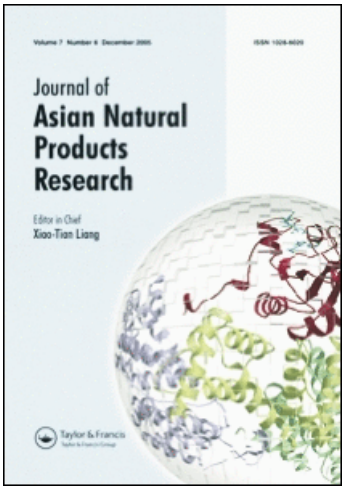

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CHEMICAL CONSTITUENTS OF *TYPHONIUM GIGANTEUM* ENGL.

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A new cerebroside, named typhonoside (**1**), was isolated from the root tuber of *Typhonium giganteum* Engl. along with three known compounds dipalmitin (**2**), α-monopalmitin (**3**) and 2,6-diamino-9-β-D-ribofuranosylpurine (**4**). The structure of **1** was determined to be 1-O-β-D-glucopyranosyl-(2S,3S,4R,8*Z*)-2-[(2′-hydroxyl-docosanoyl)amino]-8-otadecene-1,3,4-triol on the basis of spectral data.

Keywords: *Typhonium giganteum* Engl.; Cerebroside; Typhonoside (1)

INTRODUCTION

The dried root tuber of *Typhonium giganteum* Engl., a traditional Chinese medicine (Baifuzi), is recorded in Chinese Pharmacopoeia [1]. It has the effects of "dispelling wind-phlegm" and is used for the treatment of apoplexy with gurgling in the throat *etc.* To our knowledge, the investigation of the active components on the title medicine has not previously been undertaken. This paper describes the isolation and structure elucidation of a new cerebroside (**1**) as well as the identification of three known constituents from the root tuber of *T. giganteum* Engl.

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RESULTS AND DISCUSSION

Extraction of the air-dried root tubers of *T. giganteum* Engl. with 95% EtOH, and fractionation over siliceous earth column, afforded hexane, ethyl acetate and 95% EtOH fractions. The hexane and ethyl acetate extracts yielded a new cerebroside, typhonoside (**1**), and three known compounds, 1,2-di-O-palmitoyl-3-O-β-D-galactopyranosyl-sn-glycerols (**2**), α-monopalmitin (**3**), 2,6-diamino-9-β-D-ribofuranosyl-purine (**4**) by chromatography on silica gel and/or Sephadex LH-20 column, **2** has not been isolated previously from natural sources.

Compound **1** was isolated as white amorphous powder, m.p. 124 ~ 126°C. Its molecular formula was determined as $C_{46}H_{89}O_{10}N$ by HR-MS [m/z 838.6360$[M+Na]^+$ (calcd for $C_{46}H_{89}O_{10}N+Na$ m/z 838.6379)]. The IR spectrum showed bands at 3400 (OH and N—H), 1630 (amide carbonyl), 1080 (glycosidic C—O) and 720 (aliphatic long chains) cm^{-1}, which suggested the character of cerebrosides. The ^1H and ^{13}C NMR data of **1** (see Tab. I) indicated the presence of a sugar residue, an amide linkage and aliphatic long chains also suggesting the glycosphingolipid nature of **1**.

From the ^{13}C NMR of **1**, four carbon signals bearing hydroxyl group (δ70.4, 75.9, 72.6, 72.5) and one double bond (δ130.4, 130.2) were observed. The partial structure of **1** (see Fig. 2) could be deduced from the ^1H-^1H COSY and HMQC spectra. The signal at δ8.53 (N—H) gave a cross-peak with the signal at δ5.27(H-2) in the ^1H-^1H COSY spectrum of **1**, which, in turn, showed cross-peaks with the methylene protons (H-1) at δ4.53, 4.70 and 4.28(H-3). The latter correlated with the signal at δ4.21(H-4). In the same way, many correlations between δ4.21(H-4), δ1.92(H-5b) and δ1.98(H-6a); δ2.25(H-5a) and δ1.76(H-6b); δ1.76(H-6b) and δ2.23(H-7); δ2.23(H-7) and δ5.51(H-8); δ5.51(H-8) and δ5.45(H-9); δ5.45(H-9) and δ2.08(H-10) could be obtained.

From the HMBC spectra, the signal at δ8.53(N—H) gave a cross-peak with the signal at δ175.6(amide carbonyl), the later also gave a cross-peak with the signal at δ4.56(H-2′). In the ^1H-^1H COSY spectrum, the correlations between δ4.56(H-2′) and δ2.20(H-3′a), δ2.06(H-3′b); δ2.20(H-3′a), δ2.06(H-3′b) and δ1.74(H-4′a), δ1.70(H-4′b) could also be observed. Upon these, the partial structure of **1** was determined. Furthermore, in the HMBC spectrum of **1**, the signal at δ26.8(C-6) gave two cross-peaks with the signals at δ2.25(H-5a) and δ2.23(H-7) also supported the partial structure.

The length of the long-chain base (LCB) and the fatty acid (FA) was determined by EIMS (see Fig. 3). EI-MS fragments at m/z 280 and 535 are the two main ions of compound **1** by Mclafferty rearrangement. So, the

TABLE I NMR data of compound **1**(500 MHz for ^{1}H and 125 MHz for ^{13}C NMR in C_5D_5N)

Position	^{1}H *NMR(J in Hz)*	^{13}C *NMR*	*COSY correlation*	*HMBC*
1	4.70(1H, dd, 11, 6.5)	70.4	H-1b, H-2	
	4.53(1H, dd, 11, 6.5)		H-1a, H-2	
2	5.27(1H, m)	51.8	H-1, H-3, N—H	
3	4.28(1H, brs)	75.9	H-4, H-2	
4	4.21(1H, brs)	72.6	H-5, H-3	
5	2.25(1H, m)	34.0	H-5b, H-6b	C-6
	1.92(1H, m)		H-5a, H-4, H-6a	
6	1.98(1H, m)	26.8	H-5b, H-7, H-6b	C-8, C-9
	1.76(1H, m)		H-5a, H-7, H-6a	
7	2.23(2H, m)	27.9	H-6a, H-6b, H-8	C-6, C-8, C-9
8	5.51(1H, dt, 9, 5)	130.4	H-7, H-9	C-7
9	5.45(1H, dt, 9, 5)	130.2	H-8, H-10	C-10
10	2.08(2H, m)	27.6	H-9, H-11	C-8, C-9
11 – 15	1.30(10H)	29.9		
16	1.26(2H)	32.1		
17	1.25(2H)	22.9		
18	0.86(3H, t, 6.5)	14.2		
1′		175.6		
2′	4.56(1H, m)	72.5	H-3′a, H-3′b	C-1′
3′	2.20(1H, m)	35.6	H-2′, H-3′b, H-4′a	
	2.06(1H, m)		H-2′, H-3′a, H-4′b	
4′	1.74(1H, m)	25.2	H-3′a, H-3′b, H-5′	
	1.70(1H, m)			
5′ – 19′	1.30(30H)	29.9		
20′	1.26(2H)	32.1		
21′	1.25(2H)	22.9		
22′	0.86(3H, t, 6.5)	14.2		
1″	4.94(1H, d, 7.5)	105.6	H-2″	
2″	4.00(1H, m)	75.1	H-1″, H-3″	
3″	4.18(1H, m)	78.5	H-2″, H-4″	
4″	4.18(1H, m)	71.6	H-3″, H-5″	
5″	3.85(1H, m)	78.5	H-4″, H-6″a, H-6″b	
6″	4.48(1H, m)	62.7	H-5″, H-6″b	
	4.33(1H, m)		H-5″, H-6″a	
N—H	8.53(1H, d, 9)		H-2	C-1′

Assignments are supported by ^{1}H-^{1}H COSY and HMQC data.

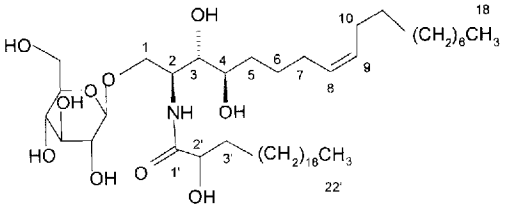

FIGURE 1 Structure of compound **1**.

location of the double bond must be in the LCB. In addition, two fragment ions at m/z 428 and 225 are from m/z 653 by α-cleavage. Then, the number of carbons in LCB and FA was determined to be 18 and 22 respectively.

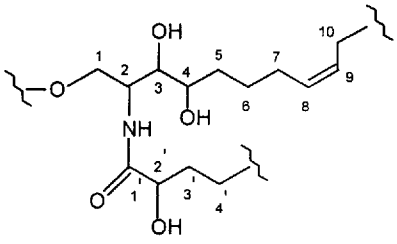

FIGURE 2 Partial structure of compound **1**.

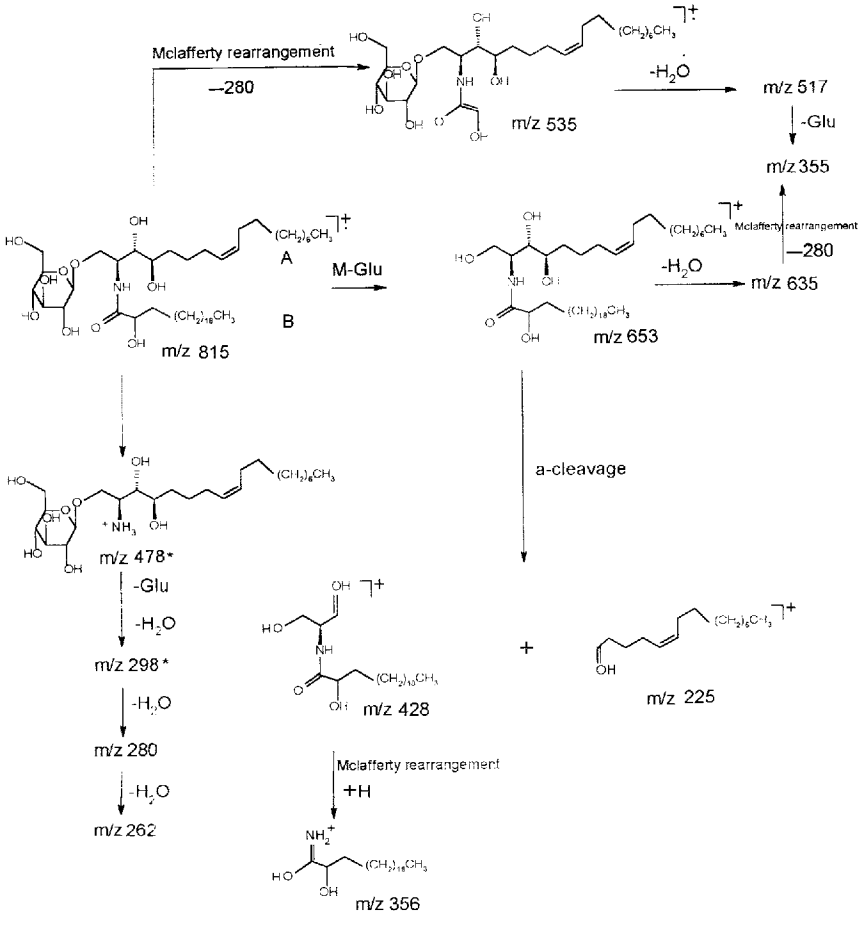

FIGURE 3 Possible EI-MS fragments of typhonoside (**1**) *peaks have not been observed.

The signals at δ 105.7, 75.1, 78.5, 78.5, 71.5 and 62.8 in the ^{13}C NMR spectrum suggested the sugar moiety in **1** was a β-glucopyranoside. The coupling constant between H-1″[δ 4.94(1H, *d*, 7.5 Hz)] and H-2″[δ 4.00(1H, *d*, 7.5 Hz)] also supported the β-D-configuration of the sugar. The 8,9 alkenyl bond was found to be *cis*, as evidence by the vicinal coupling constants ($J_{8,9} = 9$ Hz). The *cis* relationship of the double bond was also supported by the chemical shifts of C_7, C_{10} (27.9, 27.6). Usually, the signals of carbons next to a *cis* double bond appeared at δ 27–28, while those of a *trans* double bond appeared at δ 32–33 [2]. The chemical shift of H-2(δ 5.27) and the carbon chemical shifts at δ 70.4(C-1), 51.8(C-2), 75.9(C-3), 72.6(C-4), 175.6(C-1) and 72.5(C-2′) in **1** were virtually identical with those of the reported data of other (2S,3S,4R)-phytosphingosine moieties [3,4]. Thus, the structure of **1** was determined to be 1-O-β-D-glucopyranosyl-(2S,3S,4R,8E)-2-[(2′-hydroxydocosanoyl)amino]-8-octadecene-1,3,4-triol, named typhonoside.

Compound **2** was obtained as an amorphous powder and analyzed for $C_{41}H_{78}O_{10}$ (TOF 753 $[M+Na]^+$), m.p. 91 ~ 92°C. Its IR spectrum showed bands at 2870, 2840, 1720 and 720 cm^{-1}, which showed the character of glycolipid. This character could also be obtained from the indication of the ^1H and ^{13}C NMR spectra of **2** (one sugar, one glycerol and aliphatic long chains). The signals at δ 105.8, 75.0, 73.9, 72.3, 72.3, 64.5 suggested that the sugar moiety in **2** was a β-D-galactopyranoside. The coupling constant of H-1[δ 4.78 (1H, *d*, 8.0 Hz)] also suggested the β-configuration of the sugar. The length of the fatty acid was deduced by the molecular weight, 730(MW)-163(Sugar) -89(glycerol) = 478(part of fatty acid, PFA). As there are two signals at δ 173.6 in the ^{13}C NMR spectrum, the fatty acid in **2** must be two molecules. So PFA was composed by two long fatty acids and these are palmitic acids(MW 256). The structure of **2** was identified to be 1,2-di-O-palmitoyl-3-O-β-D-galactopyranosyl-sn-glycerols, which has been synthesized [5].

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were measured on a Fisher-Johns apparatus and are uncorrected. The IR spectra were obtained on a Perkin-Elmer 983G spectrometer. TOF-MS was obtained in a glycerol matrix in a positive ion mode on a Diflex III spectrometer. EIMS spectra were obtained on a

ZabSpecE mass spectrometer. NMR spectra were measured on a Bruker AM-500(500 MHz) instrument, and chemical shifts were referenced to TMS.

Plant Material

The root tubers of *T. giganteum* Engl. were collected in September, 1999, in Yuxian county, Henan Province, China. A voucher specimen (No. YBF 9901) is preserved in the Herbarium of our Institute.

Extraction and Isolation

The pieces of air-dried root tubers of *T. giganteum* Engl. (20 kg) were extracted with 95% EtOH (4 × 5 l) for 3 h each under reflex. The extraction was concentrated *in vacuo* to yield a syrup-like residue(310 g), which was mixed with siliceous earth(80–100 mesh, 400 g) and eluted with hexane, EtOAc, 95% EtOH to give three fractions.

The hexane fraction (75 g) was subjected to column chromatography over silica gel (6 × 60 cm, 100–200 mesh, 750 g) eluted with a solvent of petroleum ether-Me_2CO-MeOH gradient (500 ml each eluent) yielding eight crude fractions monitored by TLC. The fraction 2 yielded a white powder, which was crystallized to obtain compound **3** (100 mg). Fraction 4 was eluted with petroleum-ether-Me_2CO (75 : 25) as solvent to afford compound **2** (15 mg). Fraction 6 (2.8 g) was chromatographed over silica gel column eluted with $CHCl_3$-MeOH (95 : 5) to collect 44 fractions (100 ml each) and fractions 42–44 were further purified by Sephadex LH-20 using $CHCl_3$-MeOH (1 : 1) as eluent to give compound **1**(4 mg).

The EtOAc fraction (10 g) was chromatographed on a middle pressure silica gel (Silica G 60, 200 g) column eluted with $CHCl_3$–MeOH gradient (100 ml each eluent) to collect 20 fractions. Fractions 8–10 yielded compound **4** (5 mg) by crystallization from Me_2CO.

Typhonoside **(1)** m.p. 124–126°C. $[\alpha]_D^{20}$ + 35.8 (c 0.75, C_5H_5N). HR MALDI-FT-ICRMS m/z 838.6360$[M+Na]^+$ (calcd for $C_{46}H_{89}O_{10}N$+Na 838.6379). IR(KBr) $\upsilon_{max}(cm^{-1})$: 3400, 1630, 1080 and 720. ^1H NMR, ^{13}C NMR and ^1H-^1H COSY spectral data (see Tab. I). TOFMS, 838.81 $[M+Na]^-$; EI-MS. m/z(%) 797(6) [M—H_2O], 692(10), 635(34), 535(3), 517(10), 428(28), 410(24), 398(32), 356(78), 355(50), 280(62), 262(49), 225(19), 83(100).

1,2-di-O-palmitoyl-3-O-(β-D-galactopyranosyl)-sn-glycerols **(2)** m.p. 90–92°C. IR(KBr) $\upsilon_{max}(cm^{-1})$. 2870, 2840, 1720, 720. TOFMS, 753$[M+Na]^+$.

^{13}C NMR(pyridine-d_5, 125 MHz) δ ppm: 173.6(two carbons), 105.81, 75.0, 73.9, 72.3, 70.0, 69.1, 66.6, 64.5, 34.4(two carbons), 32.1(two carbons), 29.8(many carbons), 25.3(two carbons), 22.9(two carbons), 14.3(two carbons). ^{1}H NMR (pyridine-d_5, 500 MHz) δ ppm: 4.90(1H, *dd*, $J = 5,8$ Hz), 4.87(1H, *d*, $J = 8$ Hz), 4.80(1H, m), 4.61(1H, *dd*, $J = 4.5$, 11 Hz), 4.57(1H, *dd*, $J = 6$, 11 Hz), 4.49(2H, m), 4.41(1H, m), 4.17(1H, *d*, $J = 7.5$ Hz), 4.15(1H, m), 4.11(1H, m), 2.40(2H, *t*, $J = 7.5$ Hz), 2.33(2H, *t*, $J = 7.5$ Hz), 1.65(4H, m), 1.26(48H), 0.85(6H, *t*, $J = 7$ Hz).

α-monpalmitin (**3**) m.p. 56–58°C. IR(KBr) υ_{max}(cm^{-1}): 3210, 2920, 2852, 1700, 1435, 1360, 1180; EIMS m/z(%): 330(M^+)(2.0), 313(2.0), 312(2.0), 299(9.8), 257(15.3), 239(51.2), 227(3.1), 213(5.3), 134(28.7), 98(64.3), 57(97.8), 43(100).

2,6-diamino-9-β-D-ribofuranosylpurine (**4**) m.p. 220–222°C. FAB MS: 282(M^+). ^{13}C NMR(pyridine-d_5, 125 MHz) δ ppm: 157.7, 153.3, 151.4, 140.6, 121.6, 90.9, 87.8, 75.5, 72.4, 63.1.

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