Isolation and Structure of a Galactocerebroside Molecular Species from the Starfish *Culcita novaeguineae*[1)]

Masanori Inagaki, Tomohide Nakata, and Ryuichi Higuchi*

Faculty of Pharmaceutical Sciences, Kyushu University; 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan.
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A galactocerebroside molecular species, CNC-2, has been isolated from the less polar lipid fraction of a chloroform/methanol extract of the starfish *Culcita novaeguineae*. The structure of this galactocerebroside molecular species was determined on the basis of chemical and spectroscopic evidence. CNC-2 is a phytosphingosine-type galactocerebroside molecular species with nonhydroxylated and hydroxylated fatty acyl moieties. The isolation of a galactocerebroside from echinoderms is rare.

Key words glycosphingolipid; galactocerebroside; echinoderms; starfish; *Culcita novaeguineae*

In our continuing research on biologically active glycosphingolipids (GSLs) from echinoderms, a series of studies on the isolation and structural elucidation of the GSLs from starfish species have been performed in our laboratory.[2—21)] In continuation of previous studies of starfish species, the isolation and characterization of the cerebroside from the starfish *Culcita novaeguineae* (Manjyuuhitode in Japanese) are being conducted in the hope of discovering biologically active compounds from marine natural products. In this paper, the isolation and structure determination of a galactocerebroside molecular species from the whole bodies of *C. novaeguineae* are described.

The less polar lipid fraction, which was obtained from the chloroform/methanol extract of the whole bodies of *C. novaeguineae*, was subjected to repeated silica gel column chromatography to give a cerebroside molecular species, CNC-2, showing a single spot on silica gel thin-layer chromatography (TLC).

In the IR and positive-ion FAB mass spectra of CNC-2, strong hydroxy and amide absorptions and a series of molecular ion peaks were observed. Its ^{13}C-NMR spectra (Fig. 1, Table 1) exhibit the characteristic signals of a phytosphingosine-type β-galactocerebroside possessing mainly 2-hydroxy fatty acid. Therefore, CNC-2 is suggested to be a mixture of galactocerebrosides, namely molecular species. Its structure shown in Fig. 1 was characterized by comparison of its ^{13}C-NMR spectral data (Table 1) and optical rotation (+2.1°) with those of the known β-D-galactocerebroside (+3.6°) composed of (2*S*,3*S*,4*R*)-phytosphingosines and (2*R*)-2-hydroxy fatty acids obtained from the starfish *Stellaster equestris*,[12)] and on the basis of the results of its methanolysis, followed by the GC-MS analysis of the methanolysis products, fatty acid methyl ester (FAM), and long-chain base (LCB) (Experimental). The absolute configuration of its galactose moiety (D-form) was determined by the Hara method[22)] (Experimental).

Although the isolation of galactocerebroside from echinoderms is second to that of the starfish *Stellaster equestris*,[12)] the ceramide moieties of CNC-2 differ from the galactocerebroside of *S. equestris*. Since the existence of galactocerebroside in echinoderms is very rare, its isolation and characterization at this time are worth noting. Recently, the growth inhibitory activities of galactocerebrosides from a marine mollusk against cancer cell lines were reported.[23)] The biological activities of galactocerebrosides from echinoderms will be examined in the future.

Experimental

Optical rotation was measured with a Jasco Dip-370 digital polarimeter at 25 °C. IR spectrum was obtained on a Jasco FT/IR-410 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Jeol GX-270 spectrometer (270, 67.8 MHz). Positive-ion FAB-MS spectrum was acquired with a Jeol JMS-SX102 mass spectrometer (xenon atom beam; matrix, TEG). GC-MS was conducted with a Shimadzu QP-5050A [EI mode; ioniz-

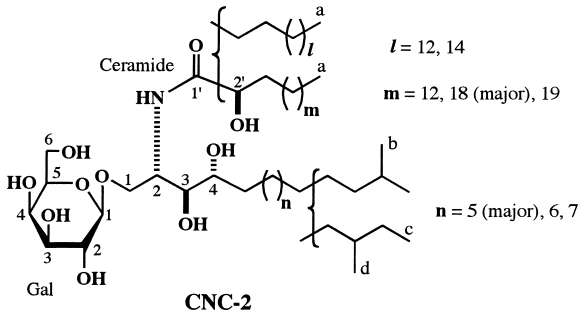

Fig. 1. Structure of CNC-2

Table 1. ^{13}C-NMR Spectral Data (δ Values) of CNC-2 in C_5D_5N

C		CNC-2
Ceramide		
1	(t)	70.5
2	(d)	51.7
3	(d)	75.9
4	(d)	72.6[e)]
1′	(s)	175.7
2′	(d)	72.4[e)]
CH_3[a)]	(q)	14.2
CH_3[b)]	(q)	22.7
CH_3[c)]	(q)	11.5
CH_3[d)]	(q)	19.3
Gal		
1	(d)	106.1
2	(d)	72.6[e)]
3	(d)	75.0
4	(d)	70.3
5	(d)	77.1
6	(t)	62.4

a)—*d*) Terminal methyl groups in the normal, iso and *ante*–iso type of side chain (see Fig. 1). *e*) Assignments may be interchanged.

* To whom correspondence should be addressed. e-mail: rhiguchi@ phar.kyushu-u.ac.jp

ing potential, 70 eV; separator and ion-source temperature 250 °C; column, TC-1701 (0.25 mm×30 m, GL Science Inc.); carrier gas, He].

Separation of CNC-2 Whole bodies of the starfish *Culcita novaeguineae* (wet weight 5.6 kg, collected at Kerama, Okinawa Prefecture, Japan) were chopped and extracted with $CHCl_3$/MeOH [1 : 2, 6 l and 1 : 1, 6 l (two times)]. The combined extracts were concentrated *in vacuo* to give an extractive, which was partitioned between H_2O (1.4 l) and AcOEt/*n*-BuOH (3 : 1, 1 l) (three times). The organic layer was concentrated *in vacuo*, and the residue (less polar lipid fraction, 38.6 g) was chromatographed on silica gel (solvent $CHCl_3$–MeOH–H_2O, 9 : 1.5 : 0.05) to give five fractions. Successive column chromatography of fraction 3 (silica gel, solvent $CHCl_3$–MeOH–H_2O, 9 : 1 : 0.05) afforded seven fractions. A quarter of the crude cerebroside fraction (fraction 4 in the seven fractions) was purified by silica gel column chromatography (solvent $CHCl_3$–MeOH–H_2O, 9 : 0.9 : 0.05) to give five fractions. Fraction 4 (13 mg) of the five fractions was CNC-2 [(*Rf*=0.26) (silica gel TLC, solvent $CHCl_3$–MeOH–H_2O, 9 : 0.9 : 0.05)].

CNC-2: Amorphous powder, $[\alpha]_D$ +2.1° (*c*=1.0, pyridine). IR (KBr) cm^{-1}: 3411 (OH), 1637, 1534 (amide). Positive-ion FAB-MS *m/z* : 750—850 $[M+H]^+$ series, 790 $[M+H]^+$ of the major component. ^{1}H-NMR (C_5D_5N) δ: 0.85 (9H, m, terminal methyl groups), 4.86 (1H, d, *J*=7.5 Hz, galactose H-1), 8.53 (1H, d, *J*=9.0 Hz, NH). ^{13}C-NMR: See Table 1.

Methanolysis of CNC-2 CNC-2 (2 mg) was heated with 5% HCl in MeOH (1.6 ml) at 80 °C for 12 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 concentrated by N_2 stream to give a mixture of LCB and methyl glycoside.

GC-MS Analysis of FAM from CNC-2 A FAM mixture from CNC-2 was subjected to GC-MS [column temperature: 180—250 °C (rate of temperature increase 5 °C/min)]. The results were as follows: methyl hexadecanoate, t_R [min] (ratio of peak areas)=7.8 (10.6), *m/z*: 270 (M^+), 227 $(M-43)^+$; methyl 2-hydroxyhexadecanoate, t_R=10.4 (12.9), *m/z*: 286 (M^+), 227 $(M-59)^+$; methyl octadecanoate, t_R=10.9 (4.3), *m/z*: 298 (M^+), 255 $(M-43)^+$; methyl 2-hydroxydocosanoate, t_R=21.7 (54.4), *m/z*: 370 (M^+), 311 $(M-59)^+$; methyl 2-hydroxytricosanoate, t_R=24.7 (17.8), *m/z*: 384 (M^+), 325 $(M-59)^+$.

GC-MS Analysis of TMS Ethers of LCB from CNC-2 A mixture of LCB and methyl glycoside from CNC-2 was heated with 1-(trimethylsilyl) imidazole–pyridine (1 : 1) for 20 min at 80 °C, and the reaction mixture (TMS ethers) was analyzed by GC-MS [column temperature: 180—250 °C (rate of temperature increase 5 °C/min)]. The results were as follows: 2-amino-1,3,4-trihydroxy-hexadecane, t_R [min] (ratio of peak areas)=14.8, 15.7 (62.1), *m/z*: 312 $(M-193)^+$, 271 $(M-234)^+$, 132; 2-amino-1,3,4-trihydroxy-heptadecane, t_R=16.3, 17.3 (28.6), *m/z*: 326 $(M-193)^+$, 285 $(M-234)^+$, 132; 2-amino-1,3,4-trihydroxy-octadecane, t_R=17.5 (9.3), *m/z*: 340 $(M-193)^+$, 299 $(M-234)^+$, 132.

Analysis of TMS Ethers of Methyl Glycoside from CNC-2 The mixture of TMS ethers of LCB and methyl glycoside was analyzed by GC-MS [column temperature: 100—250 °C (rate of temperature increase 5 °C/min)]: t_R [min]=21.1 and 22.0 (methyl galactopyranosides).

Determination of Absolute Configuration of Galactose Moiety of CNC-2 (Hara Method) CNC-2 (1 mg) was heated with 2 N HCl (1 ml) at 100 °C for 24 h in a sealed vial. The reaction mixture was then extracted with *n*-hexane, and the acidic aqueous phase was concentrated by N_2 stream. The residue (sugar fraction) was heated with L-cysteine methyl ester hydrochloride (1 mg) and pyridine (0.05 ml) at 60 °C for 1 h. Then, 0.05 ml of 1-(trimethylsilyl) imidazole was added, and the mixture was heated at 60 °C for a further 20 min to yield a trimethylsilyl ether of the methyl (4*R*)-thiazolidine-4-carboxylate derivative. The derivative was analyzed by GC-MS [column temperature: 180—250 °C (rate of temperature increase 2.5 °C/min)]; t_R=24.6 min (derivative of D-galactose, 24.6 min; L-galactose, 25.5 min).

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