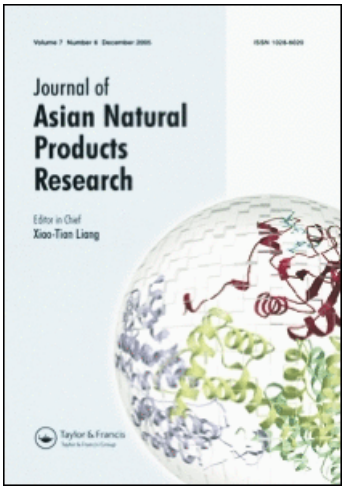

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SPHINGOSINE DERIVATIVES FROM THE SEEDS OF *ALLIUM TUBEROSUM*

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A new ceramide, named tuber-ceramide (**2**), along with a known cerebroside (**1**) were isolated from the seeds of *Allium tuberosum*. The structure of tuber-ceramide was determined on the basis of spectral data as N-(2′,3′-dihydroxy-tetracosenoyl)-2-amino-1,3,4-trihydroxy octadecane (**2**). This is the first report of sphingosine derivatives isolated from the genus *Allium*.

Keywords: *Allium tuberosum*; Sphingosine derivative; Liliaceae; Cerebroside; Ceramide; Tuber-ceramide

INTRODUCTION

Allium tuberosum is widely cultivated and used as food in China, whose seeds have been used as a traditional Chinese medicine for treating impotence and noctural emission [1]. Species of genus *Allium* are famous for their sulfur-containing biologically active natural products. With regard to the saponin constituents of the genus *Allium*, many steroidal saponins have been reported [2]. Previously, we reported the isolation and structure determination of nine constituents including two steroidal saponins from the seeds of *A. tuberosum* [3]. In our further search for potential bioactive

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components, two sphingosine derivatives, a new ceramide (tuber-ceramine, **2**) and a known cerebroside (soya-cerebroside I, **1**) were also isolated from this plant. The present paper deals with the isolation and structural elucidation of the new compound.

RESULTS AND DISCUSSION

The $CHCl_3$ soluble part of the ethanol extract from the seeds of *A. tuberosum* was obtained as previously described [2] and subjected to silica gel column chromatography to give compounds **1** and **2**.

Compound **1**, a white amorphous powder, showed a $[M+H]^+$ ion at *m/z* 714 in the FABMS, corresponding to a molecular formula of $C_{40}H_{75}NO_9$. The ^{1}HNMR and ^{13}CNMR spectra (see Table I) of **1** were in agreement with those of 1-O-β-D-glucopyrannosyl-(4E,8Z)-2-N-(2′-hydroxypalmitoyl) octadecasphinga-4,8-dienine [4] (soya-cerebroside I), previously isolated from the seeds of *Glycine max* [5].

Compound **2** was obtained as a white amorphous powder. The positive HRFAB-MS of **2** displayed a molecular ion peak at *m/z* 698.6277 $[M+H]^+$, consisting with the molecular formula $C_{42}H_{83}NO_6$ (calcd. 698.6298). The IR data at 2918, 2850, 1468 and 720 cm^{-1}, and an intense proton signal at δ 1.24–1.38 as well as two terminal methyl signals at δ 0.87 (t, $J=7.1$ Hz) and 0.86 (t, $J=7.0$ Hz) in the ^{1}HNMR spectrum (see Table I) indicated the presence of two long-chain aliphatic moieties. The ^{13}CNMR spectrum of **2** (see Table I) also displayed the characteristic of the aliphatic long-chain. The presence of the amide linkage in compound **2** was suggested by the observation of the amide band at 1639 cm^{-1} in the IR spectrum. The amide carbonyl signal was observed at δ 176.4 in the ^{13}CNMR spectrum, while the signal at δ 53.1 was attributed to the carbon attached to nitrogen. All of the above spectral informations were similar to those of compound **1**, which revealed that **2** was also a ceramide derivative.

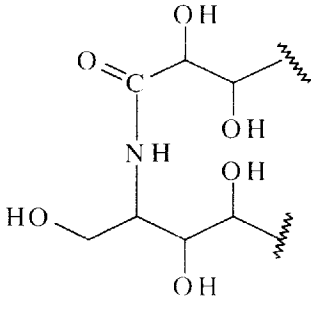
The ^{13}CNMR spectrum of **2** displayed a signal of secondary carbon at δ 61.9 supported the presence of a hydroxymethylene group at the C-1 of the sphingosine moiety. In addition, four methine carbon signals at δ 76.8, 76.3, 73.7, 72.9 indicated the presence of four additional hydroxyl groups in the molecule. The corresponding carbinylic proton signals were also observed at δ 4.35, 4.77, 4.56, 4.30, respectively. The locations of these hydroxyl groups were established by the ^{1}H–^{1}H COSY and HMBC spectral data. In the ^{1}H–^{1}H COSY spectrum, the H-2 multiplet at δ 5.15 showed coupling with three double doublets at δ 4.50, 4.44 and 4.35, which can be assigned to

TABLE I The ^{1}H- (500 MHz) and ^{13}CNMR (125 MHz) spectral data of compound **1** (CD_3OD) and **2** (pyridine-d5)

C	1		C	2	
	δ_C	δ_H		δ_C	δ_H
Sphingosine moiety			*Sphingosine moiety*		
1	69.7	4.05dd, 10.4, 5.4	1	61.9	4.50dd, 10.6, 4.5
		3.65dd, 10.4, 3.5			4.44dd, 10.6, 5.2
2	54.6	3.93m	2	53.1	5.15m
3	72.8	4.07t	3	76.8	4.35dd, 6.7, 4
4	131.3	5.42dd, 15.4, 7.2	4	72.9	4.30dt, 8.7, 6.7
5	134.4	5.67dt, 15.4, 7.2	5	34.3	2.27m
6,7	33.7, 33.3	2.01br.s			1.92m
8	131.9	5.34m	6	26.6	1.82–1.62
9	130.7	5.34m	7–15	30.4–29.5	1.38–1.24
10	33.7	1.92m	16	32.1	1.38–1.24
11–15	30.8–30.3	1.26s	17	22.9	1.38–1.24
16,17	33.1, 23.7	1.26s	18	14.3	0.86t, 7.1
18	14.5	0.84t, 7.1, 6.8	NH		6.71br.s
Fatty acid moiety			*Fatty acid moiety*		
C=O	177.2		C=O	176.4	
2′	73.1	3.93m	2′	76.3	4.77d, 4.0
3′	35.9	1.65m	3′	73.7	4.56ddd, 8.3, 4.2, 4.0
4′	26.3	1.49m			
5′–13′	30.8–30.3	1.26s	4′	32.6	2.06m
14′,15′	33.1, 23.7	1.26s	5′	26.6	1.38–1.24
16′	14.5	0.84t, 7.1, 6.8	6′	30.4–29.5	1.38–1.24
Sugar moiety			$\underline{C}H_2$–CH=	33.0	2.22m
1″	104.7	4.21d, 7.9	=CH–$\underline{C}H_2$	33.0	2.0m
2″	75.0	3.13dd, 9.1, 7.9	CH=$\underline{C}$H	130.8	5.55dd, 15.2, 5.8
3″	78.0	3.30t, 9.1		130.7	5.49dd, 15.2, 5.8
4″	71.6	3.21d, 6.8	22′	32.1	1.38–1.24
5″	77.9	3.25m	23′	22.9	1.38–1.24
6″	62.9	3.80d, 12.0	24′	14.3	0.87t, 7.0
		3.61dd, 12.0, 5.2	Others	30.4–29.5 × 16	

protons H-1a, H-1b and H-3, respectively. The H-3 proton showed a cross peak at δ 4.30 attributed to the H-4 proton, which in turn showed coupling with the multiplets at δ 2.27 and 1.92 assigned to the H-5 protons. The above $^1H-^1H$ correlation suggested the placement of a hydroxymethylene group and two hydroxyl groups in the long-chain moiety of **2**. The other two hydroxyl groups should be located at C-2′, C-3′ in the fatty acid moiety. Because, the carbinylic signal at δ 4.77 (t, $J = 4.0$ Hz) showed only one cross peak to another carbinylic signal at δ 4.56 (ddd, $J = 8.3, 4.2, 4.0$ Hz), which in turn showed coupling with the methylene protons at δ 2.06 (m) in the ^1H ^1H COSY spectrum of **2**. Additionally, the carbonyl carbon at δ 176.4 showed long-range correlation with H-2 proton at δ 5.15 and H-2′ proton at δ 4.77 in the HMBC spectrum. All of the observation suggested the presence of the partial structure A (Fig. 1) in the molecule of **2**, which was also supported by the fragment ions at *m/z* 148, 118, 100, 82 in the EI-MS spectrum (Fig. 2).

In the ^{13}CNMR spectrum, two CH carbon signals observed at δ 130.8 and 130.7 suggested compound **2** possessed a double bond. The corresponding olefinic protons appeared at δ 5.55 (dd, $J = 15.2, 5.8$ Hz) and 5.49 (dd, $J = 15.2, 5.8$ Hz) were observed and the large coupling constant of 15.2 Hz indicated the double bond with a *trans* configuration for the vicinal protons. The most prominent fragment observed at *m/z* 375, arising by the McLafferty rearrangement of the amide group in the EI-MS spectrum, indicated the double bond should be at the N-acyl moiety. However, all spectral data of **2** could not determine the exact location of the double bond. In addition, chemical methods were also unsuccessful due to scarcity of the sample



A

FIGURE 1 Partial structure of tuber-ceramine (**2**).

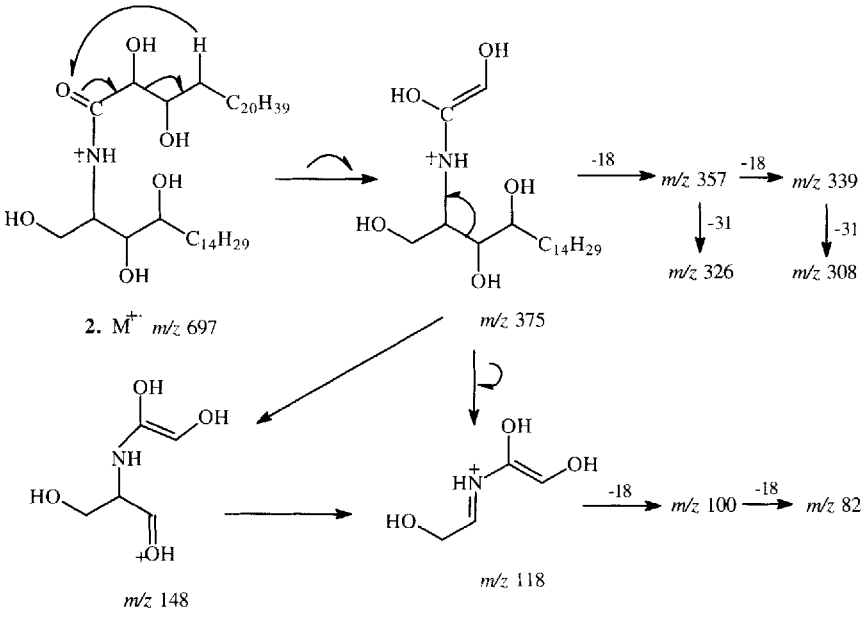


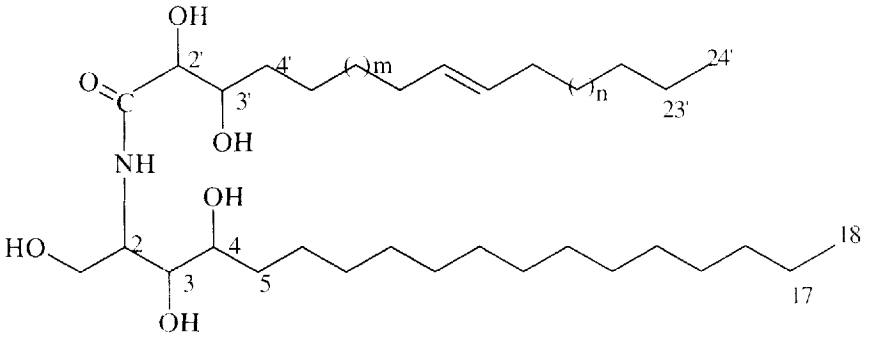
FIGURE 2 The fragmentation pattern of EI-MS for tuber-ceramide (**2**).

and its poor solubility in the usual solvents. Thus, the structure of compound **2** (Fig. 3) was tentatively proposed as N-(2′,3′-dihydroxy-tetracosenoyl)-2-amino-1,3,4-trihydroxy octadecane. To our knowledge, it is a new sphingosine derivative, here named as tuber-ceramide.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were obtained on a Boetius micro-melting apparatus and are uncorrected. Optical rotations were measured with Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 683 instrument. The ^{1}HNMR spectra (500 MHz) and ^{13}CNMR (125 MHz) were recorded on a Bruker AM-500 spectrometer, and the chemical shifts are reported in ppm using the solvent as reference. EI-MS was obtained on a VG Zab-2F and FABMS on a micromass AutoSpec-Ultima TOF mass spectrometer.



2. m + n = 12

FIGURE 3 Structure of compound **2**. $m + n = 12$.

Plant Material

The seeds of *A. tuberosum* were purchased from Beijing Tong-Ren-Tang Group, China, and were identified by Mr. Wei-Ze Liu, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation

Air-dried and powdered seeds of *A. tuberosum* (9.5 kg) were defatted by percolation with petroleum ether followed by extraction with hot 75% ethanol. The combined EtOH extracts were suspended in H_2O and partitioned with $CHCl_3$ and n-BuOH, respectively.

The $CHCl_3$ fraction was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH gradient to give five fractions (S_1–S_5). The fraction S_4 was repeatedly chromatographed over silica gel column using $CHCl_3$–MeOH as solvent to afford compound **1** (38 mg). The fraction S_5 was chromatographed over silica gel eluted with AcOEt–MeOH to afford compound **2** (18 mg).

Soya-cerebroside I (**1**) $C_{40}H_{75}NO_9$, white amorphous powder, m.p. 213–214°C, $[\alpha]_D^{21}$ +23(c 0.05, MeOH). FAB-MS *m/z* 714 $[M + H]^-$, 696 $(M + H - H_2O)$, 534 $(M + H - H_2O - Glu)$, 264. IR (KBr) ν_{max}: 3364, 2918, 2850, 1645, 1557, 1468, 1082, 720 cm^{-1}. ^{1}HNMR and ^{13}CNMR data were showed in Table I.

Tuber-ceramide (**2**) $C_{42}H_{83}NO_6$, white amorphous powder, m.p. 148–149°C, $[\alpha]_D^{21}$ +28(c 0.02, MeOH). HRFAB-MS *m/z* 698.6277 $[M+H]^+$ (calcd. 698.6298); EI-MS *m/z* 662 ($M+H-2H_2O$, 1), 375(4), 357($375-H_2O$, 6), 339($375-2H_2O$, 3), 326(4), 278(5), 268(6), 148(1), 131(20), 118(47), 100(50), 82(80), 57(85), 43(100). IR (KBr) ν_{max}: 3344, 2918, 2851, 1639, 1468, 1076, 720 cm^{-1}. ^{1}HNMR and ^{13}CNMR data were showed in Table I.

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