Two New Ceramides from the Marine Sponge *Ircinia fasciculata*

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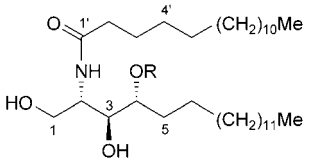
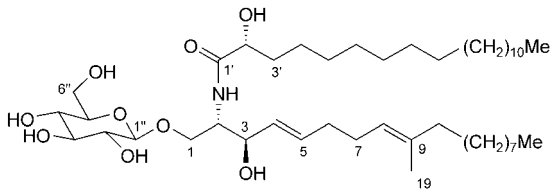
A new 4-sulfated ceramide, ircisulfamide (= *N*-[(1*S**,2*S**,3*R**)-2-hydroxy-1-(hydroxymethyl)-3-(sulfooxy)-heptadecyl]hexadecanamide; **1**), and a new glycosphingolipid, ircicerebroside (= (2*R**)-*N*-{(1*S**,2*R**,3*E*,7*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxy-8-methylheptadeca-3,7-dienyl}-2-hydroxyeicosanamide; **2**), were isolated from the aqueous EtOH extract of the marine sponge *Ircinia fasciculata* (PALLAS). The structures of the new compounds were elucidated on the basis of spectroscopic analysis and by means of chemical methods.

Introduction. – Different sphingolipids such as ceramide, sphingosine, and sphingosine-1-phosphate have been isolated from marine vertebrates, invertebrates, algae, and fungi *etc.* [1–3]. These compounds have received increasing attention in the last years as some of their derivatives act as endogenous cell-function modulators and secondary messengers [4]. Sphingolipids have been shown to be potent and specific inhibitors of protein kinase C [5], Na- and K-ATPase, and calmodulin kinase, and to activate phospholipase C, phospholipase D, casein kinase II, tyrosine kinase, and DG kinase [6]. In addition, sphingolipids can also induce Ca^{2+} release [7], proliferate cells [8], protect human keratinocytes from apoptosis [9], suppress colon carcinogenesis in rats [10], and enhance apoptosis of radiation-resistant prostate cancer cells [11].

In continuation of our search for potent bioactive secondary metabolites from the marine invertebrates [12–14], a new 4-sulfated ceramide, ircisulfamide (**1**), and a new glycosphingolipid, ircicerebroside (**2**), were isolated from the aqueous EtOH extract of the marine sponge *Ircinia fasciculata*. The structures of compounds **1** and **2** were elucidated on the basis of spectroscopic analysis and chemical methods.



1 R = SO_3H
1a R = H



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Results and Discussion. – Compound **1** was isolated as a colorless, amorphous, optically active powder, with $[\alpha]_D^{20} = +16.7$ ($c = 0.24$, $CHCl_3$). From HR-FAB-MS experiments, the molecular formula $C_{34}H_{69}NO_7S$ was deduced, corresponding to three degrees of unsaturation. The IR absorption at 1234 cm^{-1} was attributed to a sulfate functionality, as further confirmed by elemental analysis. The IR absorption band at 3383 cm^{-1} indicated OH and NH groups, and the typical absorptions at 1645 and 1545 cm^{-1} suggested that **1** is a secondary amide, which was supported by NMR experiments (δ(H) 5.97 (*d*, $J = 6.0$ Hz, NH); δ(C) 51.9 (N–C); δ(C) 174.0 (C=O)) (see *Table 1*). The ^{1}H-NMR spectrum of **1** revealed the presence of two Me groups at δ(H) 0.88 (2*t*), an N–CH function at δ(H) 4.38, two O–CH groups at δ(H) 3.97 and 3.70, an O–CH_2 moiety at δ(H) 4.18 and 3.51, and a series of overlapped CH_2 resonances at δ(H) 1.20–1.34 (*Table 1*). The ^{13}C-NMR spectrum revealed the presence of two oxygenated C-atoms at δ(C) 70.4 (*t*) and 75.2 (*d*). Besides, a low-field signal at δ(C) 85.2 (*d*) suggested the presence of a sulfate group. All the spectroscopic data confirmed that **1** was a ceramide derivative.

Table 1. *NMR Data for Ircisulfamide* (**1**). In $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering.

	^{13}C	^{1}H	^{1}H,^{1}H-COSY
H_a–C(1)	70.4 (*t*)	4.18 (*dd*, $J = 9.0, 7.0$)	H–C(2)
H_b–C(1)	–	3.51 (*dd*, $J = 9.0, 7.0$)	–
H–C(2)	51.9 (*d*)	4.38 (*m*)	H–C(1), H–C(3), NH
H–C(3)	75.2 (*d*)	3.97 (*dd*, $J = 6.0, 4.0$)	H–C(2), H–C(4)
H–C(4)	85.2 (*d*)	3.70 (*dd*, $J = 11.5, 6.0$)	H–C(3), H–C(5)
CH_2(5)	33.4 (*t*)	1.56 (*m*)	H–C(4)
CH_2(6–16)	29.3–29.7 (*t*)	1.20–1.34	–
CH_2(17)	22.7 (*t*)	–	–
Me(18)	14.1 (*q*)	0.88 (*t*, $J = 7.0$)	–
NH	–	5.97 (*d*, $J = 6.0$)	H–C(2)
C(1′)	174.0 (*s*)	–	–
CH_2(2′)	36.7 (*t*)	2.22 (*t*, $J = 7.5$)	H–C(3′)
CH_2(3′)	25.8 (*t*)	1.63 (*m*)	H–C(2′)
CH_2(4′–14′)	29.3–29.7 (*t*)	1.20–1.34	–
CH_2(15′)	22.7 (*t*)	–	–
Me(16′)	14.1 (*q*)	0.88 (*t*, $J = 7.0$)	–

^{1}H,^{1}H-COSY Correlations revealed two different networks, as shown in the *Figure*. In the HMQC spectrum, the signal at δ(H) 3.70 (*m*, H–C(4)) gave a cross-peak with the signal at δ(C) 85.2, indicating that the OSO_3H group was linked at C(4)[1]). The HMBC correlations between C(2)/NH and C(1′)/NH not only confirmed the position of the NH group, but also connected the two partial structures *via* an amide bond.

Methanolysis [15] of **1** afforded methyl palmitate, which was identified by GC/MS analysis (*m*/*z* 270 (95%)), implying the presence of a hexadecanoyl group. As the formula was $C_{34}H_{69}NO_7S$, the structure of **1** was, assigned as *N*-[2-hydroxy-1-(hydroxymethyl)-3-(sulfooxy)heptadecyl]hexadecanamide, and named *ircisulfamide*.

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[1]) Arbitrary atom numbering.

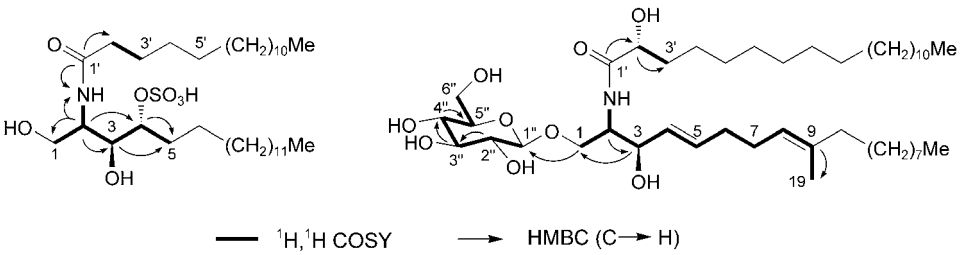

Figure. *^{1}H,^{1}H-COSY and key HMBC correlations for* **1** *and* **2**

By considering biogenetic [16] and steric factors, and from the NMR chemical shifts of H–C(2) and those of C(2) and C(3), the relative configuration of **1** could be derived [3]. Solvolysis [17] of **1** afforded the desulfated ceramide **1a**, which has been reported before [15]. Its resonances at δ(C) 62.0 (C(1)) 52.8 (C(2)), 76.7 (C(3)), and 72.3 (C(4)) were very similar to the ones reported (61.9, 52.7, 76.5, and 71.9, resp.) [15], indicating the same relative configuration, *i.e.*, (2*S*,3*S*,4*R*)[1]). This was further confirmed by comparison of the optical rotations, with an $[\alpha]_D^{20}$ value of + 19.3 ($c = 0.01$, $CHCl_3$) for **1a**.

Whereas 1-sulfated ceramides [17–19] and 4-sulfated sphingosine [20] have been reported, ircisulfamide (**1**) is, to the best of our knowledge, the first 4-sulfated ceramide.

Compound **2** was obtained as a colorless, amorphous powder, with an $[\alpha]_D^{20}$ value of + 9.1 ($c = 0.02$, MeOH). HR-FAB-MS furnished the molecular formula $C_{45}H_{85}NO_9$, with four degrees of unsaturation. The IR absorption band at 3378 cm^{-1} indicated the presence of OH and amide groups. The typical IR absorptions at 1643 and 1536 cm^{-1} again suggested a secondary amide, as supported by the presence of NMR signals at δ(H) 7.73 (*d*, $J = 8.0$ Hz, NH), δ(C) 54.5 (C–N), and δ(C) 177.1 (C=O) (see *Table 2*). In the ^{1}H-NMR spectrum, one olefinic signal at δ(H) 5.14 (*m*, H–C(8)), assignable to a trisubstituted C=C bond, and two olefinic signals (δ(H) 5.47 (*dd*, $J = 15.5$, 7.5 Hz, 1 H); 5.73 (*dt*, $J = 15.5$, 6.0 Hz, 1 H)), attributable to a disubstituted C=C bond, were observed. The ^{1}H-NMR spectrum also showed the presence of three Me groups (δ(H) 1.59 (*s*, Me(19)); 0.90 (*s*, Me(18), Me(20′))). As for **1**, two regions corresponding to aliphatic CH_2 groups at δ(H) 1.20–1.35 were observed. However, in the case of **2**, an additional glucopyranosyl (Glc) moiety could be distinguished.

Methanolysis of **2** afforded methyl 2-hydroxyicosanoate, which was identified by GC/MS analysis (*m*/*z* 342 (93%)), together with methyl glucopyranoside [21]. The optical rotation of the latter, $[\alpha]_D^{20} = +76.2$ ($c = 0.02$, MeOH), was close to that of an authentic D-configured sample. The anomeric signal at δ(C) 104.6 (*d*) confirmed that **2** was a monoglycoside, and the signals at δ(C) 104.6, 74.9, 77.8, 71.5, 77.9, and 62.6 suggested a β-D-glucopyranoside. The coupling constant between the anomeric H–C(1″) resonance at δ(H) 4.26 (*d*, $J = 9.0$ Hz) and the vicinal H–C(2″) signal at δ(H) 3.19 (*d*, $J = 9.5$ Hz) further supported β-configuration.

^{1}H,^{1}H-COSY Experiments revealed three distinct networks in **2** (see the *Figure*), and their linkages were resolved by means of HMBC analysis. The large vicinal coupling constant J(4,5) (15.5 Hz) clearly indicated an (*E*)-configured C=C bond in 4-position. The second unsaturation at C(8) was also assigned (*E*)-configuration on the

Table 2. *NMR Data for Ircicerebroside* (**2**). In CD_3OD; δ in ppm, J in Hz. Arbitrary atom numbering.

	^{13}C	^{1}H	$^{1}H,^{1}H$-COSY
$H_a-C(1)$	69.7 (*t*)	4.10 (*dd*, *J* = 10.0, 5.0)	$H_b-C(1)$, H−C(2)
$H_b-C(1)$	–	3.69 (*dd*, *J* = 10.0, 3.5)	$H_a-C(1)$
H−C(2)	54.5 (*d*)	3.99 (*m*, 1 H)	H−C(1), H−C(3), NH
H−C(3)	72.8 (*d*)	4.14 (*dd*, *J* = 7.5, 5.0)	H−C(2), H−C(4)
H−C(4)	131.1 (*d*)	5.47 (*dd*, *J* = 15.5, 7.5)	H−C(3), H−C(5), H−C(6)
H−C(5)	134.6 (*d*)	5.73 (*dt*, *J* = 15.5, 6.0)	H−C(4), H−C(6)
$CH_2(6)$	33.8 (*t*)	2.06 (*m*)	H−C(5), H−C(7)
$CH_2(7)$	35.4 (*t*)	2.04 (*m*)	H−C(6), H−C(8)
H−C(8)	124.8 (*d*)	5.14 (*t*, *J* = 6.5)	H−C(7), H−C(19)
C(9)	136.6 (*s*)	–	H−C(8), H−C(10)
$CH_2(10)$	40.8 (*t*)	1.98 (*t*, *J* = 7.5)	–
$CH_2(11-16)$	30.4–30.8 (*t*)	1.20–1.35	–
$CH_2(17)$	23.7 (*t*)	1.20–1.35	–
Me(18)	14.5 (*q*)	0.90 (*t*, *J* = 7.0)	–
Me(19)	16.2 (*q*)	1.59 (*s*)	–
NH	–	7.73 (*d*, *J* = 8.0)	–
C(1′)	177.1 (*s*)	–	–
H−C(2′)	73.0 (*d*)	3.97 (*m*)	–
$H_a-C(3')$	35.8 (*t*)	1.55 (*m*)	H−C(2′)
$H_b-C(3')$	–	1.69 (*m*)	H−C(2′), $H_a-C(3')$
$CH_2(4')$	26.2 (*t*)	1.38 (*m*)	–
$CH_2(5'-18')$	30.4–30.8 (*t*)	1.20–1.35	–
$CH_2(19')$	23.7 (*t*)	1.20–1.35	–
Me(20′)	14.4 (*q*)	0.90 (*t*, *J* = 7.0)	H−C(2′), $H_b-C(3')$
H−C(1″)	104.6 (*d*)	4.26 (*d*, *J* = 9.0)	–
H−C(2″)	74.9 (*d*)	3.19 (*dd*, *J* = 9.5, 8.0)	H−C(2″)
H−C(3″)	77.8 (*d*)	3.36 (*dd*, *J* = 9.5, 8.0)	H−C(1″), H−C(3″)
H−C(4″)	71.5 (*d*)	3.26 (*m*)	H−C(2″), H−C(4″)
H−C(5″)	77.9 (*d*)	3.31 (*m*)	H−C(3″), H−C(5″)
$H_a-C(6'')$	62.6 (*t*)	3.87 (*dd*, *J* = 10.5, 2.0)	H−C(4″), H−C(6″)
$H_b-C(6'')$	–	3.67 (*dd*, *J* = 10.5, 3.5)	H−C(5″), $H_b-C(6'')$

basis of the upfield-shifted ^{13}C-NMR signal for Me(19) [22]. By comparing the NMR chemical shifts of H−C(2) and C(1) to C(3), C(1′), and C(2′) with those of known glucosphingolipids [3][21], the relative configuration of **2** was determined as (2*S*,3*R*,2′*R*)[1]). Compound **2** was, thus, assigned the structure (2*R**)-*N*-{(1*S**,2*R**,3*E*,7*E*)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxy-8-methylheptadeca-3,7-dienyl}-2-hydroxyeicosanamide, and named *ircicerebroside*.

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Experimental Part

General. Vacuum liquid chromatography (VLC): silica gel *60 H* (*Qingdao Marine Chemical Plant*). TLC: precoated silica gel GF_{254} (*Qingdao Marine Chemical Plant*), visualization by spraying with 1% vanillin in conc. H_2SO_4, followed by heating at 105° for 1–2 min. Optical rotation: *Schmidt & Haensch Polartronic hnqw5* polarimeter. IR Spectra: *Bruker EQUINOX-55* IR spectrophotometer, with KBr pellets; in cm^{-1}. NMR Spectra: *Varian Unity INOVA-500* spectrometer operating at 500 (^{1}H) and 125 (^{13}C) MHz, in $CDCl_3$; chemical

shifts δ in ppm rel. to Me_4Si (= 0 ppm), coupling constants J in Hz. FAB-MS: *VG ZAB-HS* and *VG Autospec-500* mass spectrometers; in m/z. GC/MS: *Finnigan Voyager* GC/MS apparatus (*DB-5 MS* column, 30 m × 0.25 mm × 0.25 μm). Elemental analysis: *Elementar Vario EL* CHNS elemental analyzer.

Animal Material. The marine sponge *Ircinia fasciculata* (PALLAS) was collected from Wei-zhou Island, Beihai, P. R. China, during September 2001, and authenticated by Mr. *Zhi-Can Tang* (Institute of Oceanology, Chinese Academy of Sciences). A voucher specimen (01-WZ-02) was deposited at the Research Center of Organic Natural Products, Sun Yat-Sen (Zhongshan) University.

Extraction and Isolation. The dried marine sponge (500 g) was extracted with 95% aq. EtOH at r.t. (3 ×). The EtOH solns. were evaporated *in vacuo* to afford 40.5 g of residue. The latter was suspended in H_2O, and successively extracted with AcOEt and BuOH. The AcOEt soluble fraction (28.6 g) was subjected to VLC using a step gradient of hexane/AcOEt. The fraction eluted with hexane/AcOEt 8 : 2 afforded **1** (5.4 mg), and the one eluted with hexane/AcOEt 6 : 4 yielded **2** (3.2 mg).

Ircisulfamide (= N-*[(1S*,2S*,3R*)-2-Hydroxy-1-(hydroxymethyl)-3-(sulfooxy)heptadecyl]hexadecanamide*; **1**). Colorless, amorphous powder. $[\alpha]_D^{20} = +16.7$ ($c = 0.24$, $CHCl_3$). IR (KBr): 3383, 3312, 2919, 2850, 1645, 1545, 1470, 1382, 1234, 1082, 718. ^{1}H- and ^{13}C-NMR[1]): see *Table 1*. FAB-MS: 636 ($[M+H]^+$). HR-FAB-MS: 636.4865 ($[M+H]^+$, $C_{34}H_{70}NO_7S^+$; calc. 636.4873).

Desulfation of Ircisulfamide (**1**). Compound **1** (4.0 mg) was dissolved in a mixture of anh. pyridine (0.5 ml) and 1,4-dioxane (0.5 ml), and heated at 120° for 18 h. The mixture was cooled to r.t. and then partitioned between $CHCl_3$ and H_2O. The org. layer afforded the desulfated product **1a** (1.5 mg) as an amorphous powder. $[\alpha]_D^{20} = +19.3$ ($c = 0.01$, $CHCl_3$). ^{13}C-NMR ($CDCl_3$)[1]): 174.5 (C(1′)); 76.5 (C(3)); 72.0 (C(4)); 62.1 (C(1)); 53.1 (C(2)); 36.3 (C(2′)); 33.4 (C(5)); 29.7–29.4 (*n* CH_2); 25.8 (C(3′)); 22.7 (C(17), C(15)); 14.1 (C(18), C(16′)). FAB-MS: 557 ($[M+H]^+$).

Ircicerebroside (= *(2R*)*-N-*{(1S*,2R*,3E,7E)-1-[(β-D-Glucopyranosyloxy)methyl]-2-hydroxy-8-methylheptadeca-3,7-dienyl}-2-hydroxyeicosanamide*; **2**). Colorless, amorphous powder. $[\alpha]_D^{20} = +9.1$ ($c = 0.02$, MeOH). IR (KBr): 3378, 2921, 2851, 1643, 1536, 1456, 1080, 1036. ^{1}H- and ^{13}C-NMR[1]): see *Table 2*. FAB-MS: 784 ($[M+H]^+$), 605 ($[M+H-C_6H_{12}O_6]^+$). HR-FAB-MS: 784.6280 ($[M+H]^+$, $C_{45}H_{86}NO_9^+$; calc. 784.6303).

Methanolysis. Compounds **1** or **2** (1.1 mg) in a mixture of 1N aq. HCl (5 ml) and MeOH (15 ml) was heated at reflux for 15 h with magnetic stirring. Then, H_2O (25 ml) was added, and the mixture was extracted with hexane (3 ×). The combined org. layer was concentrated under N_2 to yield a colorless solid, which was identified by GC/MS analysis. In the case of **2**, the aq. layer was removed under N_2, and purified on a *C18* reversed-phase column to afford methyl glucopyranoside (0.2 mg). $[\alpha]_D^{20} = +76.2$ ($c = 0.02$, MeOH).

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