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Structure determination and synthesis of a new cerebroside isolated from the traditional Chinese medicine *Typhonium giganteum* Engl.

Xuesong Chen,[a,b] Yu-Lin Wu[a,*] and Dihua Chen[b,*]

[a]*State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China*

[b]*Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, 100 Dongbeiwang Road, Beijing 100094, China*

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Abstract—A new cerebroside with C18-4,8-sphingadienine as the long chain base has been isolated from the traditional Chinese medicine *Typhonium giganteum* Engl., and its structure was determined by 2D NMR and MS methods. It was then synthesized using D-xylose and ascorbic acid as the chiral starting materials. © 2002 Elsevier Science Ltd. All rights reserved.

The dried root tuber of *Typhonium giganteum* Engl. is recorded in Chinese pharmacopoeia as a traditional Chinese medicine and named Baifuzi in Chinese.[1] It has the effect of 'dispelling wind-phelgm' and has been used for the treatment of cerebral apoplexy for a long time in China. However, the active components of the medicine were not clear until now.

In our research searching for the biologically active constituents from *T. giganteum* Engl., we have isolated eight cerebrosides for the first time from this plant. It has been reported that cerebrosides act by signal transduction through cell membranes and exhibit significant activities. In 1991, Harouse[2a] reported that galactosyl ceramide inhibited the entry of HIV-1 in neural cell lines. In 1990, Kitagawa reported[2b] that cerebrosides from soybean, the seeds of *Glycine max* Merrill, showed ionophoric activity for Ca^{2+} ions. There are many reviews on the biological activities of cerebrosides, sphingosine and sphingolipids.[3] A significant amount of synthetic work[4] on cerebrosides has been reported. Due to their scarcity in natural sources and their interesting biological activities, in addition to isolation and structure determination we also began total syntheses of these new cerebrosides. Herein, we report the structure determination and the synthesis of typhoniside A (Fig. 1), a representative of these cerebrosides.

The EtOH extract (310 g) of *T. giganteum* Engl. (20 kg) collected from Yu county Henan province, China, was divided into three fractions using hexane, EtOAc and 95% EtOH. The hexane fraction (75 g) was subjected to column chromatography over silica gel (eluent: P.E./Me_2CO/MeOH) to give eight crude fractions. Fraction 6 (2.8 g) was chromatographed using a silica gel column (eluent: $CHCl_3$/MeOH, 95:5) to afford 200 mg of a

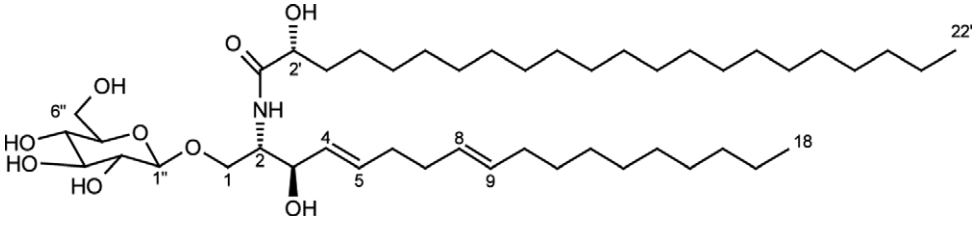

Figure 1. The structure of typhoniside A (**1**).

Keywords: *Typhonium giganteum* Engl.; cerebroside; typhoniside.

* Corresponding authors. Fax: 86 10 67635913; e-mail: ylwu@pub.sioc.ac.cn

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mixture of cerebrosides, which was purified further to give eight cerebrosides by HPLC (eluent: MeOH/H_2O, 96:4) including typhoniside A (6.0 mg).

Typhoniside A was a white amorphous powder with mp 172–174°C. Its molecular formula was determined as $C_{46}H_{87}O_9N$ by HRMS m/z 798.6451 $[M+H]^+$ (calcd for $C_{46}H_{88}O_9N$ m/z 798.6453). Its IR spectrum (3400, 1630, 1080, 720 cm^{-1}) and ^1H and ^{13}C NMR spectra (Table 1) suggested the glycosphingolipid nature.

The signals at δ 105.6, 78.5, 71.7, 78.5 and 62.8 in the ^{13}C NMR spectrum suggested that the sugar moiety in **1** was a β-glucopyranoside. The coupling constant between H-1″ [δ 4.91 (1H, d, 7.7 Hz)] and H-2″ [δ 4.03 (1H, t, 7.7 Hz)] supported the β-D-configuration of the sugar. The 4,5 alkenyl bond was found to be *trans*, as evidenced by the vicinal coupling constant ($J_{4,5}=15.3$ Hz). The 8,9 alkenyl bond was also found to be *trans*, as evidenced by the chemical shifts of C-7 and C-10 (32.88, 32.75). Usually the signals of the carbons next to a *trans* double bond appear at δ 32–33.[5] With this information we determined that **1** is a glucosylceramide.

The length of the long chain base (LCB) and the fatty acid (FA) were determined by HREIMS. Two diagnostic fragment ions at m/z 311.3259 (calcd for $C_{21}H_{43}O$: 311.3314) and at m/z 306.2489 (calcd for $C_{19}H_{32}O_2N$: 306.2433) were observed for part of the α-hydroxydocosanoyl acid and the fragment of the α-hydroxydocosanoyl acid due to a Mclafferty rearrangement. The number of carbons in the LCB and FA were determined to be 18 and 22, respectively.

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

Position	^1H NMR (J in Hz)	^{13}C NMR	HMBC
1	4.70 (1H, dd, 10.3, 5.8)	70.1	1″
	4.25 (1H, dd, 10.3, 4.0)		
2	4.81 (1H, m)	54.7	1
3	4.76 (1H, t, 6.1)	72.594[a]	1, 2
4	5.93 (1H, dd, 15.3, 6.1)	132.112[b]	3, 4
5	6.00 (1H, dt, 15.3, 5.3)	131.988[b]	4, 6
6	2.16 (2H, s-like)	32.93[c]	5, 7
7	2.01 (2H, s-like)	32.88[c]	6, 8
8	5.49 (1H, s-like)	129.96	7, 9
9	5.49 (1H, s-like)	131.14	8, 10
10	2.16 (2H)	32.75[c]	9
11–15	1.25–1.30 (10H)	29.51–30.18	
16	1.25–1.30 (2H)	32.1	
17	1.25–1.30 (2H)	22.9	
18	0.86 (3H, t, 6.0)	14.2	
1′		175.6	
2′	4.57 (1H, dd, 7.7, 3.6)	72.396[a]	1
3′	2.04 (2H)	35.7	
4′	1.78 (2H)	25.9	
5′–19′	1.25–1.30 (30H)	29.5–30.2	
20′	1.25–1.30 (2H)	32.1	
21′	1.25–1.30 (2H)	22.9	
22′	0.86 (3H, t, 6.0)	14.2	
1″	4.91 (1H, d, 7.7)	105.6	1
2″	4.03 (1H, t, 7.7)	75.1	3″
3″	4.24 (1H, m)	78.5	4″
4″	4.24 (1H, m)	71.7	5″
5″	3.90 (1H, m)	78.5	6″
6″	4.52 (1H, dd, 12.0, 1.9)	62.8	5″
	4.34 (1H, dd, 12.0, 5.4)		
N–H	8.35 (1H, d, 9)		1′

Note: a, b, c indicates that the chemical shifts are interchangeable.

The carbon chemical shifts at δ 70.1 (C-1), 54.7 (C-2), 72.6 (C-3), 175.6 (C-1′) and 72.4 (C-2′) were in agreement with those reported for other (2*S*,3*R*,2′*R*) sphingosine moieties.[6] Thus, the structure of **1** was assigned as 1-*O*-β-D-glucopyranosyl-(2*S*,3*R*,4*E*,8*E*)-2-[2′(*R*)-hydroxydocosanoylamino]-4,8-octadecadiene-1,3-diol.

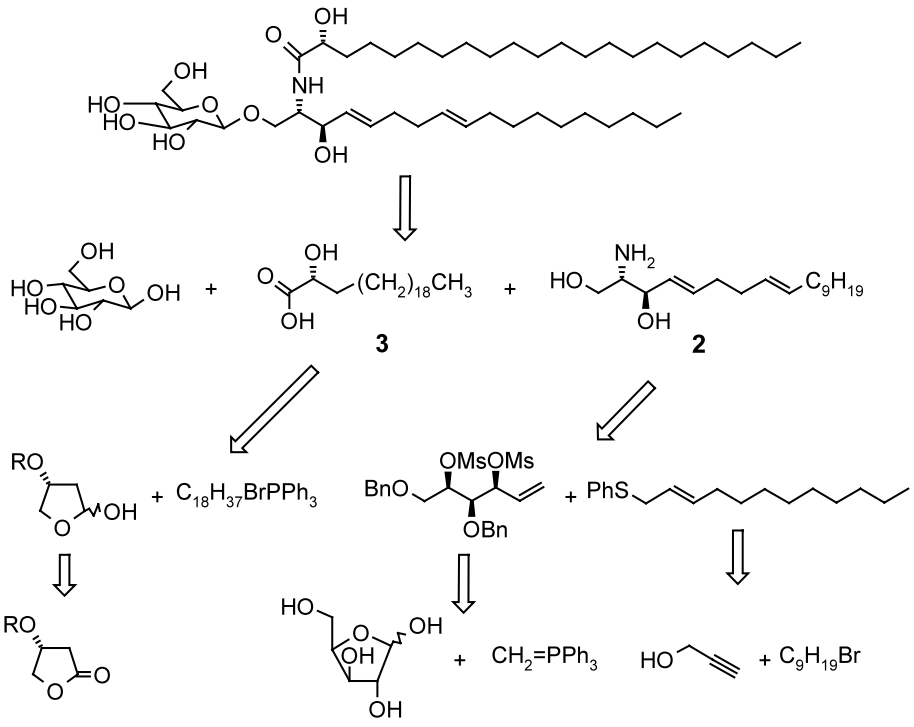
After the structure had been determined we designed a facile and convergent approach for the synthesis of typhoniside A to confirm the structure and try to find another way to provide this cerebroside for biological investigation.

Based on the retrosynthetic analysis depicted in Scheme 1, the molecule **1** was divided into three fragments. The sphingadiene fragment **2** was synthesized from D-xylose via a S_N2' type of reaction mediated by a thioether carbanion as in our previous report.[7] The chirally pure FA part **3**, (*R*)-α-hydroxydocosanoyl acid, was prepared from (*R*)-4-hydroxytetrahydrofuran-2-one **4**, which in turn could be obtained from L-ascorbic acid.[8] The synthesis from **4** to **3** is shown in Scheme 2.

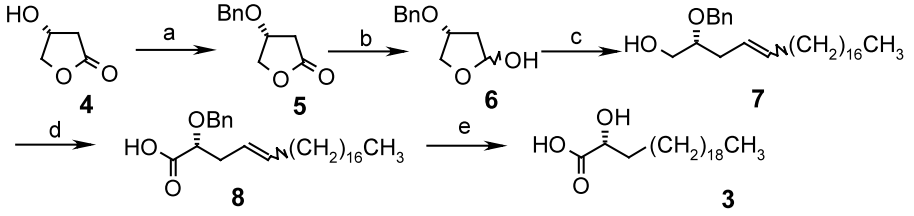
Treatment of (*R*)-4-hydroxytetrahydrofuran-2-one **4**[8] with a catalytic amount (20 mol%) of trimethylsilyl trifluoromethanesulfonate (TMS-OTf) and 1.5 equivalents of benzyl trichloroacetimidate prepared by sodium hydride catalyzed addition of benzyl alcohol to trichloroacetonitrile[9] yielded **5**, $[\alpha]_D^{20}$ +35.9 (*c* 0.6, CH_2Cl_2). Lactone **5** was converted to hemiacetal **6** by DIBAL-H reduction. The Wittig reagent generated from the octadecanyl bromide phosphonium salt reacted with hemiacetal **6** to give alcohol **7**, which was oxidized to afford acid **8**. Removal of the benzyl protecting group and hydrogenation of the double bond gave **3**, $[\alpha]_D^{20}$ +12 (*c* 0.93, pyridine).

Thereafter ceramide **12** was synthesized as shown in Scheme 3. Acylation of **3** with Ac_2O yielded **9**, $[\alpha]_D^{20}$ +6.6 (*c* 0.5, $CHCl_3$).[10] Acid **9** was treated *N*-hydroxysuccinimide in the presence of DCC to give amide **10**, which was reacted with sphingadiene **2** in the presence of DMAP and Et_3N to yield **11**, $[\alpha]_D^{20}$ +10.7 (*c* 0.5, $CHCl_3$). Removal of the protecting group with K_2CO_3 gave ceramide **12**. The ceramide **12** was converted to the glycosyl acceptor **13**, $[\alpha]_D^{20}$ +6.6 (*c* 0.5, $CHCl_3$), using a conventional method[10] in 77% overall yield.

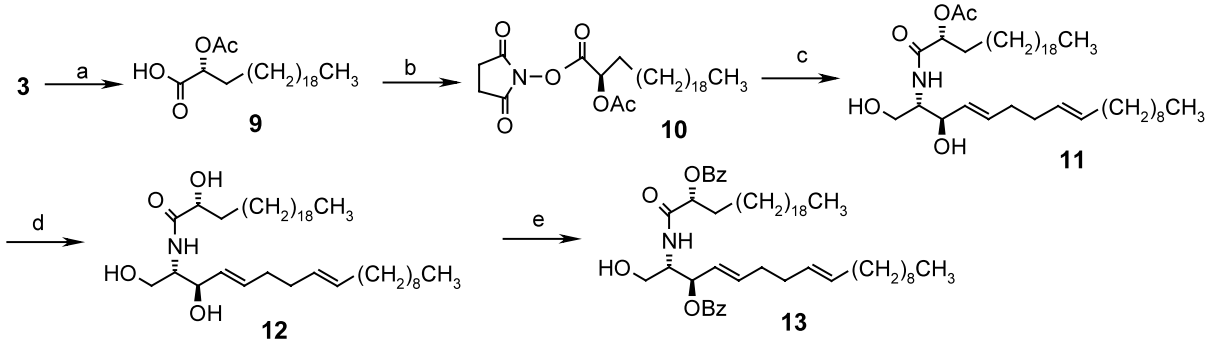
The final glycosylation was performed in the usual way. Thus ceramide **13** was treated with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimedate **14**[11] in the presence of a catalytic amount of TMSOTf (0.05 equiv.) in CH_2Cl_2 to provide the 1-*O*-glucosylated product **15**, $[\alpha]_D^{20}$ +20.8 (*c* 1.3, $CHCl_3$). Deprotection of the alcohol groups in **15** yielded **1**, $[\alpha]_D^{20}$ +4.0 (*c* 0.28, $CHCl_3$/MeOH 1:1). All the spectroscopic properties of



Scheme 1. Retrosynthetic analysis of typhoniside A.



Scheme 2. *Reagents and conditions*: (a) TMSOTf, $CCl_3C(NH)OBn$, cyclohexane: CH_2Cl_2 (2:1), 71%; (b) DIBAL-H, CH_2Cl_2, 74%; (c) BuLi, $C_{18}H_{37}PPh_3Br$, THF, 0°C, 95%; (d) PDC, DMF, rt 32 h, 68%; (e) 10% Pd/C, cyclohexane:EtOH (1:2), 40°C, 20 h, 88%.



Scheme 3. *Reagents and conditions*: (a) Ac_2O, DMAP, CH_2Cl_2, overnight, pyridine, rt, 100%; (b) *N*-hydroxysuccinimide, DCC, CH_2Cl_2, 5 h, 80%; (c) **2**, DMAP, Et_3N, THF, 8 h, 95%; (d) K_2CO_3, MeOH, 90%; (e) (i) TrCl, pyridine, DMAP, 80°C; (ii) BzCl, pyridine, 14 h; (iii) *p*-TsOH, CH_2Cl_2:MeOH (2:1) 8 h, 77% over the three steps.

the product were in agreement with those of the isolated natural material (Scheme 4).[12]

In conclusion, a new cerebroside, typhoniside A (**1**) has been isolated from the traditional Chinese medicine *Typhonium giganteum* Engl., and was synthesized via the chiron approach.

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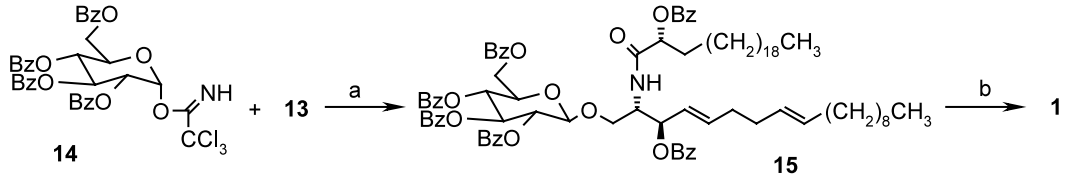

Scheme 4. *Reagents and conditions*: (a) TMSOTf, CH_2Cl_2, 65%; (b) NaOMe, MeOH, 80%.

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