JOURNAL OF NATURAL PRODUCTS

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J. Nat. Prod., **1994**, 57 (1), 138-141• DOI: 10.1021/np50103a020 • Publication Date (Web): 01 July 2004

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Journal of Natural Products
Vol. 57, No. 1, pp. 138-141, January 1994

NEW CEREBROSIDES FROM *EURYALE FEROX*

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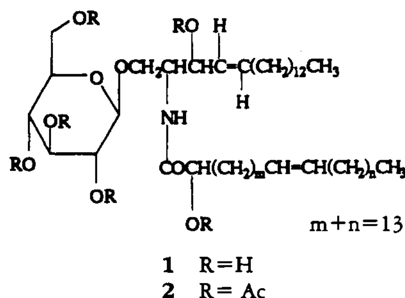
ABSTRACT.—The structure of a novel cerebroside, isolated from the rhizome with adventitious roots of *Euryale ferox* (Nymphaeaceae), has been elucidated by spectroscopic methods and characterized as an isomeric mixture of *N*-α-hydroxyl-*cis*-octadecaenoyl-1-*O*-β-glucopyranosylsphingosine [**1**] and its trans isomer.

The traditional Chinese medicinal herb, *Euryale ferox* Salisb. (Nymphaeaceae), is a water lily. The herb is considered to be a tonic and has also been recommended to treat pyodermas, hernias, and leucorrhea (1). In an investigation of the novel constituents of this medicinal herb, a cerebroside **1**, as an isomeric mixture of *N*-α-hydroxy-*cis*-octadecaenoyl-1-*O*-β-glucopyranosylsphingosine and its trans isomer, was obtained from the rhizome and adventitious roots. The structure was established by spectral analysis including ms and 2D nmr spectroscopy.

The fabms of **1** showed ions at *m/z* 725 (0.9%) $[M-H_2O+2H]^+$ and 724 (1.1%) $[M-H_2O+H]^+$ in the high mass region. The presence of two diagnostically useful fragments ions at *m/z* 562 (0.7%) $[M-C_6H_{11}O_6]^+$ and 561 (0.7%) $[M-C_6H_{12}O_6]^+$, formed by cleavage of the glucose moiety of the molecule, indicated a molecular weight of 741 for **1**.



The peak at *m/z* 443 (1.0%) $[724-281]^+$, formed by elimination of a fatty acyl group from the base moiety, established that the mol wt of the fatty acid is 282 and that the base is sphingosine. The eims of **1** and of its peracetylated derivative **2** both gave an ion peak at *m/z* 262 (11 and 12%) (Figure 1), which confirmed that the base moiety of the cerebroside is sphingosine. The eims of **2** also gave the fragments of the sugar moiety, *m/z* 331 (25%), 271 (39%), 229 (4%), 211 (45%), 169 (100%), and 109 (40%), due to an acetylated glucopyranoside (2).

Acetylation of **1** to **2** also improved the quality of the ^{1}H-nmr spectra. The ^{1}H nmr spectrum of **2** (Table 1), recorded in $CDCl_3$ with two drops of C_6D_6 added, displayed six acetyl signals (δ 2.07, 1.97, 1.96, 1.91, 1.87, and 1.86), indicating that **1** has six OH groups, together with a group of signals corresponding to a β-glucopyranose and a group corresponding to a sphingosine moiety. The proton at δ 6.32 (d, J=9.0 Hz) assigned to H-N correlated on the 2D-COSY spectrum with H-2 of the sphingosine. Knowing H-2, identification of H-3 (δ 5.32) and then of C-3 (δ 73.2) was straightforward using homo- and heteronuclear 2D nmr methods. The olefinic protons from the sphingosine consisted of a multiplet centered at δ 5.41 arising from H-4 and a multiplet centered at δ 5.81 from H-5. A

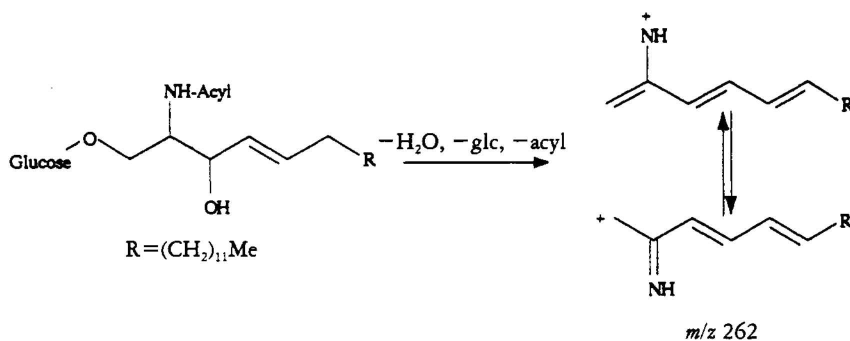


FIGURE 1. Fragmentation of **1** by eims.

coupling constant of 16 Hz between these two protons indicated a trans geometry for the double bond.

For characterization of the stereospecificity of the sphingoid base, we subjected **1** to ^{13}C-nmr measurement in $CDCl_3/CD_3OH$ solvent and compared the data with those of glucosyl-*erythro*-ceramide and glucosyl-*threo*-ceramide (3). Since the significant shift difference between the two configurations was observed for sphingosine C-3, the erythro configuration of the base in **1** was directly demonstrated (Table 3).

In the ^{13}C-nmr spectrum of **2** (Table 2), another CH appeared at δ 74.0 (C-2 in acyl moiety), its proton at δ 5.16 (dd, 1H, J=5 and 7 Hz, H-2), coupling with a methylene group. The acyl moiety is hence an α-hydroxyl linear acyl chain without any branch. The ^{1}H-nmr spectrum of **2** also showed two proton reso-

TABLE 1. ^{1}H Assignments of the Main Signals of Acetylated Cerebroside **2**.[a]

Proton	ppm			
Sphingosine moiety				
H_a-1	3.61	dd	1H	J=10, 3 Hz
H_b-1	3.94	dd	1H	J=10, 3 Hz
H-2	4.31	m	1H	
H-3	5.32	t	1H	J=6 Hz
H-4	5.41	dd	1H	J=6, 16 Hz
H-5	5.81	tt	1H	J=6, 16 Hz
H-6	2.1	m	2H	
NH	6.32	d	1H	J=9 Hz
H-18	0.89	t	3H	J=8 Hz
β-Glucosyl moiety				
H-1	4.48	d	1H	J=9 Hz
H-2	4.95	t	1H	J=9 Hz
H-3	5.20	t	1H	J=9 Hz
H-4	5.09	t	1H	J=9 Hz
H-5	3.70	ddd	1H	J=2,5,9 Hz
H_a-6	4.14	dd	1H	J=2, 12 Hz
H_b-6	4.24	dd	1H	J=5, 12 Hz
N-Acyl moiety				
H-2	5.16	dd	1H	J=5, 7 Hz
H-3	1.8	m	2H	
CH=CH	5.40–5.27	m	2H	
H-18	0.89	t	3H	J=8 Hz

[a]Measured at 500 MHz in $CDCl_3$; residual $CHCl_3$ used as internal standard; assignments are based on ^{1}H-^{1}H COSY.

TABLE 2. ^{13}C Chemical Shift Assignments of Acetylated Cerebroside **2**.[a]

Carbon	Structural moiety			
	Sphingosine	N-Acyl[b]		Glucosyl
C-1	67.0	170.3		100.5
C-2	50.9	74.0		71.2
C-3	73.2	31.8		71.7
C-4	125.1, 125.2	24.8		68.2
C-5	136.2, 136.3			72.0
C-6	32.6			61.7
		cis	trans	
		27.4	32.4	
		128.4	131.3	
		128.8	131.7	
		26.6	31.8	
C-16	31.9	31.9		
C-17	22.7	22.7		
C-18	14.1	14.1		
Ac×6	169.8–168.9	20.3–20.8		

[a]Spectrum measured at 125 MHz in $CDCl_3$ containing 2 drops of C_6D_6, using solvent as internal reference. Assignments are based on DEPT, ^{13}C-^{1}H COSY. All signals for unresolved methylene carbons appear as a large signal at 29–30 ppm.
[b]Signals assigned to vinyl carbons may be exchanged.

nances at δ 5.3 (m, 1H) and 5.4 (m, 1H), which were assigned to a double bond occurring in the acyl chain. Four CH signals were observed for this double bond at δ 128.4, 128.8, 130.7, and 131.3 in the DEPT spectrum of **2** together with four CH peaks (δ 26.6, 27.4, 31.8, and 32.4) assigned to two allylic methylene groups. From this, it could be assumed that the double bond occurs in both cis and trans form, since the chemical shift values for the trans vinylic methylene are around δ 33 and δ 27 for the cis form (4). This was further confirmed by the chemical shifts of the two vinyl carbons (C-4, C-5) of the sphingosine residue of **2**, which resonated at δ 125.1 (C-4), 125.2 (C-4), 136.2 (C-5), and 136.3 (C-5). These slight

TABLE 3. ^{13}C Chemical Shift Assignments of Sphingosine Moieties in **1** for Comparison with Those of Glucosyl-*erythro*-ceramide and Glucosyl-*threo*-ceramide.

Carbon	Compound		
	Glucosyl-*erythro*-ceramide[a]	*Glucosyl-threo*-ceramide[a]	**1**[b]
C-1	69.10	69.55	68.5
C-2	53.84	53.74	53.1
C-3	72.58	70.70	72.2
C-4	134.72	133.48	129.0[c]
C-5	129.75	129.50	133.9[c]
C-6	32.71	32.76	32.4
C-7–C-15	30.37–29.38	30.37–29.38	30–29
C-16	32.26	32.31	32.0
C-17	22.96	22.97	22.6
C-18	14.22	14.22	13.9

[a]Data in these columns are from Sarmientos *et al.* (3).
[b]Spectrum measured at 22.5 MHz in $CDCl_3/CD_3OH$, using solvent as internal reference.
[c]Assignments are based on Table 2.

shift effects are due to the different influence from the acyl isomers. Ir spectra showed two absorptions at 720 and 960 cm^{-1}, also indicating the cis and trans forms.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: ^1H and ^{13}C nmr on a Bruker AM-500 and an FX-90Q; fabms on a ZAB-HS; eims on a Nicolet-2000; ir on a Shimadzu IR-400.

PLANT MATERIAL.—The rhizomes with roots of *E. ferox* were collected from eastern China (Nanjing). Voucher specimen (No. r-1) is available for inspection at the Department of Phytochemistry, China Pharmaceutical University.

ISOLATION OF THE COMPOUND.—Air-dried and powdered plant material (2 kg) was extracted with 90% aqueous EtOH, and 20 g of residue was obtained. The extract was chromatographed on a Si gel column with CH_2Cl_2, CH_2Cl_2-EtOH (96:4), and $CHCl_3$-MeOH (93:7) as eluents. The most polar fraction was purified by recrystallization in $CHCl_3$/MeOH and afforded cerebroside **1**.

Cerebroside **1**.—Colorless amorphous powder: Molisch reaction positive; fabms see text; eims *m/z* (rel. int. %) 262 (11), 152 (3), 135 (6), 113 (10), 95 (30), 81 (50), 67 (70), 60 (80), 55 (80); ir ν max (KBr) 3300, 2950, 2900, 2800, 1640, 1630, 1535, 1460, 1370, 1080, 1040, 960, 720 cm^{-1}; ^1H nmr ($CDCl_3$ and pyridine-d_5, 500 MHz) 0.78 (6H, t, J=8 Hz), 1.17 (42H, m), 1.33 (2H, m), 1.51 (1H, m), 1.70 (1H, m), 1.90 (4H, m), 3.32 (2H, m), 3.51 (2H, m), 3.76 (1H, dd, J=12, 5 Hz), 3.86 (3H, m), 4.04 (2H, m), 4.10 (1H, m), 4.30 (1H, d, J=9 Hz), 5.25 (2H, m), 5.42 (1H, dt, J=15, 7 Hz), 5.59 (1H, m), 7.60 (1H, t, J=8 Hz); ^{13}C nmr ($CDCl_3$ and CD_3OH, 22.5 MHz) 176.0, 133.9, 131.0, 130.6, 129.0, 128.8, 128.4, 103.0, 76.0, 76.0, 73.3, 72.2, 72.2, 69.5, 68.5, 53.1, 34.5, 32.5, 32.4, 32.0, 31.8, 29–30 (a large peak), 27.2, 25.2, 22.6 13.9.

Peracetylated cerebroside **2**.—A solution of **1** in a mixture of CH_2Cl_2 and 4-dimethylaminopyridine was treated with an excess of Ac_2O, then diluted with CH_2Cl_2 and washed with brine. The dried CH_2Cl_2 layer was evaporated to dryness under reduced pressure to give the peracetylated cerebroside **2**: white powder; found C 65.60, H 9.61, N 1.40 ($C_{54}H_{91}NO_{15}$ required C 65.26, H 9.16, N 1.41%); eims *m/z* (rel. int. %) 513 (2), 331 (25), 272 (5), 271 (39), 262 (12), 229 (4), 211 (45), 169 (100), 109 (40), 97 (8), 93 (3), 91 (5), 79 (7), 77 (4); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

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Received 22 March 1993