Ceramides and Cerebrosides from *Ligusticum chuanxiong* HORT.

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Two new ceramides, (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1,3,4-trihydroxypentadec-8-en-2-yl]heptacosanamide (**1**) and (2*R*)-2-hydroxy-*N*-{(3*S*,4*S*,5*S*)-4-hydroxy-5-[(4*E*)-undec-4-en-1-yl]tetrahydrofuran-3-yl}heptacosanamide (**2**), were isolated from the aerial parts of *Ligusticum chuanxiong*, along with one known ceramide, (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1,3,4-trihydroxyicos-8-en-2-yl]tetracosanamide (**3**), and two known cerebrosides, (2*R*)-*N*-[(2*S*,3*R*,4*E*,8*E*)-1-(β-D-glucopyranosyloxy)-3-hydroxydodeca-4,8-dien-2-yl]-2-hydroxydocosanamide (**4**) and (2*R*)-*N*-[(2*S*,3*S*,4*R*,8*E*)-1-(β-D-glucopyranosyloxy)-3,4-dihydroxyoctadec-8-en-2-yl]-2-hydroxyhexadecanamide (**5**). Their structures were elucidated by extensive spectroscopic analysis, including 1D- and 2D-NMR, as well as HR-ESI-MS experiments.

Introduction. – Ceramides and cerebrosides, two families of sphingolipids, are important components of a wide variety of tissues and organs in biological systems. Chemically, ceramide usually consists of a long-chain sphingosine or sphingol and an amide-linked long-chain fatty acid, cerebrosides are composed of a hexose and a ceramide moiety. Biologically, cerebrosides have been proven to serve as structural support and texture determinants of cell membranes, and act as mediators of biological events. In particular, the NGF (nerve growth factor)-like low molecular-weight compounds as represented by cerebrosides are thus considered to be promising for the treatment of *Alzheimer*'s disease. A growing collection of evidence has indicated that ceramides have a wide range of biological functions of regulating cell growth and variation, and participating protein secretion and immunologic process [1].

Ligusticum chuanxiong HORT. (Umbelliferae) is a common medicinal plant, growing in the southwestern China. Its dried rhizome is one of the most important crude drugs in traditional Chinese medicines and has been used to treat chest and hypochondrium, stomachache, amenia [2]. In our ongoing research on chemical constituents of the aerial parts of *Ligusticum chuanxiong*, we have reported before some phenols from its AcOEt extract [3], and we have also isolated three ceramides and two cerebrosides from its petroleum ether extract. In this paper, we deal with the isolation and structural elucidation of three ceramides, (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1,3,4-trihydroxypentadec-8-en-2-yl]heptacosanamide (**1**), (2*R*)-2-hydroxy-*N*-{(3*S*,4*S*,5*S*)-4-hydroxy-5-[(4*E*)-undec-4-en-1-yl]tetrahydrofuran-3-yl}heptacosanamide (**2**), and (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1,3,4-trihydroxyicos-8-en-2-

yl]tetracosanamide (**3**), and two cerebrosides, (2*R*)-*N*-[(2*S*,3*R*,4*E*,8*E*)-1-(*β*-D-glucopyranosyloxy)-3-hydroxydodeca-4,8-dien-2-yl]-2-hydroxydocosanamide (**4**) and (2*R*)-*N*-[(2*S*,3*S*,4*R*,8*E*)-1-(*β*-D-glucopyranosyloxy)-3,4-dihydroxyoctadec-8-en-2-yl]-2-hydroxyhexadecanamide (**5**) (*Fig. 1*). Amongst these, compounds **1** and **2** are new ceramides, compounds **3**–**5** were isolated from this genus for the first time. Their structures were elucidated by spectral and chemical means.

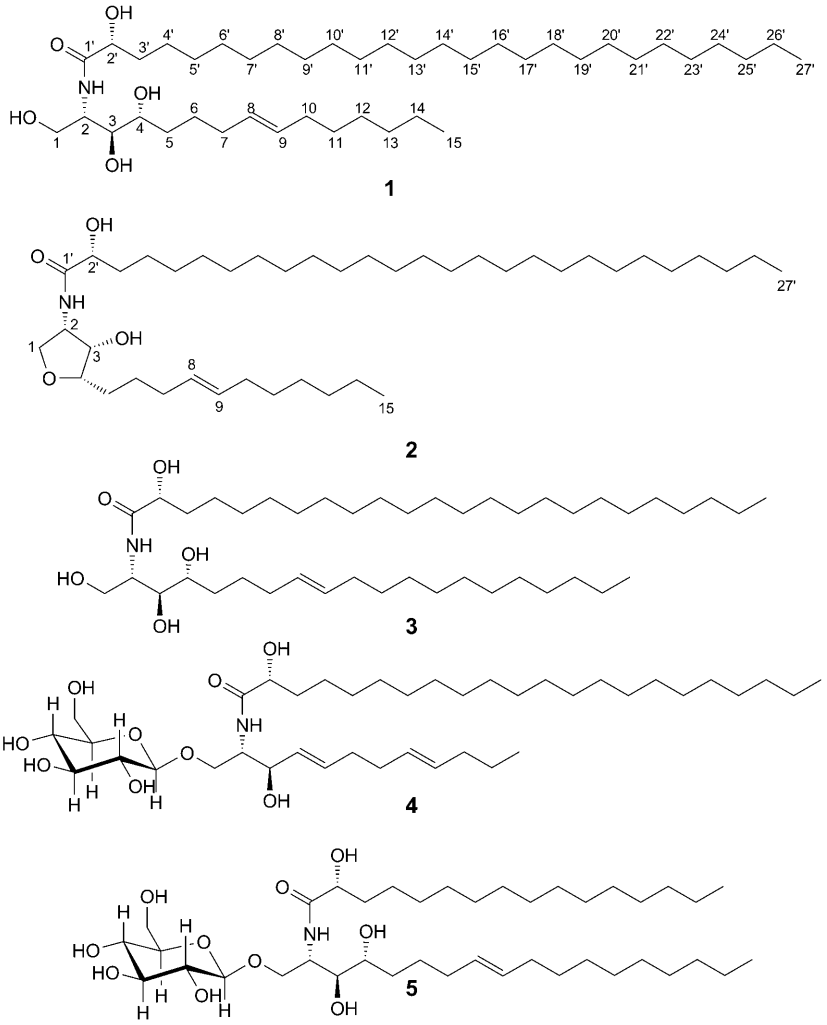

Fig. 1. *Ceramides and cerebrosides from the aerial parts of* Ligusticum chuanxiong

Results and Discussion. – The 80% EtOH extract of the aerial parts of *Ligusticum chuanxiong* HORT. was suspended in H_2O and extracted successively with petroleum ether, AcOEt, and BuOH to give the respective extracts after solvent removal. The

combined petroleum ether layers were concentrated *in vacuo* to leave the residue, which was chromatographed on SiO_2 to give compounds **1**–**5**. The known compounds **3**–**5** were identified as (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1,3,4-trihydroxyicos-8-en-2-yl]tetracosanamide (**3**) [4], (2*R*)-*N*-[(2*S*,3*R*,4*E*,8*E*)-1-(*β*-D-glucopyranosyloxy)-3-hydroxydodeca-4,8-dien-2-yl]-2-hydroxydocosanamide (**4**) [5][6], and (2*R*)-*N*-[(2*S*,3*S*,4*R*,8*E*)-1-(*β*-D-glucopyranosyloxy)-3,4-dihydroxyoctadec-8-en-2-yl]-2-hydroxyhexadecanamide (**5**) [7][8], by comparison of their spectral data with those reported in the literature.

Compound **1** was isolated as a white powder. The molecular formula of **1** was established as $C_{42}H_{83}NO_5$ by HR-ESI-MS (*m*/*z* 682.6315, $[M+H]^+$). The ESI-MS (positive mode) showed a quasi molecular ion peak at *m*/*z* 682 ($[M+H]^+$), and its MS/MS showed characteristic peaks at *m*/*z* 612 (an ion formed by the *McLafferty* rearrangement of the olefinic bond), 468 (an ion derived from the *β*-fission of the OH group), and 424 (an ion derived from the *α*-fission of the NH group; *Fig. 2*). The ^{1}H- and ^{13}C-NMR spectra (*Table*) were typical of a sphingosine-type ceramide possessing 2-hydroxy fatty acid [1]. Assignments of all H-atoms and C-atoms in **1** can be made by ^{1}H,^{1}H-COSY, HMQC and HMBC spectra (*Fig. 3*). These long range correlations allowed to clearly deduce the presence of a sphingosine-type ceramide. In the ^{1}H,^{1}H-COSY spectrum, one H-atom of the CH_2 group at *δ*(H) 4.49 correlated with the CH group at *δ*(H) 5.08–5.11, the CH group at *δ*(H) 5.08–5.11 correlated with the CH group at *δ*(H) 4.33, the CH group at *δ*(H) 4.33 correlated with the CH group at *δ*(H) 4.26–4.28, which suggested three OH groups at C(1), C(3) and C(4). An HMBC experiment was run to support these assignments. The fatty acid linked to C(2) of the sphingosine was confirmed by the correlations between the NH H-atom and the C-atom C(2) and the C=O group with the signal at *δ*(C) 175.2. HMBC Correlations of the C=O group at 175.2 with H–C(2′) which in turn showed correlations with C(2′) and

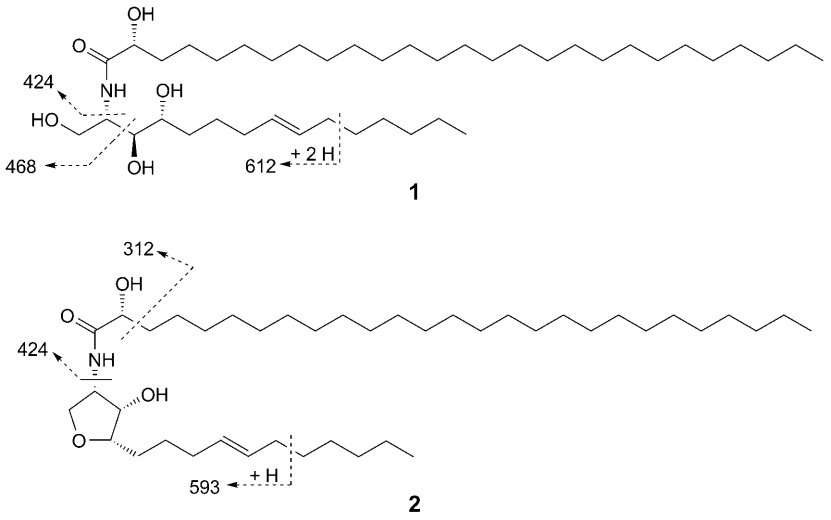

Fig. 2. *ESI-MS/MS Fragment analysis of* **1** *and* **2**

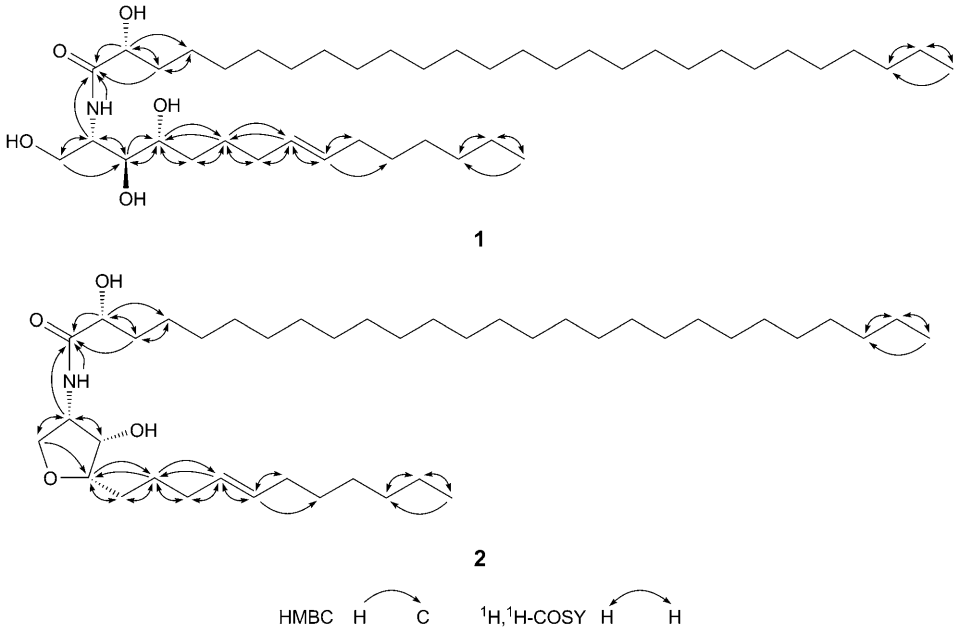

Fig. 3. *Key HMBC and ^{1}H,^{1}H-COSY data for* **1** *and* **2**

HO–C(2′) confirmed the presence of an α-OH fatty acid side chain. The structure of the α-OH fatty acid side chain in **1** was examined. When **1** was methanolyzed with methanolic HCl, a fatty acid methyl ester (FAME) was obtained together with a long-chain base (LCB). On the basis of EI-MS analysis, the FAME was characterized as methyl 2-hydroxyheptacosanoate. In the HMBC spectrum (*Fig. 3*), H–C(4) at δ(H) 4.26–4.28 correlated with C(5) at δ(C) 33.9 and a C-atom at δ(C) 27.7; the olefinic H-atoms at δ(H) 5.51–5.54 correlated with a C-atom at δ(C) 33.3 and the above mentioned C-atom at δ(C) 27.7; the H-atom at 1.65–1.70 orrelated with C(4) and the olefinic C-atom. In the HMQC spectrum, the H-atom at 1.65–1.70 correlated with the C-atom at δ(C) 27.7, the H-atom at 1.99–2.05 correlated with the C-atom at δ(C) 33.3. These data indicate that the olefinic bond in the LCB residue of **1** is located at C(8), which suggested that the LCB component was 2-amino-1,3,4-trihydroxy-8-pentadecene, and the fragment ion at *m*/*z* 612 due to elimination of pentene from the quasi molecular ion also supported this conclusion. Furthermore, the C-atom signals at δ(C) 33.3 and 33.0 ppm confirmed the *E* geometry of the olefinic bond at C(8) in the LCB. The H-atom signal at δ(H) 5.51–5.54 was assigned to the olefin H-atoms, based on the ^{1}H,^{1}H-COSY spectrum of **1**. Consideration of biogenesis and steric hindrance of sphingolipids, the chemical shift of the H–C(2) signal and the C-atom signals of C(1) to C(4), C(1′) and C(2′) of sphingolipids generally acknowledged to determine the absolute configuration of the phytosphingosine moiety. The H-atom signal at δ(H) 5.08–5.11 (H–C(2)) and the C-atom signals at δ(C) 62.1 (C(1)), 53.0 (C(2)), 76.9 (C(3)), 72.5 (C(4)), 175.2 (C(1′)), and 72.9 (C(2′)) in **1** were nearly identical with those

Table. *[1]H-NMR* (500 MHz) *and [13]C-NMR* (125 MHz) *Spectral Data of Compounds* **1** *and* **2** *in (D5)Pyridine.* δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
NH	8.55 (*d*, *J* = 9)		8.47 (*d*, *J* = 9)	
1	4.49 (*dd*, *J* = 11, 4.6, H_a), 4.41 (*dd*, *J* = 11, 5.0, H_b)	62.1	4.52 (*dd*, *J* = 15, 7, H_a), 3.93 (*dd*, *J* = 15, 7.5, H_b)	71.6
2	5.08–5.11 (*m*)	53.0	4.76–4.79 (*m*)	51.9
3	4.33 (*dd*, *J* = 4.5, 6.5)	76.9	4.32 (*t*, *J* = 6.0)	74.6
4	4.26–4.28 (*m*)	72.5	4.14 (*dt*, *J* = 6.0, 5.0)	86.1
5	2.21–2.23 (*m*, H_a), 1.94–1.98 (*m*, H_b)	33.9	2.20–2.22 (*m*, H_a), 1.93–1.97 (*m*, H_b)	32.9
6	1.65–1.70 (*m*)	27.7	1.65–1.70 (*m*)	26.4
7	1.99–2.05 (*m*)	33.3	1.99–2.05 (*m*)	32.9
8	5.51–5.54 (*m*)	130.7	5.46–5.48 (*m*)	130.4
9	5.51–5.54 (*m*)	130.7	5.46–5.48 (*m*)	130.4
10	1.88–1.91 (*m*)	33.0	1.88–1.91 (*m*)	32.2
11–14	1.26–1.37 (*m*)	30.4–29.7	1.25–1.36 (*m*)	30.2–29.4
15	0.88 (*t*, *J* = 7)	14.3	0.88 (*t*, *J* = 7)	14.3
1′		175.2		175.5
2′	4.61 (*dd*, *J* = 7.5, 3.5)	72.9	4.63 (*dd*, *J* = 7.5, 3.5)	72.6
3′a	2.15–2.20 (*m*)	35.7	2.15–2.20 (*m*)	35.6
3′b	1.99–2.05 (*m*)		1.99–2.05 (*m*)	
4′a	1.95–1.97 (*m*)	26.9	1.95–1.97 (*m*)	26.8
4′b	1.72–1.74 (*m*)		1.72–1.74 (*m*)	
5′–26′	1.26–1.37 (*m*)	30.4–29.7	1.25–1.36 (*m*)	30.2–29.4
27′	0.88 (*t*, *J* = 7)	14.3	0.88 (*t*, *J* = 7)	14.3

of (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxyoctadecane-2-yl]tetracosanamide [9–12]. Thus, the structure of compound **1** was established as (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,8*E*)-1,3,4-trihydroxypentadec-8-en-2-yl]heptacosanamide.

Compound **2** was obtained as a white powder. The molecular formula of **2** was established as $C_{42}H_{81}NO_4$ by HR-ESI-MS (*m*/*z* 664.6199 $[M+H]^+$). The ESI-MS (positive ion mode) showed a quasi molecular ion peak at *m*/*z* 664 ($[M+H]^+$), and its MS/MS showed several characteristic ions at *m*/*z* 646 (an ion due to dehydration from the *quasi*-molecular ion), 593 (an ion formed by the *McLafferty* rearrangement of the olefinic bond), 424 (an ion derived from the α-fission of the NH group), and 312 (an ion derived from the β-fission of the OH group; *Fig. 2*). A comparison of the ^{1}H- and ^{13}C-NMR spectral data (*Table*) of **2** with those of **1** showed that, their spectral data were very similar, except that the C(1) chemical shift of **2** was shifted downfield by 9.5 ppm and the C(4) signal in **2** was shifted downfield by 13.6 ppm. Furthermore, the ESI-MS spectrum (positive ion mode) showed a *quasi*-molecular ion peak $[M+H]^+$ at *m*/*z* 664, indicating a deficiency of 18 mass units in comparison with that of **1**, which suggested a 1,4-epoxy linkage in **2** due to deprivation of H_2O from **1**. A HMBC (*Fig. 3*) cross peak between H–C(1) (δ(H) 4.52) and C(4) (δ(C) 86.1) also supported this conclusion. The structure of **2** was also examined by mass spectrometry analyses of its methanolysis derivatives. 2D-NMR including HMQC and HMBC experiments allowed

us to assign all the H-atom and C-atom signals for **2**. The relative configurations of C(2), C(3), C(4) were presumed to be *S*, *S*, and *S*, respectively, which was confirmed by the coupling constants (*Table*) for the *cis*-H-atoms ($J < 7.0$ Hz) in the tetrahydrofuran ring (for *trans*-H-atoms, $J > 7.0$ Hz) [13][14]. The relative configuration of C(2′) was determined to be *R* by comparing the spectral data of H–C(2′) and C(2′) with those of **1**. Thus, compound **2** was identified as (2*R*)-2-hydroxy-*N*-{(3*S*,4*S*,5*S*)-4-hydroxy-5-[(4*E*)-undec-4-en-1-yl]tetrahydrofuran-3-yl}heptacosanamide. The ceramide with 1,4-epoxy bonds is reported for the first time.

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Experimental Part

General. Column chromatography (CC) was carried out on silica gel (SiO_2; 200–300 mesh, *Qingdao haiyang Chemical Group Co. Ltd*, P. R. China). Optical rotations: *Jasco DIP-370* digital polarimeter. IR Spectra: *Perkin-Elmer 8900* FT-IR instrument; KBr disks. 1D- and 2D-NMR spectra: in (D_5)pyridine; on a *Bruker AV 500* spectrometer. EI-MS: *QP5050A* mass spectrometer. ESI-MS: *Micromass Quattro micro ES-Cl*. HR-ESI-MS: *Bruker APEX-II* mass spectrometer.

Plant Material. The aerial parts of *Ligusticum chuanxiong* HORT. were collected from Sichuan Province, China in June 2004 and identified by *N.-Y. Y.* of Shanghai Institute of Pharmaceutical Industry. A voucher specimen (No. CX-04-00021) was deposited with the Herbarium of Shanghai Institute of Pharmaceutical Industry.

Extraction and Isolation. The aerial parts (9 kg) of *Ligusticum chuanxiong* HORT. were extracted with 80% EtOH (2×50 l) for 2 h under reflux, and the combined extracts were concentrated *in vacuo*. The resulting extract (630 g) was then suspended in H_2O and extracted successively with petroleum ether (PE), AcOEt, and BuOH to give the respective extracts after solvent removal. The combined PE layers were concentrated *in vacuo* to leave a residue (180 g), which was chromatographed on SiO_2 (2 kg) eluting with $CHCl_3$/MeOH, stepwise gradient ($100:0 \rightarrow 5:1$), and 5 fractions were collected. *Fr. 3* (15 g) was separated by SiO_2 (CH_2Cl_2/MeOH 20:1) to obtain compounds **2** (22 mg), **1** (35 mg), and **3** (20 mg). *Fr. 4* (10 g) was separated by SiO_2 (CH_2Cl_2/MeOH 10:1) to obtain compounds **4** (15 mg) and **5** (15 mg).

*(2*R*)-2-Hydroxy*-N-*[(2*S*,3*S*,4*R*,8*E*)-1,3,4-trihydroxypentadec-8-en-2-yl]heptacosanamide* (**1**). White powder. $[\alpha]_D^{20} = +10.5$ ($c = 1.04$, C_5H_5N). IR (KBr): 3400, 3210, 2920, 1623, 1538, 1475. ^{1}H- and ^{13}C-NMR: *Table*. ESI-MS/MS: 682 ($[M+H]^+$), 612, 468, 424. HR-ESI-MS: 682.6315 ($[M+H]^+$, $C_{42}H_{84}NO_5^+$; calc. 682.6344).

*(2*R*)-2-Hydroxy*-N-*{(3*S*,4*S*,5*S*)-4-hydroxy-5-[(4*E*)-undec-4-en-1-yl]tetrahydrofuran-3-yl}heptacosanamide* (**2**). White powder. $[\alpha]_D^{20} = +2.32$ ($c = 1.04$, C_5H_5N). IR (KBr): 3211, 2918, 1621, 1536, 1476. ^{1}H- and ^{13}C-NMR: *Tables*. ESI-MS/MS: 664 ($[M+H]^+$), 646, 593, 424, 312. HR-ESI-MS: 664.6199 ($[M+H]^+$, $C_{42}H_{82}NO_4^+$; calc. 664.6238).

Methanolysis of **1** *and* **2**. Compounds **1** and **2** (*ca.* 1 mg) were heated with 10% HCl in MeOH (1 ml each) at 80° for 14 h, resp. The mixture was then extracted with hexane and concentrated *in vacuo*. The residue was analyzed by EI-MS, which showed the characteristic fragment ions (*m*/*z* 440 M^+, 381 [$M-$COOMe]$^+$) of methyl 2-hydroxyheptacosanoate.

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