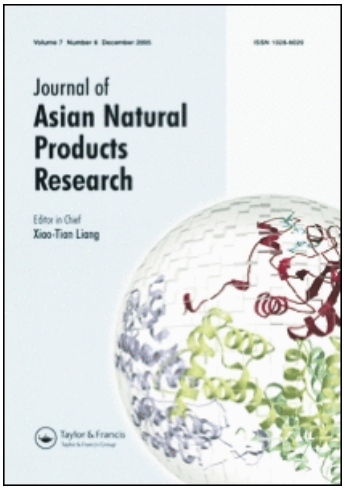

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A new cerebroside from *Uvaria tonkinensis* var. *subglabra*

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Note

A new cerebroside from *Uvaria tonkinensis* var. *subglabra*

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A new cerebroside, subglain A (**1**), together with five known compounds (**2–6**) have been isolated from the stems of *Uvaria tonkinensis* var. *subglabra*. The structure of **1** has been determined to be 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*,2′*R*)-2-[*N*-(2′-hydroxytetracosanyl)-*N*-(1″,2″-dihydroxyethyl)-amide]-8-tetradecene-1,3,4-triol by spectroscopic evidence. The known compounds were identified as schisandriside (**2**), erythritol (**3**), β-D-glucopyranose (**4**), kaempferol-3,7-*O*-α-L-dirhamnoside (**5**), and (+)-lyoniresinol (**6**).

Keywords: *Uvaria tonkinensis* var. *subglabra*; Annonaceaes; Subglain A; Cerebroside

1. Introduction

As a continuation of our work in search of anticancer constituents from plant sources [1,2], *Uvaria tonkinensis* var. *subglabra*, which is used as a folk medicine for the treatment of different diseases in China, was subjected to phytochemical studies. From the ethanolic extract, a new compound (**1**) and five known compounds (**2–6**) have been isolated.

2. Results and discussion

Compound **1** gave a quasi-molecular ion at *m/z* 848.6443 ($[M + H]^+$) by HR-FABMS, which is consistent with a molecular formula of $C_{46}H_{90}O_{12}N$. The IR spectrum shows bands at 3369 (OH), 1614 (amide carbonyl), 1080 (glycosidic C–O), and 720 (aliphatic long chains) cm^{-1}. The ^1H and ^{13}C NMR data of **1** (table I) indicate a sugar residue, an amide linkage and two aliphatic long chains. All the data suggest that **1** has a glycosphingolipid nature. Six carbon signals bearing hydroxyl groups (δ 69.9, 75.6, 73.0, 73.9, 64.4 and 73.0) besides the sugar

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data for **1** (CD_3OD, *J* in Hz).

Position	δ_H *(ppm)*	δ_C *(ppm)*	*Position*	δ_H *(ppm)*	δ_C *(ppm)*
1	3.81 (1H, dd, 10.5, 4)	69.9	3′	1.62 (1H, m)	35.7
	4.05 (1H, dd, 10.5, 6.5)			1.74 (1H, m)	
2	4.26 (1H, m)	51.7	4′	1.40 (2H, m)	26.1
3	3.58 (1H, t, 5.5)	75.6	5′-21′	1.28 (2H)	30.7
4	3.53 (1H, m)	73.0	22′	1.28 (2H)	33.0
5	1.68 (1H, m)	33.0	23′	1.28 (2H)	23.7
	1.40 (1H, m)		24′	0.89 (3H, t, 7)	14.4
6	1.58 (2H, m)	27.2	1″	3.63 (1H, m)	73.9
7	2.04 (2H, m)	28.3	2″	3.50 (1H, dd, 11, 6)	64.4
8	5.38 (1H, dt, 11, 6.5)	131.0		3.56 (1H, dd, 11, 5)	
9	5.34 (1H, dt, 11, 6.5)	130.8	1‴	4.29 (1H, d, 7.5)	104.7
10	2.07 (2H, m)	28.2	2‴	3.19 (1H, dd, 9, 7.5)	75.0
11	1.28 (2H)	30.7	3‴	3.34 (1H, m)	78.0
12	1.28 (2H)	33.0	4‴	3.27 (1H, m)	71.6
13	1.28 (2H)	23.7	5‴	3.28 (1H, m)	78.0
14	0.89 (3H, t, 7)	14.4	6‴	3.86 (1H, m)	62.0
1′		177.1		3.67 (1H, m)	
2′	4.01 (1H, dd, 7.5, 4)	73.0			

unit and one double bond (δ 131.0, 130.8) appear in the ^{13}C NMR of **1**. The signal at δ 4.26 (H-2) shows a cross-peak with the signals at δ 3.81, 4.05 (H-1) and 3.58 (H-3) in $^{1}H-^{1}H$ COSY spectrum of **1**. The latter correlates with the signal at δ 3.53 (H-4). There are also correlations between δ 3.56, 3.50 (H-2″) and 3.63 (H-1″); 1.74, 1.62 (H-3′) and 4.01 (H-2′); δ 1.40, 1.68 (H-5) and 3.53 (H-4); δ 1.58 (H-6) and 1.40, 1.68 (H-5); δ 2.02-2.08 (H-7) and 1.58 (H-6); δ 5.33–5.38 (H-8 and H-9) and 2.02–2.08 (H-7 and H-10). The partial structure of **1** was deduced by combined analysis of DEPT, HMQC and HMBC (figure 1) spectra. In the ^{1}H NMR of **1**, however, there are no signals between the δ 8–9, which indicates a tertiary amide may be present [3].

The length of the long-chain base (LCB) and the fatty acid (FA) were determined by FAB-MS (figure 2). FAB-MS fragments at *m/z* 685, 481, 480, 443, 319 and 283 are the six main ions of compound **1**, predicting 14 and 24 carbons in the LCB and FA, respectively, and that the double bond must be located in the LCB. In addition, the fragments at *m/z* 481 and 443 suggest compound **1** is a tertiary amide.

^{1}H and ^{13}C NMR spectral data (table 1) of the sugar moiety in **1** suggest a β-glucopyranoside. The coupling constant between H-1‴ [δ 4.29 (1H, d, 7.5 Hz)] and H-2‴ [δ 3.18 (1H, dd, 9, 7.5 Hz)] also support a β-configuration for the sugar. The 8,9 alkenyl bond should be *cis* according to the chemical shifts of C-7 and C-10 (δ 28.3, 28.2) [3, 4] and the coupling constant of H-8 and H-9 (11 Hz). The carbon chemical shifts at δ 69.9 (C-1), 51.7

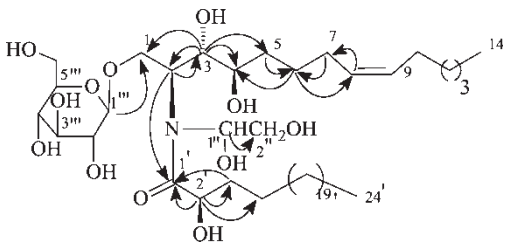

Figure 1. Structure and the key HMBC correlations of **1**.

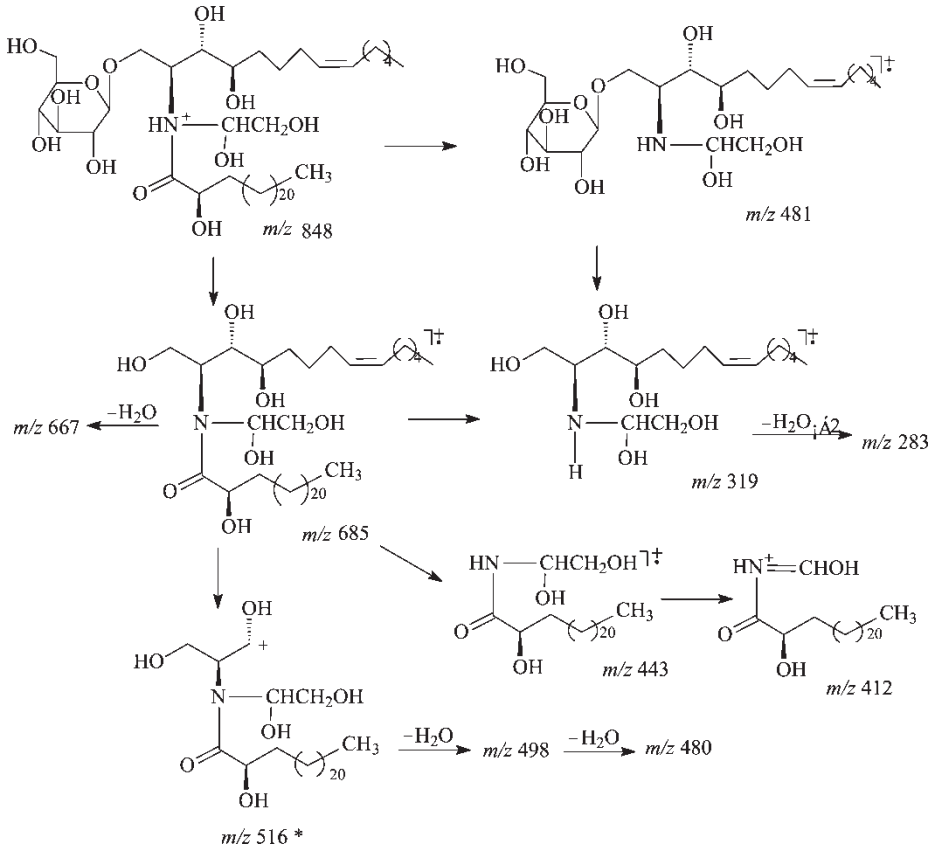

Figure 2. Possible FAB-MS fragments of **1** (* peak not observed).

(C-2), 75.6 (C-3), 73.0 (C-4), 177.1 (C-1′) and 73.0 (C-2′) in **1** are virtually identical to those of typhonoside [3]. Including biogenetic considerations, compound **1** was determined to be 1-*O*-β-D-glucopyranosyl-(2*S*,3*S*,4*R*,8*Z*,2′*R*)-2-[*N*-(2′-hydroxytetracosanoyl)-*N*-(1″,2″-dihydroxyethyl)-amino]-8-tetradecene-1,3,4-triol, named subglain A. To our knowledge, this is the first report of a cerebroside with a tertiary amide structure.

3. Experimental

3.1 General experimental procedures

Melting points (uncorrected) were determined using a Fisher-Johns melting point apparatus. IR spectra were obtained with a Perkin-Elmer 983 G spectrometer. EIMS spectra were recorded on a Zabspec E spectrometer and HR-FABFMS spectra on a ZAB-HS spectrometer. NMR spectra were taken on a Bruker AM 500 spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Separation and purification were performed on column chromatography, over silica gel (200–300 mesh, Qingdao Marine Chemical Inc. China),

TLC (silica gel GF_{254}, Qingdao Marine Chemical Inc. China) and Sephadex LH-20 (Amersham Pharmacin Biotech).

3.2 Plant material

The stems of *Uvaria tonkinensis* var. *subglabra* were collected from the Yunnan province of China in February 2002 and identified by Professor Yu-Lin Lin from the authors' institute. A voucher specimen (no. Y02021720) has been deposited in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, in Beijing.

3.3 Extraction and isolation

The dried stems of *Uvaria tonkinensis* var. *subglabra* (10 kg) were extracted with hot 95% EtOH (3 × 20 l). After evaporating EtOH *in vacuo*, the resultant dark brown residue (130 g) was chromatographed over silica gel (1.0 kg) and eluted with mixtures of light petroleum–acetone in a step gradient. Fractions obtained in the general procedure were combined into 14 groups: Fraction-1 [5 g, light petroleum 100%, 2 l], fraction-2 [1 g, light petroleum–acetone 98:2, 1.5 l], fractions-3 and 4 [0.5 g and 1 g, light petroleum–acetone 30:1, each 2 l], fraction-5 [20 g light petroleum–acetone 8:1, 5 l], fractions-6 and 7 [5 and 0.5 g, light petroleum acetone 4:1, each 2 l], fractions-8, 9 and 10 [1, 1 and 3 g, light petroleum–acetone 2:1, each 1.5 l], fractions-11 and 12 [3 and 5 g, light petroleum–acetone 1:1, each 1.5 l], fraction-13 [5 g, light petroleum–acetone 1:2, 2 l], Fractions-14 [15 g, acetone 100%, 2 l]. Fraction-6 (5 g) was passed through silica gel (150 g) chromatography (using $CHCl_3$–MeOH 15:1, 2 l) and Sephadex LH-20 (20 g, using EtOH, 300 ml) to afford **1** (5 mg, TLC, $CHCl_3$–MeOH, 15:1, R_f 0.3). Fraction-7 (0.5 g) was purified by column chromatography over silica gel (20 g) using $CHCl_3$–MeOH (9:1, 500 ml) to afford **6** (10 mg, TLC, $CHCl_3$–MeOH, 9:1, R_f 0.2). Fraction-8 (1 g) was subjected to column chromatography over silica gel (35 g) using $CHCl_3$–MeOH (7:1, 1 l) to obtain **2** (100 mg, TLC, $CHCl_3$–MeOH, 7:1, R_f 0.3). Fraction-10 (3 g) was further separated on silica gel chromatography (200 g, using $CHCl_3$–MeOH, 5:1, 4 l) and recrystallized from methanol to give **5** (100 mg, TLC, $CHCl_3$–MeOH, 5:1, R_f 0.2). Fraction-11 (3 g) was purified by chromatography on Sephadex LH-20 (100 g, using MeOH, 500 ml) to obtain **3** (15 mg, TLC, $CHCl_3$–MeOH, 5:1, R_f 0.3). Fraction-12 (5 g) was chromatographed over silica gel (200 g) and eluted with $CHCl_3$–MeOH–H_2O (8:2:0.2, 6 l) to afford **4** (200 mg, TLC, $CHCl_3$–MeOH–H_2O, 8:2:0.2, R_f 0.2).

*Subglain A (**1**)*: white amorphous powders, mp 120–122°C; $[\alpha]_D^{20}$: + 7 (*c* 0.11, MeOH); HR-FABMS *m/z*: 848.6443 ($[M + H]^+$, calcd for $C_{46}H_{90}O_{12}N$, 848.6463); IR (KBr) ν_{max} (cm^{-1}): 3369, 2918, 2850, 2484, 1614, 1468, 1080, 1038, 721; FABMS: *m/z* 848 (35), 819 (10), 699 (25), 685 (25), 667 (15), 656 (10), 638 (10), 481(100), 480 (10), 443 (10), 380 (5), 358 (5), 319 (15), 299 (5), 287 (10), 283 (10); ^1H and ^{13}C NMR data see table 1.

Along with the new compound (**1**), five known compounds (**2–6**) were also isolated from the stems of *Uvaria tonkinensis* var. *subglabra*. By comparing physical and spectroscopic data (^1H, ^{13}C NMR, DEPT and MS data) with the literature values, they were identified as schisandriside (**2**) ($[\alpha]_D^{20}$: + 28 *c* 1.2, EtOH) [5], erythritol (**3**) ($[\alpha]_D^{20}$: 0 *c* 1.0, MeOH) [6], β-*O*-D-glucopyranose (**4**) ($[\alpha]_D^{20}$: +5.6 *c* 0.059, MeOH) [7], kaempferol-3,7-*O*-α-L-dirhamnoside (**5**) [8], (+)-lyoniresinol (**6**) ($[\alpha]_D^{20}$: +49 *c* 2.1, acetone) [9].

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