A. molpadioides*.

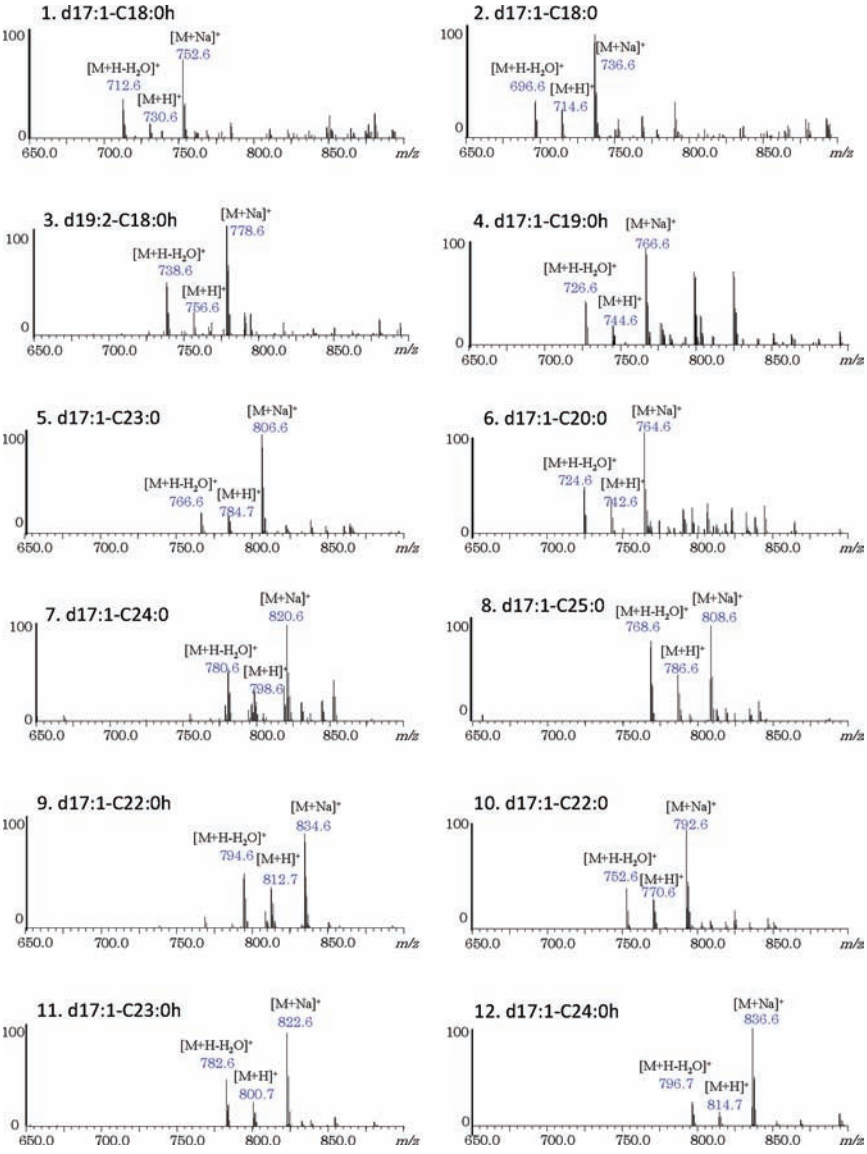

Figure 3. Mass spectra of peak components (peak nos. 1−12) of glucocerebrosides from the sea cucumber *A. molpadioides*.

Table 1. Detection and Identification of Glucocerebroside Molecular Species from the Sea Cucumber *A. molpadioides* Using LCMS-IT-TOF Analysis

peak no.	retention time (min)	precursor ion $[M + H]^+$ (m/z)	formula (M)	product ion (m/z)	molecular species
1	7.09	730.6	$C_{41}H_{80}NO_9$	250.3	d17:1-C18:0h[a]
2	7.93	714.6	$C_{41}H_{80}NO_8$	250.3	d17:1-C18:0[a]
3	8.49	756.6	$C_{43}H_{82}NO_9$	276.3	d19:2-C18:0h[a]
4	8.80	744.6	$C_{42}H_{82}NO_9$	250.3	d17:1-C19:0h[a]
5	11.58, 24.90	784.6	$C_{46}H_{90}NO_8$	250.3	d17:1-C23:0[a]
6	12.36	742.6	$C_{43}H_{84}NO_8$	250.3	d17:1-C20:0[a]
7	13.42	798.6	$C_{47}H_{92}NO_8$	250.3	d17:1-C24:0[a]
8	16.66	812.7	$C_{48}H_{94}NO_8$	250.3	d17:1-C25:0[a]
9	17.26	786.6	$C_{45}H_{88}NO_9$	250.3	d17:1-C22:0h[a]
10	19.49	770.6	$C_{45}H_{88}NO_8$	250.3	d17:1-C22:0[a]
11	21.70	800.6	$C_{46}H_{90}NO_9$	250.3	d17:1-C23:0h[a]
12	27.59	814.7	$C_{47}H_{92}NO_9$	250.3	d17:1-C24:0h[a]

[a] Tentative new compound.

Table 2. Detection and Identification of Glucocerebroside Molecular Species from the Sea Cucumber *C. frondosa* Using LCMS-IT-TOF Analysis

peak no.	retention time (min)	precursor ion $[M + H]^+$ (m/z)	formula (M)	product ion (m/z)	molecular species
1	6.02	724.6	$C_{42}H_{77}NO_8$	260.2	d18:3-C18:0[a] a
2	6.13	724.6	$C_{42}H_{77}NO_8$	262.3	d18:2-C18:1 a
3	6.86	726.6	$C_{42}H_{79}NO_8$	262.3	d18:2-C18:0
4	7.28	738.6	$C_{43}H_{79}NO_8$	274.2	d19:3-C18:0[a]
5	7.63	714.6	$C_{41}H_{79}NO_8$	250.2	d17:1-C18:0[a]
6	8.63	808.6	$C_{48}H_{89}NO_8$	260.2	d18:3-C24:0[a] b
7	9.22	796.6	$C_{47}H_{89}NO_8$	262.3	d18:2-C23:0[a]c
8	10.78	780.6	$C_{46}H_{85}NO_8$	260.2	d18:3-C22:0[a]
9	11.10	784.6	$C_{46}H_{89}NO_8$	250.3	d17:1-C23:0[a]
10	11.30	784.6	$C_{45}H_{85}NO_9$	262.3	d18:2-C21:0h[a]
11	11.49	810.6	$C_{47}H_{87}NO_9$	262.3	d18:2-C23:1h[a] d
12	12.04	742.6	$C_{43}H_{83}NO_8$	278.3	d19:1-C18:0[a]
				250.3	d17:1-C20:0[a]
13	12.45	822.6	$C_{49}H_{91}NO_8$	260.2	d18:3-C25:0[a]
14	13.27	794.6	$C_{47}H_{87}NO_8$	262.3	d18:2-C23:1[a]
15	13.85	798.6	$C_{47}H_{91}NO_8$	264.3	d18:1-C23:0 e
				250.3	d17:1-C24:0[a] e
16	14.36−15.49	824.6	$C_{48}H_{89}NO_9$	262.2	d18:2-C24:1h
17	14.92	782.6	$C_{46}H_{87}NO_8$	250.2	d17:1-C23:1[a] f
18	15.60	798.6	$C_{46}H_{87}NO_9$	262.2	d18:2-C22:0h
19	15.77	782.6	$C_{46}H_{87}NO_8$	264.3	d18:1-C22:1 f
				250.3	d17:1-C23:1[a] f
20	16.02−17.52	812.7	$C_{48}H_{93}NO_8$	250.3	d17:1-C25:0[a]
21	16.98	786.6	$C_{45}H_{87}NO_9$	262.3	d18:2-C21:1h[a]
22	17.08	808.6	$C_{48}H_{89}NO_8$	262.3	d18:2-C24:1 b
23	17.10	782.6	$C_{46}H_{87}NO_8$	262.3	d18:2-C22:0 f
24	18.19	796.7	$C_{47}H_{89}NO_8$	264.3	d18:1-C23:1[a] c
				250.3	d17:1-C24:1[a] c
25	18.53	770.6	$C_{43}H_{79}NO_{10}$	264.3	d18:1-C21:0
			$C_{41}H_{71}NO_{12}$	268, 250.3	t17:0-C20:1h[a]
26	18.88	770.6	$C_{44}H_{83}NO_9$	262.2	d18:2-C20:0h
27	19.37	812.7	$C_{47}H_{89}NO_9$	262.3	d18:2-C23:0h
28	19.85−20.63	826.7	$C_{49}H_{95}NO_8$	278.3	d19:1-C24:0[a] g
				264.3	d18:1-C25:0 g
29	20.87	800.6	$C_{46}H_{89}NO_9$	264.3	d18:1-C22:0h
				250.3	d17:1-C23:0h
30	22.62	810.7	$C_{47}H_{87}NO_9$	290.3	d20:2-C21:1h[a] d
31	23.11	784.6	$C_{46}H_{89}NO_8$	264.3	d18:1-C22:0 h
				250.3	d17:1-C23:0[a] h
32	23.58	784.7	$C_{45}H_{85}NO_9$	262.2	d18:2-C21:0h[a]
33	24.12	826.7	$C_{48}H_{91}NO_9$	262.3	d18:2-C24:0h
34	25.02	840.7	$C_{50}H_{97}NO_8$	278.3	d19:1-C25:0[a]
35	26.20	814.7	$C_{47}H_{91}NO_9$	264.3	d18:1-C23:0h
				250.3	d17:1-C24:0h
36	28.44	824.7	$C_{49}H_{93}NO_8$	278.3	d19:1-C24:1[a]
37	29.53	798.7	$C_{47}H_{91}NO_8$	278.3	d19:1-C22:0[a] e
				264.3	d18:1-C23:0 e
				250.3	d17:1-C24:0[a] e
38	31.68	854.7	$C_{51}H_{99}NO_8$	292.3	d20:1-C25:0[a]
39	32.93	828.7	$C_{48}H_{93}NO_9$	278.3	d19:1-C23:0h
				264.3	d18:1-C24:0h
40	41.45	842.7	$C_{49}H_{95}NO_9$	278.3	d19:1-C24:0h[a]

[a] Tentative new compound. Letters a−h indicate isomers.

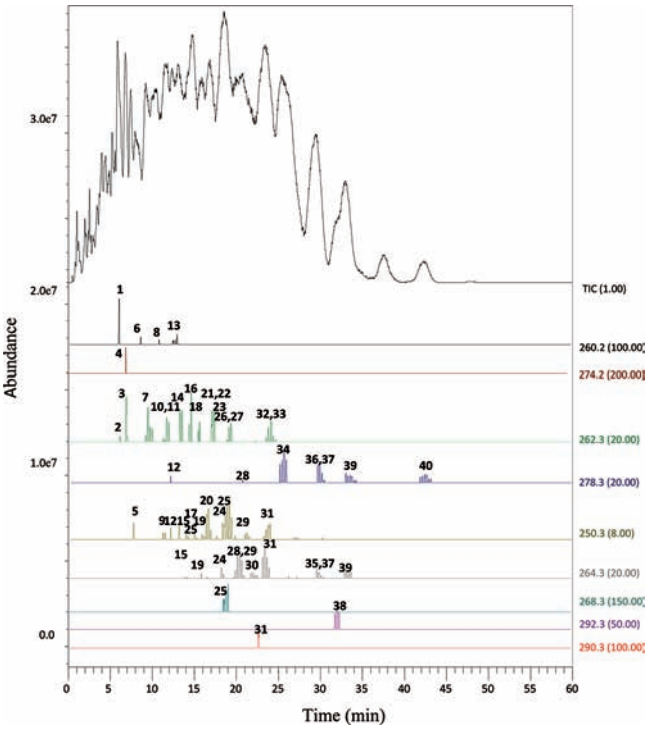

Figure 4. Total ion and nine selected product ion chromatograms of glucocerebrosides from the sea cucumber *C. frondosa*.

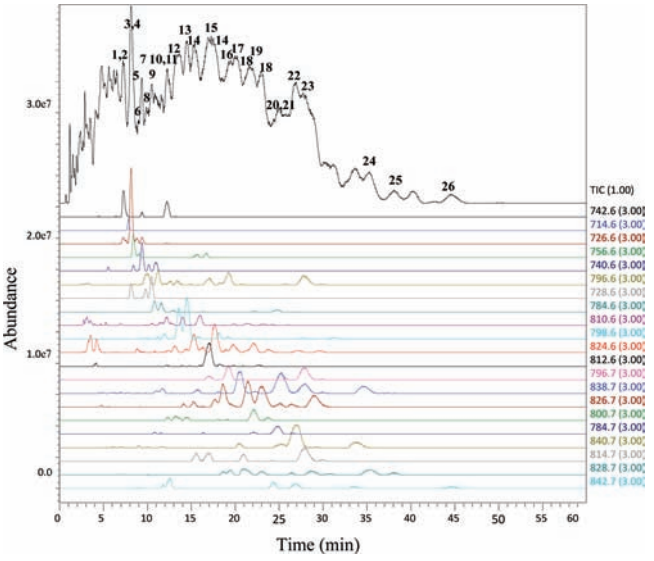

Figure 5. Total ion chromatograms of glucocerebrosides from the sea cucumber *A. japonicus*.

(peak no. 37, $C_{47}H_{91}NO_8$), respectively. Among these 40 peaks, there are 31 tentative new compounds and 22 glucocerebroside isomers.

The total ion chromatogram and 21 selected ion chromatograms of glucocerebrosides from *A. japonicus* are shown in Figure 5. For *A. japonicus*, 26 glucocerebroside species, including nine isomers, were clearly identified (Table 3). The molecular mass distribution ranged from m/z 714.6 to m/z 838.7 ($[M + H]^+$). The bases d17:1, d18:2, d18:1, and d19:2 were the major long-chain bases present, and glucocerebroside molecules consisting of d16:1 and d16:2 were also detected in *A. japonicus*. In our previous report, the long-chain bases of the glucocerebrosides from the sea cucumber *Stichopus variegatus* (Japan) were identified as d17:1, d18:1, d18:2, d18:3, d19:1, d19:2, and d19:3, and the fatty acid moieties were mainly identified as nonhydroxy saturated fatty acids.[28] The glucocerebrosides of *A. japonicus* were similar to those of *Stichopus variegates* and to those of *Stichopus japonicus* (Japan) as identified using GC-MS and NMR by Kisa et al.[25]

Comparison of Glucocerebrosides from Three Sea Cucumbers and Other Dietary Sources. Sphingolipids in plants primarily contain d18:1[8], d18:2[4,8], and 4-hydroxy-8-sphingenine (t18:1[8]) as sphingoid bases. Sphingolipid Δ4-desaturase and sphingolipid Δ8-desaturase have been identified in plants.[29] It is known that the major sphingoid backbone in fungal sphingolipids is a unique 9-methyl branched sphingoid base (9-methyl-4,8-sphingadienine, d19:2).[6,30] Our previous investigation indicated that the molecular structures of rice glucocerebrosides are similar to those of maize, with the molecular species of the glucocerebrosides in rice consisting of d18:2 and t18:1 acylated to form hydroxy fatty acids with 18−26 carbon atoms. Glucocerebrosides containing d19:2 acylated to form hydroxy fatty acids with 14−24 carbon atoms were identified in maitake.[28] It has been reported that the long-chain bases in marine invertebrates are notably different from those in mammals and plants.[14,31] Triene bases conjugated to dienes, such as 2-amino-4,8,10-octatriene-1,3-diol (d18:3) and 2-amino-9-methyl-4,8,10-octatriene-1,3-diol (d19:3), have been identified in marine invertebrates, including edible squid[31] and starfish.[32] In addition, we have reported that sea cucumber glucocerebrosides contain long-chain bases with three double bonds.[30]

Vesper et al.[4] have summarized the amount of sphingolipids in foods, including dairy, meat, and egg products, and estimated that sphingolipid consumption was 300−400 mg/day in the United States. We previously reported that the estimated daily intake of plant-derived glucocerebrosides in Japan was 50 mg based on the glucocerebroside contents of foodstuffs,[14,33] and we have investigated the digestion and absorption of plant-derived sphingolipids.[12] Our findings indicate that the metabolic fate of plant-derived long-chain bases, such as d18:2, within enterocytes differs from that of d18:1. Sphingoid bases, except for d18:1, appear to be transported out of cells across the apical membranes of enterocytes by P-glycoprotein after absorption, and consequently, their intestinal uptake is quite poor.[34] Thus, the determination of sphingolipid structures, including variations in the sphingoid backbone, from dietary sources is important in understanding the functional and nutritional significance of these compounds.

From the present results, we can conclude that structural information and mass data for glucocerebrosides can be obtained in a single experiment using LCMS-IT-TOF. Fragmentation data successfully led to the correct assignment of glucocerebrosides with similar chemical formulas. The complexity of the molecular species profile of glucocerebrosides from three edible sea cucumbers, *A. molpadioides*, *C. frondosa*, and *A. japonicus*, is evidently different. Many novel glucocerebroside structures were found, although the locations of the double bonds in the long-chain bases and fatty acids chains cannot be identified by this method. To our knowledge, only common C_{18}-sphingoid bases are found in plants, mammals, and fungi, although long-chain bases of different chain lengths occur in minor amounts. Also, d17:1, a typical predominant sphingoid base in sea cucumber

Table 3. Detection and Identification of Glucocerebroside Molecular Species from the Sea Cucumber *A. japonicus* Using LCMS-IT-TOF Analysis

peak no.	retention time (min)	precursor ion $[M + H]^+$ (m/z)	formula (M)	product ion (m/z)	molecular species
1	7.22	742.6	$C_{42}H_{79}NO_9$	262.3	d18:2-C18:0h
2	7.88	714.6	$C_{41}H_{79}NO_8$	250.3	d17:1-C18:0[a]
3	8.06−8.29	726.6	$C_{42}H_{79}NO_8$	262.3	d18:2-C18:0
4	8.44	756.6	$C_{43}H_{81}NO_9$	276.3	d19:2-C18:0h
5	9.31	740.6	$C_{43}H_{82}NO_8$	276.3	d19:2-C18:0
6	9.77, 11.16	796.6	$C_{47}H_{89}NO_8$	262.2	d18:2-C23:0[a] a
7	10.04	796.6	$C_{47}H_{89}NO_8$	234.2	d16:2-C25:0[a] a
8	10.41	728.6	$C_{42}H_{81}NO_8$	264.3	d18:1-C18:0
9	10.81	784.6	$C_{46}H_{89}NO_8$	250.2	d17:1-C23:0[a] a
10	11.46−11.69	784.6	$C_{46}H_{89}NO_8$	236.2	d16:1-C24:0[a] b
11	12.10−12.17	810.6	$C_{48}H_{91}NO_8$	262.3	d18:2-C24:0
12	13.37−13.64	798.6	$C_{47}H_{91}NO_8$	250.3	d17:1-C24:0[a] b
13	14.13−14.88	798.6	$C_{47}H_{91}NO_8$	236.2	d16:1-C25:0[a] c
14	15.05−15.55, 18.00	824.7	$C_{49}H_{93}NO_8$	262.3	d18:2-C25:0[a]
15	16.64−17.39	812.6	$C_{48}H_{93}NO_8$	250.3	d17:1-C25:0[a]
16	19.04−19.60	796.7	$C_{47}H_{89}NO_8$	250.2	d17:1-C24:1[a]
17	20.44−20.72	838.7	$C_{50}H_{95}NO_8$	276.3	d19:2-C25:0[a]
18	21.15−21.68, 22.78−23.65	826.7	$C_{49}H_{95}NO_8$	264.3	d18:1-C25:0
19	21.85−22.42	800.7	$C_{46}H_{89}NO_9$	250.3	d17:1-C23:0h
20	24.68	784.7	$C_{46}H_{89}NO_8$	264.3	d18:1-C22:0 c
21	26.44−27.00	840.7	$C_{50}H_{97}NO_8$	278.3	d19:1-C25:0[a]
22	27.47−28.50	814.7	$C_{47}H_{91}NO_9$	250.3	d17:1-C24:0h
23	28.69−29.51	826.7	$C_{48}H_{91}NO_9$	262.3	d18:2-C24:0h[a]
24	35.51	828.7	$C_{48}H_{93}NO_9$	278.3	d19:1-C23:0h d
25	38.28	828.7	$C_{48}H_{93}NO_9$	264.3	d18:1-C24:0h d
26	44.41	842.7	$C_{49}H_{95}NO_9$	278.3	d19:1-C24:0h[a]

[a] Tentative new compound. Letters a−h indicate isomers.

glucocerebrosides, is not widely found in plants, mammals, or fungi. Further work may serve to determine if there is a close relationship between the geographical area of the habitat of the sea cucumbers and the molecular species of glucocerebrosides and long-chain bases present in these organisms.

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ABBREVIATIONS USED

LCMS-IT-TOF, liquid chromatography−ion trap−time-of-flight mass spectrometry; CDL, curved desolvation line; CID, collision-induced dissociation; d17:1, 2-amino-1,3-dihydroxy-4-heptadecene; d18:0, dihydrosphingosine; d18:1, sphingosine; d18:2, 4,8-sphingadienine; d18:3, 2-amino-1,3-dihydroxy-4,8,10-octatriene; t18:0, 4-hydroxysphinganine (phytosphingosine); t18:1, 4-hydroxy-8-sphingenine; d19:2, 9-methyl-4,8-sphingadienine; d19:3, 2-amino-1,3-dihydroxy-9-methyl-4,8,10- octatriene

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