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华麻花头根中的神经酰胺成分

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摘要：从菊科麻花头属植物华麻花头 (*Serratula chinensis* S. Moore) 根中分离得一组神经酰胺类化合物，经光谱和化学方法分析，鉴定为(2S, 3S, 4R, 8E)-8, 9-二脱氢植物鞘氨醇 (2R)-2-羟基脂肪酰胺，其脂肪酸链主要由十六，二十二，二十三，二十四和二十五烷酸组成，其中 2-羟基十六碳酸组成的神经酰胺(2S, 3S, 4R, 8E)-2-[(2R)-2-羟基棕榈酰胺]-8-十八碳烯 -1, 3, 4-三醇为一新化合物。高锰酸钾氧化法被应用于判断单不饱和长链中双键的位置。

关键词：华麻花头；麻花头属；菊科；神经酰胺类化合物；高锰酸钾氧化法
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Ceramides from the Roots of *Serratula chinensis*

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Abstract: A mixture of five ceramides was isolated from the roots of *Serratula chinensis* (Compositae). The structures of these compounds were elucidated as (2S, 3S, 4R, 8E)-2-amino-8-octadecene-1, 3, 4-triol with five 2-hydroxy fatty acids of varying chain lengths (C_{16}, C_{22-25}) linked to the amino group (**1a**–**1e**, respectively). Ceramide **1a** was a new compound. The structures were established by spectroscopic and chemical means. The $KMnO_4$ oxidation method was applied to locate the double bond position in monounsaturated long chain bases.
Key words: *Serratula chinensis*; *Serratula*; Compositae; Ceramides; $KMnO_4$ oxidation

Serratula chinensis S. Moore (Compositae) is a perennial herbaceous plant growing mainly in south China[1]. Its roots have long been used as a folk medicine for treatment of pharyngitis and morbilli in China[2]. Our previous paper had reported the isolation of seven ecdysteroids[3]. In continuation of our investigation of bioactive natural products from the roots of this plant, a group of ceramides were isolated and characterized. This paper deals with the isolation and structure elucidation of these compounds.

1 Results and Discussion

The 95% EtOH extract of the powdered dry roots of *S. chinensis* was successively fractionated with petroleum ether (60–90℃), $CHCl_3$ and *n*-BuOH. The petroleum ether fraction was subjected to silica gel column chromatography (CC) to give compound **1** (Fig. 1).

The positive ESI-MS of **1** displayed a series of $[M + Na]^+$ peaks at *m/z* 592, 676, 690, 704, and 718, in

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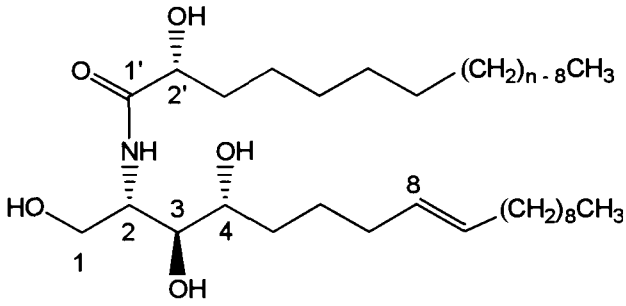

1a n = 16
1b n = 22
1c n = 23
1d n = 24
1e n = 25

Fig. 1 The structures of compound **1**

accordance with the molecular formula $C_{34}H_{67}NO_5$, $C_{40}H_{79}NO_5$, $C_{41}H_{81}NO_5$, $C_{42}H_{83}NO_5$, and $C_{43}H_{85}NO_5$, respectively. The IR spectrum of **1** showed absorption bands for hydroxyl, amide and $(CH_2)n$ functionalities. The ^1H and ^{13}C NMR spectra of **1** (Table 1) indicated the presence of a secondary amide linkage (δ_H 8.58, 1H, d, J = 8.8 Hz; δ_C 175.3) and two long chain aliphatic moieties, suggesting the structural nature of ceramides. Methanolysis of **1** yielded a fraction of fatty acid methyl esters (FAMEs) and a long chain base (LCB). The fraction of FAMEs was determined to be a mixture of methyl 2-hydroxypalmitate (1.2%), methyl 2-hydroxybehenate (14.3%), methyl 2-hydroxytricosanoate (20.5%), methyl 2-hydroxytetracosanoate (55.3%), methyl 2-hydroxypentacosanoate (8.7%) by GC-MS analysis. After having excluded the signals due to the fatty acid moieties, the remaining ^1H and ^{13}C signals were indicative of a monounsaturated phytosphingosine moiety because of the presence of an oxymethylene (δ_H 4.50 and 4.40, δ_C 62.0), an amidomethine (δ_H 5.11, δ_C 53.0), two oxymethines (δ_H 4.33 and 4.28, δ_C 76.8 and 72.9), and two olefinic methines (δ_H 5.51 and 5.49, δ_C 130.9 and 130.7). In the $^1H-^1H$ COSY spectrum of **1**, the amidomethine proton gave cross peaks with the amido proton at δ 8.58, the oxymethylene protons, and the oxymethine proton at δ 4.33. The latter correlated with the oxymethine proton at δ 4.28. The EI-MS and the positive ESI-MS of the LCB obtained from the methanolysis of **1** gave a $[M]^+$ ion at *m/z* 315 and a $[M+H]^+$ peak at *m/z* 316, respectively. These findings indicated that the phytosphingosine moiety was 2-amino-octadecene-1, 3, 4-triol. In order to determine the position of the double bond in the phytosphingosine moiety, the $KMnO_4$ oxidation was performed on the LCB. The oxidation afforded *n*-decanoic acid which was determined by GC-MS analysis. This allowed the location of the double bond at C-8. The *trans* (*E*) configuration of the double bond was evidenced by the coupling constant (J = 16.0 Hz) between H-8 and H-9 in the ^1H NMR spectrum, and the chemical shifts of the carbons next to the double bond at δ 33.4 and 33.1 (C-7 and C-10) in the ^{13}C NMR spectrum[4-6]. The 2'R, 2S, 3S, 4R configuration of the ceramide was also indicated by the ^{13}C NMR chemical shifts of C-1' (δ 175.3), C-2' (δ 72.5), C-1 (δ 62.0), C-2 (δ 53.0), C-3 (δ 76.8), and C-4 (δ 72.9)[7-9]. Based on the above evidence, **1** was deduced to be comprised of a common long chain base linked to varying chain lengths of 2-hydroxy fatty acid residue. Therefore, **1** was established as a mixture of (2S, 3S, 4R, 8E)-2- [(2R)-2-hydroxypalmitoylamino] -8-octadecene-1, 3, 4-triol (**1a**), (2S, 3S, 4R, 8E)-2-[(2R)-2-hydroxybehenoylamino]-8-octadecene - 1, 3, 4- triol (**1b**)[10], (2S, 3S, 4R, 8E)-2-[(2R)-2-hydroxytricosanoylamino]-8-octadecene-1, 3, 4-triol (**1c**)[10], (2S,3S, 4R, 8E) - 2 -[(2R)- 2 -hydroxytetracosanoylamino]-8-octadecene-1, 3, 4-triol (**1d**)[10], and (2S, 3S, 4R, 8E)-2-[(2R)-2-hydroxypentacosanoylamino]-8-octadecene-1, 3, 4-triol (**1e**)[10]. Among them, ceramide **1a** was found to be a new compound.

The O_3 oxidation[11, 12], $NaIO_4$-$AgNO_3$ oxidation[11], $KMnO_4$ - $NaIO_4$ oxidation[13], and DMDS/GC - MS detection[14] have been previously applied to locate the

double bond position in monounsaturated phytosphingosines and dihydrosphingosines. In our present study, the $KMnO_4$ oxidation reaction was used and achieved the same satisfying results. This method is more convenient and easier to operate, and under less harsh reaction condition in comparison with the previous ones.

2 Experimental

2.1 General

Optical rotations were measured with a Perkin Elmer 343 spectropolarimeter. The IR spectra were taken in KBr on a WQF-410 FT-IR spectrophotometer. The ^1H NMR (400 MHz), ^{13}C NMR (100 MHz), and 2D NMR spectra were recorded on a Bruker DRX-400 instrument. Chemical shifts were expressed in ppm (δ) with TMS as an internal standard. The positive ESI-MS data were obtained with a MDS SCIEX API 2000 LC/MS/MS system by direct inlet using MeOH as solvent. The GC-MS analyses were performed with a Shimazu QP-5000 instrument [GC conditions: DB-1 capillary column (30 m × 0.25 mm); column temperature, 60→260℃; rate of temperature increase, 10℃ min^{-1}; injector temperature, 270℃; He at 15 ml min^{-1}]. Silica gel 60 (200 –300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Develosil ODS (5 μm, Nomura Chemical Co. Ltd., Japan) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. TLC was performed on precoated plates (Kieselgel $60GF_{254}$, Merck) with detection effected by exposure to iodine vapor.

2.2 Plant material

The roots of *S. chinensis* were collected in Lechang County, Guangdong Province, China, in Autumn 2001, and identified by Prof. Zexian Li, South China Botanical Garden, the Chinese Academy of Sciences. A voucher sample (No. 621633) is deposited at the Herbarium of South China Botanical Garden, the Chinese Academy of Sciences (IBSC).

2.3 Extraction and isolation

The ground dry roots (8 kg) of *S. chinensis* were extracted with 95% EtOH by percolation at room temperature. The EtOH percolate was concentrated to syrup (500 g) *in vacou*. This syrup was suspended in

Table 1 ^1H (400 MHz) and ^{13}C (100 MHz) NMR data of 1 (in pyridine-d_5)

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
1a	4.50 dd (10.4, 4.4)	70.4	16	1.23 – 1.30	32.2
1b	4.40 dd (10.4, 4.4)		17	1.23 – 1.30	23.0
2	5.11 m	54.6	18	0.85 t (7.2)	14.4
3	4.33 m	71.3	NH	8.58 d (8.8)	
4	4.28 m	34.9	1′		175.3
5	2.19 m, 1.93 m	25.9	2′	4.61 dd (7.2, 3.6)	72.5
6	1.78 m	29.7 – 30.5	3′	2.19 m, 2.00 m	35.7
7 and 10	2.19 m	33.4	4′	2.00 m, 1.78 m	26.8
	2.00 m	33.1	5′ - (n ? 3)′	1.23 – 1.30	29.7 – 30.1
8 and 9	5.51 dt (16.0, 5.6)	130.9	(n ? 2)′	1.23 – 1.30	32.2
	5.49 dt (16.0, 5.6)	130.7	(n ? 1)′	1.23 – 1.30	23.0
11–15	1.23 – 1.30	29.7 – 30.1	n′	0.85 t (7.2)	14.4

n = 16, 22 – 25

H_2O and the aqueous suspension was successively extracted three times each with petroleum ether, $CHCl_3$, and *n*-BuOH. The petroleum ether extract, upon concentration under lower pressure, afforded a dark syrup (25 g). This syrup was subjected to a silica gel CC using $CHCl_3$-MeOH (98:2) as the eluant, yielding compound **1** (600 mg).

2.4 Ceramide 1

White amorphous powder. $[\alpha]_D^{25}$ + 9.20 (*c* 0.174, pyridine). IR (KBr) cm^{-1}: 3430 (OH), 1633 (C=O), 1509, 1428, 1369, 1261, 721. ^1H and ^{13}C NMR data: see Table 1. Positive ESI-MS *m/z*: 592 $[C_{34}H_{67}NO_5 + Na]^+$, 676 $[C_{40}H_{79}NO_5 + Na]^+$, 690 $[C_{41}H_{81}NO_5 + Na]^+$, 704 $[C_{42}H_{83}NO_5 + Na]^+$, 718 $[C_{43}H_{85}NO_5 + Na]^+$.

2.5 Methanolysis of 1

Mixture **1** (24 mg) was refluxed with 0.9 mol/L HCl in 82% aqueous MeOH (15 ml) for 18 h[15]. The resulting solution was extracted three times with *n*-hexane. The *n*-hexane solution was dried over anhydrous Na_2SO_4 and then concentrated to yield a fraction of FAMEs (14.5 mg). The H_2O layer, after evaporation of MeOH, was basified to pH 9 with ammonia liquor and extracted with Et_2O. The Et_2O layer was dried over anhydrous Na_2SO_4 and evaporated to yield a LCB (8.0 mg). The fraction of FAMEs, white amorphous powder, $[\alpha]_D^{25}$ −5.0 (*c* 0.1, $CHCl_3$), was analyzed by GC-MS. Peak 1 (t_R 14.28 min, 1.2%, 2-hydroxypalmitic acid methyl ester), EI-MS *m/z*: 286 $[M]^+$ (2), 268 $[M - H_2O]^+$ (0.5), 227 $[M - CH_3OCO]^+$ (8), 208 (1), 182 $[M-CH_3OCO - CH_2OHCH_2]^+$ (0.5), 159 (1), 145 (2), 127 $[C_9H_{19}]^+$ (3), 125 (4), 111 (6), 97 (20), 90 $[CH_3OC(OH)=CHOH]^+$ (21), 83 (27), 69 (30), 57 (81). Peak 2 (t_R 21.94 min, 14.3%, 2-hydroxybehenic acid methyl ester), EI-MS *m/z*: 370 $[M]^+$ (5), 352 $[M - H_2O]^+$ (0.5), 311 $[M - CH_3OCO]^+$ (7), 292 (1), 266 $[M - CH_3OCO - CH_2OHCH_2]^+$ (0.5), 250 (0.2), 236 (0.4), 224 (0.4), 197 $[C_{14}H_{29}]^+$ (0.5), 127 (4), 111 (10), 97 (21), 90 $[CH_3OC(OH)=CHOH]^+$ (22), 83 (27), 69 (33), 57 (86). Peak 3 (t_R 24.67 min, 20.5%, 2-hydroxytricosanoic acid methyl ester), EI-MS *m/z*: 384 $[M]^+$ (6), 366 $[M - H_2O]^+$ (0.5), 325 $[M-CH_3OCO]^+$ (6), 306 (1), 280 $[M - CH_3OCO - CH_2OHCH_2]^+$ (0.5), 263 (0.6), 238 (0.5), 183 $[C_{13}H_{27}]^+$ (0.1), 159 (1), 145 (2), 125 (5), 111 (10), 97 (24), 90 $[CH_3OC(OH)=CHOH]^+$ (25), 83 (29), 69 (36), 57 (89). Peak 4 (t_R 28.56 min, 55.3%, 2-hydroxytetracosanoic acid methyl ester), EI-MS *m/z*: 398 $[M]^+$ (10), 380 $[M - H_2O]^+$ (0.2), 339 $[M - CH_3OCO]^+$ (8), 320 (1), 294 $[M - CH_3OCO - CH_2OHCH_2]^+$ (0.5), 278 (0.6), 252 (0.2), 238 (0.1), 183 $[C_{13}H_{27}]^+$ (0.5), 159 (2), 145 (3), 127 $[C_9H_{19}]^+$ (7), 111 (15), 97 (35), 90 $[CH_3OC(OH)=CHOH]^+$ (35), 83 (40), 69 (45), 57 (90). Peak 5 (t_R 32.16 min, 8.7%, 2-hydroxypentacosanoic acid methyl ester), EI-MS *m/z*: 412 $[M]^+$ (3), 353 $[M - CH_3OCO]^+$ (2), 334 (1), 308 $[M - CH_3OCO - CH_2OHCH_2]^+$ (0.2), 252 (0.4), 226(0.6), 207 (0.5), 183 $[C_{13}H_{27}]^+$ (0.4), 159 (1), 145 (2), 127 (5), 111 (10), 97 (21), 90 $[CH_3OC(OH)=CHOH]^+$ (20), 83 (24), 69 (30), 57 (89). The LCB, 2-amino-octadec-8-ene-1, 3, 4-triol: EI-MS *m/z*: 315 $[M]^+$ (0.1), 279 $[M - 2H_2O]^+$ (13), 261 $[M - 3H_2O]^+$ (0.5), 223 (5), 167 $[C_{12}H_{23}]^+$ (29), 149 (100), 113 (9), 104 (10), 71 (13), 57 (31). Positive ESI-MS *m/z*: 316 $[M + H]^+$, 298 $[M - H_2O + H]^+$, 280 $[M - 2H_2O + H]^+$, 262 $[M - 3H_2O + H]^+$.

2.6 Oxidation of the LCB from 1

The LCB (2.0 mg) from methanolysis of **1** was dissolved in 10% H_2SO_4 and acetone (5 ml each). $KMnO_4$ (100 mg) was added and stirred overnight at room temperature. The reaction was then quenched with aqueous $Na_2S_2O_3$ (5%). The reaction mixture, after removal of acetone, was extracted with Et_2O. The Et_2O layer was dried over Na_2SO_4, and concentrated, to give *n*-decanoic acid, GC-MS: GC t_R 12.07 min, EI-MS *m/z* 172 $[M]^+$ (4), 155 $[M - OH]^+$ (3), 143 (5), 129 (28), 87 (11), 73 (70), 60 $[CO_2H_2]^+$ (100).

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