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TWO NEW CERAMIDES FROM *Zephyranthes candida*

Zhiping Wu,[1,2] Yu Chen,[2] Xu Feng,[2*] Bing Xia,[2] Ming Wang,[2] and Yunfa Dong[2]

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Two new ceramides were isolated from the bulbs of Zephyranthes candida. *Their structures were established as (2*S*,3*S*,4*R*,13*E*)-1,3,4-trihydroxy-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-13-octadecene, named zephyranamide A (**1**) and (2*S*,3*S*,4*R*)-1,3,4-trihydroxy-2-octacosanoylaminohexadecene, named zephyranamide B (**2**). The structures of the new compounds were elucidated by spectral techniques including ^{1}H NMR, ^{13}C NMR, as well as HSQC, HMBC, DEPT, and COSY.*

Key words*:* Amaryllidaceae, *Zephyranthes candida*, ceramide, zephyranamide A, zephyranamide B.

Zephyranthes candida belongs to the Amaryllidaceae family. It is mainly distributed in the temperate zone of Western Hemisphere and is used as an ornamental and medicinal plant in China. The genus *Zephyranthes* is widely used as folk medicine in many countries. *Z. candida* has been employed in Africa as a treatment for diabetes mellitus, and *Z. parulla* appears in the history of Peru as a treatment for tumors [1]. Z. *rosea* and *Z. flava* are used for a variety of therapeutic purposes in India [2–4]. Herein we describe the isolation and structure elucidation of two new ceramides, named zephyranamide A (**1**) and zephyranamide B (**2**), from the bulbs of this plant. To the best of our knowledge, this is the first isolation of ceramides from a plant in the genus *Zephyranthes* and even the family Amaryllidaceae.

The molecular formula of zephyranamide A (**1**) was established to be $C_{42}H_{83}NO_5$ by its positive ESI-MS data for the $[M+H]^+$ at *m/z* 682. The IR spectrum showed absorption bands at 3334 and 3202, 2902 and 1623, 1527, and 1482, and 710 cm^{-1} indicating the presence of hydroxyl, amide carbonyl functions, and olefinic carbons, respectively, and the ^{1}H and ^{13}C NMR spectrum suggested that **1** and **2** have the ceramide nature [5, 6]. The ^{1}H NMR spectrum of **1** (Table 1) displayed a downfield doublet at δ_H 8.55 (1H, d, J = 8.9 Hz, NH), six olefinic, oxygenated, or other heteroatomized protons between δ_H 4.25–5.52, as well as the signals of six methyl protons at δ_H 0.87 (6H, br.t, J = 6.5 Hz, H-18 and H-24′) and a very strong aliphatic methylene band at δ_H 1.25–1.36. Signals of a *trans*-olefinic bond at δ_H 5.52 (1H, dt, J = 14.6, 8.8 Hz, H-13) and δ_H 5.49 (1H, dt, J = 14.6, 8.8 Hz, H-14) and five characteristic signals of geminal protons to hydroxyl groups were observed at δ_H 4.61 (1H, m, H-2′), 4.41 (1H, dd, J = 10.5, 4.7 Hz, H-1a), 4.48 (1H, dd, J = 10.5, 4.6 Hz, H-1b), 4.34 (1H, dd, J = 5.2, 3.7 Hz, H-3), and 4.25 (1H, m, H-4). Another signal at low field was observed at δ_H 5.08 (1H, m, H-2) for a methine proton vicinal to the nitrogen atom of an amide linkage. The ^{13}C NMR (Table 1) and DEPT spectral data of **1** showed one quaternary carbon at δ_C 175.2 (C-1′), two olefinic methine carbons at δ_C 130.2 (C-13 or C-14) and 130.8 (C-13 or C-14), as well as five oxygenated or other heteroatomized carbons at δ_C 76.9 (C-3), 73.1 (C-4), 72.5 (C-2′), 62.1 (C-1), and 53.1 (C-2), aliphatic methylenes between δ_C 29.6–30.4, and two terminal methyls at δ_C 14.3 (C-18 and C-24′). The low-field doublet at δ_H 8.55 (NH) was deuterium-exchangeable, and there was no correlation from this signal to any carbon in the HSQC spectrum of **1**. On the other hand, a correlation from δ_H 8.55 (NH) to δ_H 5.08 (1H, m, H-2), and correlations from δ_H 8.55 (NH) to δ_C 62.1 (C-1), 53.1 (C-2), 76.9 (C-3), 175.2 (C-1′), and 72.5 (C-2′) were observed in the ^{1}H–^{1}H COSY and HMBC spectra of **1**, respectively. The chemical shifts of the allylic methylene carbons in **1** were assigned at δ_C 33.2 (C-12 or C-15) and 33.2 (C-12 or C-15) based on the clearly observed HMBC correlations from the olefinic signals at δ_H 5.52 and 5.49 (2H, m, H-13 and H-14) to these two carbon signals (Fig. 1).

1) College of Life Science, Nanjing Agricultural University, Nanjing 210095, P. R. China; 2) Jiangsu Center for Research & Development of Medicinal Plants, Jiangsu Institute of Botany, Chinese Academy of Sciences/Nanjing Botanical Garden Mem. Sun Yat-Sen, Nanjing, 210014, P. R. China, fax: +86 25 84347084, e-mail: fengxu@mail.cnbg.net. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 695–698, November–December, 2009. Original article submitted May 30, 2008.

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TABLE 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) as well as COSY Spectral Data of Compound **1** in C_5D_5N (δ, ppm, J/Hz)

C atom	δ_H (HSQC)	δ_C	^{1}H–^{1}H COSY
1a	4.41 (dd, J = 10.5, 4.7)	62.1	H-2
1b	4.48 (dd, J = 10.5, 4.6)	62.1	
2	5.08 (m)	53.1	H-1, H-3, NH
3	4.34 (dd, J = 5.2, 3.7)	76.9	H-2, H-4
4	4.25 (m)	73.1	H-3, H-5
5a, 5b	1.92, 2.25 (m)	34.2	H-4, H-6
6	1.92 (m)	26.7	H-5, H-7
7–11	1.25–1.36 (br.s)	29.6–30.4	
12	2.15 (m)	33.2	H-11, H-13
13	5.52 (dt, J = 14.6, 8.8)	130.2	H-12, H-14
14	5.49 (dt, J = 14.6, 8.8)	130.8	H-13, H-15
15	1.98 (m)	33.2	H-14, H-16
16	1.22 (m)	32.2	
17	1.22 (br.s)	23.0	
18	0.87 (t, J = 6.5)	14.3	
NH	8.55 (d, J = 8.9)	–	H-2
1′	–	175.2	H-3′
2′	4.61 (m)	72.5	H-2′, H-4′
3′a, 3′b	2.21 (m), 2.02 (m)	35.8	H-3′, H-5′
4′	1.92 (m)	26.7	H-4′, H-(6′–22′)
5′	1.75 (m)	25.9	
6′–22′	1.25–1.36 (br.s)	29.6–30.4	
23′	1.22 (br.s)	23.0	
24′	0.87 (t, J = 6.5)	14.3	H-(6′-22′)

TABLE 2. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) as well as COSY Spectral Data of Compound **2** in C_5D_5N (δ, ppm, J/Hz)

C atom	δ_H (HSQC)	δ_C	^{1}H–^{1}H COSY
1a	4.49 (dd, J = 10.9, 4.9)	62.3	H-2
1b	4.45 (dd, J = 11.7, 5.8)	62.3	
2	5.06 (m)	53.8	H-1, H-3, NH
3	4.37 (dd, J = 5.9, 5.2)	76.8	H-2, H-4
4	4.24 (m)	73.1	H-3, H-5
5	1.92 (m)	34.1	H-4
6	1.78 (m)	26.7	
7–13	1.23–1.44 (br.s)	29.7–30.4	
14	1.24 (m)	23.0	
15	1.22 (m)	32.2	
16	0.87 (t, J = 6.7)	14.3	
NH	8.39 (d, J = 8.5)	–	H-2
1′	–	173.3	
2′	2.45 (t, J = 7.5)	36.9	H-3′
3′	1.78 (m)	26.3	H-2′
4′–25′	1.23–1.44 (br.s)	29.7–30.4	
26′	1.24 (m)	23.0	
27′	1.22 (m)	32.2	
28′	0.87 (t, J = 6.7)	14.3	

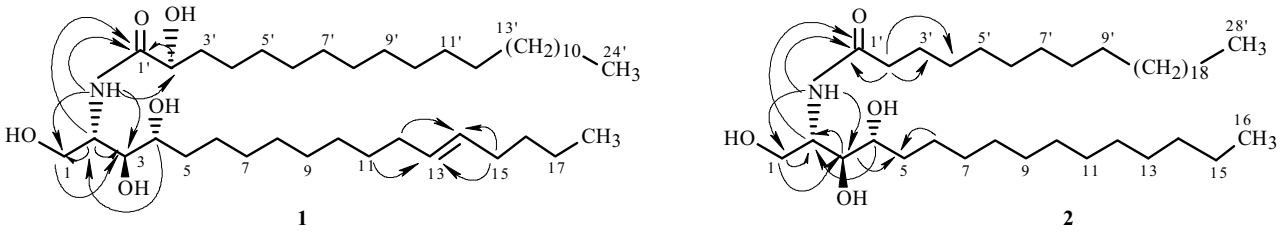

Fig. 1. Structures and key HMBC correlations (H→**C**) of **1** and **2**.

Since the chemical shifts of allylic methylene carbons are different when alkene double bonds are *cis*-oriented ($\delta_C \approx 27$ ppm) compared with when they are *trans*-oriented ($\delta_C \approx 32$ ppm) [7, 8], the 13,14 alkenyl bond in compound **1** was assigned to the *E* configuration. The presence of 2-amino and 1,2′,3,4 tetrahydroxyl groups as well as the 13,14 double bond in the main chain was established from the ESI-MS fragmentation pattern analysis [9–11]. The positive ESI-MS of **1** showed a quasimolecular ion at *m/z* 682 $[M+H]^+$, and the number of carbons in the long chain base (LCB) and the fatty acid (FA) were determined to be 18 and 24, respectively. Further investigation of the ESI-MS showed some important fragment ions at *m/z* 664, 640, 625, 599, 382, 367, 309, 282, 268, and 255, suggesting the double bond to be located at C-13 and C-14. This was confirmed in the ESI-MS of **1**, which displayed the characteristic peaks at *m/z* 625 $[M+H–C_4H_9]^+$ and 599 $[M+H–C_6H_{11}]^+$. On the other hand, the ESI-MS spectrum of compound **1** additionally displayed a small but crucially important peak at *m/z* 640 $[M+H–C_3H_6]^+$ formed by elimination of propylene through McLafferty rearrangement [12, 13], which further confirmed the position of the double bond (Fig. 2). Based on the ^{1}H NMR chemical shifts of the chiral center and the data of optical rotation compared with those of the natural and synthetic sphingolipids [14, 16], compound **1** has a sphingosine moiety with (2*S*,3*S*,4*R*,13*E*) geometry. Based on this information, the structure of compound **1** was determined to be (2*S*,3*S*,4*R*,13*E*)-1,3,4-trihydroxy-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-13-octadecene, named zephyranamide A.

Zephyranamide B (**2**) has the molecular formula $C_{44}H_{89}NO_4$, determined by positive ESI-MS data for the $[M+Na]^+$ at *m/z* 718. The IR absorption bands data at 3340, 2906, 1620, 1465, and 715 cm^{-1} indicated the presence of hydroxyls, amide groups, and long aliphatic chains. The NMR spectra of **2** (Table 2) showed an amide NH signal (1H, δ_H 8.39, d, J = 8.5 Hz), a carbonyl (δ_C 173.3, C-1′) and a group of proton signals (66H, δ_H 1.23–1.44, br.s), and two terminal methyls (6H, δ_H 0.87, t), which indicated that **2** might belong to the ceramide class [17, 18]. The characteristic signals of 2-amino-1,3,4-triol of the hydrocarbon chain were observed at δ_H 5.06 (1H, m, H-2), 4.49 (1H, dd, J = 10.9, 4.9 Hz, H-1a), 4.45 (1H, dd, J = 11.7, 5.8 Hz, H-1b), 4.37 (1H, dd, J = 5.9, 5.2 Hz, H-3), 4.24 (1H, m, H-4) in the ^{1}H NMR spectra, and at δ_C 53.8 (C-2), 62.3 (C-1), 76.8 (C-3), 73.1 (C-4) in the ^{13}C NMR spectra. The ^{1}H NMR showed signals corresponding to six methyl protons at δ_H 0.87 (6H, t, J = 6.7 Hz, H-16 and H-28′) and a very strong aliphatic methylene band at δ_H 1.23–1.44. The ^{13}C NMR spectrum of **2** showed one quaternary carbon at δ_C 173.3 (C-1′) and two terminal methyl groups at δ_C14.3. The presence of a triplet signal at δ_H 2.45 (2H, t, J = 7.5 Hz, H-2′) due to the methylene protons connected to amide carbonyl indicated that the *N*-acyl moiety in **2** was a nonhydroxy fatty acid [19]. The structural elucidation and assignments of complete proton and carbon signals were achieved by 2D NMR (HSQC, HMBC, and ^{1}H–^{1}H COSY) techniques and chemical methods. The ^{1}H–^{1}H COSY spectra showed the C_1-C_2-C_3-C_4-C_5 connectivity. The carbonyl group position was assigned by the long-range HMBC correlations between H-3 at δ_H 4.37 and C-2 at δ_C 53.8, between H-1 at δ_H 4.49, 4.45 and C-2, and between H-4 at δ_H 4.24 and C-2.

The length of the long-chain base (LCB) and the fatty acid (FA) was determined by ESI-MS (Fig. 2). The positive ESI-MS of **2** showed a quasimolecular ion at *m/z* 718 $[M+Na]^+$, and the significant fragment ions at *m/z* 317 $[M+H–C_{27}H_{55}]^+$, 330 $[M–C_{26}H_{53}]^+$, 540 $[M–C_{11}H_{23}]^+$, 478 $[M–C_{12}H_{25}–CHOH–H_2O]^+$, and 422 $[M–LCB]^+$ in the ESI-MS indicated the presence of an amino alcohol chain of 16 carbon atoms. Moreover, fragment ions at *m/z* 678, 664, 646, 407, 379, 273, 229, and 169 are the main ions of compound **2**, which predicts 16 and 28 carbons in the LCB and FA, respectively.

The relative stereochemistry of **2** at C-2, C-3, and C-4 was proposed to be 2*S*, 3*S*, and 4*R*, since the ^{1}H NMR spectral data of **2** [H-1a at δ_H 4.49 (dt, J = 10.9, 4.9 Hz), H-1b at δ_H 4.45 (dt, J = 10.8, 5.8 Hz), H-2 at δ_H 5.06 (m), H-3 at δ_H 4.37 (dd, J = 5.9, 5.2 Hz), and H-4 at δ_H 4.24 (m)] were in good agreement with those of natural and synthetic ceramides reported in the literature [16, 20]: (2*S*,3*S*,4*R*)-2-(2′-hydroxytetracosanoylamino)hexadecane-1,3,4-triol isolated from the starfish *Acanthaster planci* [H-1a at δ_H 4.52 (dd, J = 10.7, 4.5 Hz), H-1b at δ_H 4.43 (dd, J = 10.6, 5.0 Hz), H-2 at δ_H 5.12 (m), H-3 at δ_H 4.36 (dd, J = 4.6, 6.6 Hz), and H-4 at δ_H 4.29 (m)]. Based on the ^{1}H NMR chemical shifts of the chiral center and the data of optical rotation compared with those of the reported ceramides, (2*S*,3*S*,4*R*)-2-(2′-hydroxytetracosanoylamino)hexadecane-1,3,4-triol [16, 20], compound **2** has the same absolute configuration at asymmetric centers 2, 3, and 4. Including biogenetic considerations, compound **2** was assigned to be (2*S*,3*S*,4*R*)-1,3,4-trihydroxy-2-octacosanoylaminohexadecane, named zephyranamide B.

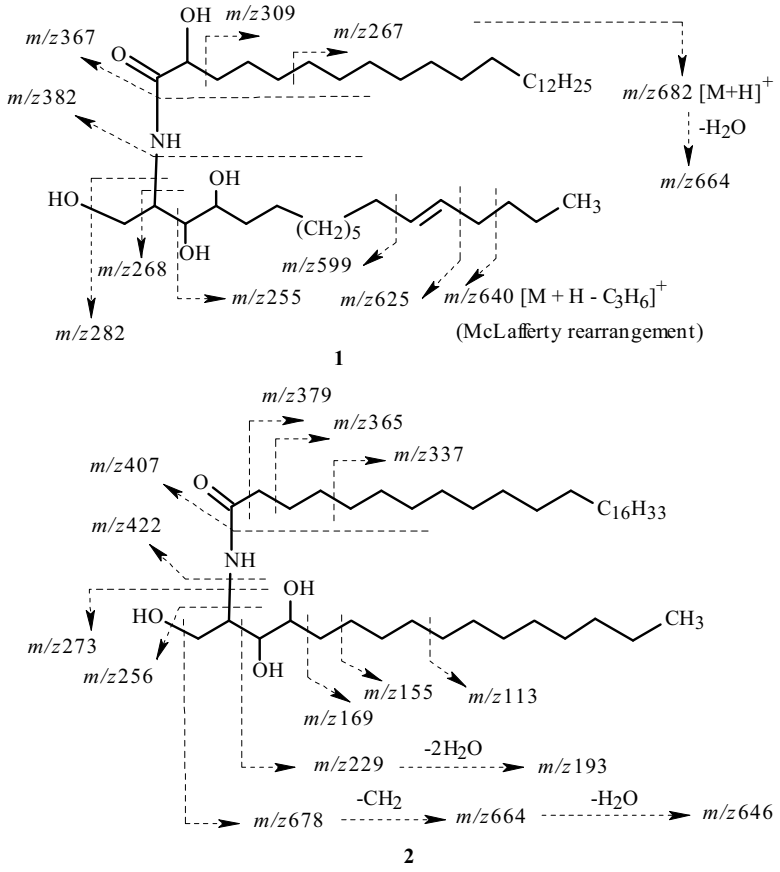

Fig. 2. ESI-MS fragment analysis of compounds **1** and **2**.

EXPERIMENTAL

General Methods. ^{1}H NMR and ^{13}C NMR, COSY, HSQC, and HMBC spectra: Bruker spectrometers operating at 500 MHz; ESI-MS: Agilent 1100 LC/MSD SL; IR spectra: IMPACT 400 (KBr); JASCO P-1020 optical rotation apparatus.

Plant Material. Bulbs of *Zephyranthes candida* were collected from Nanjing Agricultural University, Jiangsu province, P. R. China, in August, 2006. A voucher specimen documenting the collection was authenticated at the Herbarium, Institute of Botany (Nanjing Botanical Garden Mem. Sun Yat-Sen), Jiangsu province and Chinese Academy of Sciences, where it was deposited.

Extraction and Purification. Air-dried, powdered bulbs of *Zephyranthes candida* (10.2 kg) were extracted three times with 95% EtOH at room temperature for two weeks. The aqueous ethanol phase was evaporated under reduced pressure and the initial residue dried in *vacuo*. The resulting residue was dissolved in H_2O and re-extracted with petroleum ether, $CHCl_3$, and then $CHCl_3$–MeOH (3:2). The extracts portion of $CHCl_3$ and $CHCl_3$–MeOH (3:2) were combined and evaporated, and subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH [99:1→97:3→95:5→9:1→8:2→7:3→6:4→1:1] to furnish eight fractions (fractions 1 to 8). Fraction 2 was then chromatographed on silica gel eluting with petroleum ether–EtOAc [100:0→90:10→80:20→70:30] to furnish four fractions (fractions 1 to 4); the second fraction was further purified using $CHCl_3$–MeOH as eluent with gradient polarity to afford **1** (12.0 mg) and **2** (8.0 mg).

Zephyranamide A (1), white amorphous powder, mp 141–143°C ($CHCl_3$–MeOH), $[\alpha]_D^{25}$ –3.20° (*c* 0.05, pyridine). IR (ν_{max}, KBr, cm^{-1}): 3334, 3202, 2902, 1623, 1527, 1482, and 710. ESI-MS: *m/z* 682 $[M+H]^+$.

Zephyranamide B (2), white amorphous powder, mp 117–118°C ($CHCl_3$–MeOH), $[\alpha]_D^{25}$ –3.38° (*c* 0.13, pyridine). IR (ν_{max}, KBr, cm^{-1}): 3340, 2906, 1620, 1465, and 715. ESI-MS: *m/z* 718 $[M+Na]^+$. For the ^{1}H NMR and ^{13}C NMR as well as HSQC, HMBC, and COSY spectral data of **1** and **2**, see Tables 1 and 2 and Fig. 1.

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