Two new ceramides from the radix of *Angelica sinensis*

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Phytochemical investigation of the radix of *Angelica sinensis* has led to the isolation two new ceramides, angelicamide A (**1**) and B (**2**), along with one known ceramide, (2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-2'-hydroxytetracosanoyl]-8-octadecene-1,3,4-triol (**3**). Their structures were determined by spectroscopic methods including IR, NMR (1D and 2D NMR) and MS. This is the first report on the occurrence of ceramides in the *Angelica* genus.

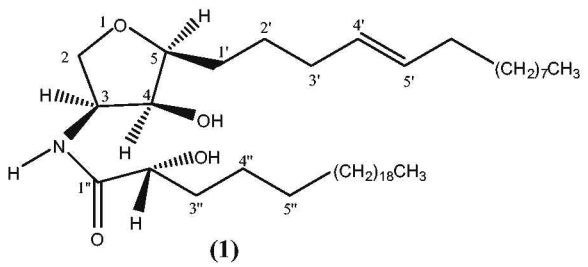
Keywords: *Angelica sinensis*, Umbelliferae, ceramides

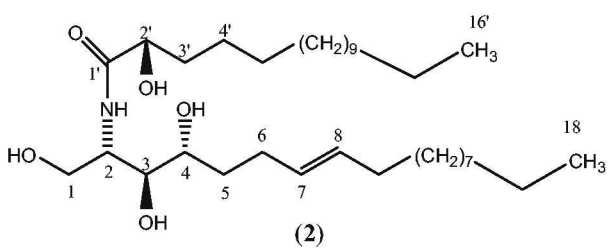
Angelica sinensis (Oliv.) Diels (Umbelliferae) is known worldwide as a medicinal plant that is found in Gansu, Yunnan and Sichuan provinces of China.[1] The radix of *A. sinensis*, called Danggui, is a popular tonic which was first recorded in "*Shen Nong Ben Cao Jing*" in the Han Dynasty (AD 25–225). It is recommended as a tonic, haematopoietic and anti-inflammatory agent for the treatment of menstrual disorders, such as amenorrhea and dysmenorrhoea.[2-4] Moreover it is also used as a health food product for women in Asia[5] and as a dietary supplement in Europe and America.[6,7]

Previous studies on the chemical constituents of *A. sinensis* revealed the presence of phthalides, organic acids, amino acids and polysaccharides.[7-12] While investigating the chemical constituents of the radix of *A. sinensis*, two new ceramides, Angelicamide A (**1**) and Angelicamide B (**2**), were isolated along with a known ceramide (2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-2'-hydroxytetracosanoyl]-8-octadecene-1,3,4-triol (**3**). Their structures were determined by spectroscopic measurements. To our knowledge, this is the first report about the occurrence of ceramides in *Angelica* species (Fig. 1).

Angelicamide A (**1**) was obtained as white amorphous powder, m.p. 100.6–102.1 °C. The HR–APCI–MS showed the molecular formula to be $C_{42}H_{81}NO_4$ (*m/z* 664.6237 $[M + H]^+$, Calcd 664.6238). IR absorption at 3397 cm^{-1} indicated the presence of hydroxyl groups. A secondary amide carbonyl group was deduced from the IR absorption at 1662 and 1526 cm^{-1} and the signals at δ 51.8 (C-3) and δ 174.6 (C-1") in the ^{13}C NMR spectrum (Table 1). The ^1H NMR spectrum exhibited a doublet at δ 6.86 (1H, d, J = 7.0 Hz) due to an NH proton; The ^1H NMR spectrum also revealed a pair of olefinic protons at δ 5.40 (1H, dt, J = 6.0, 15.0 Hz, H-5') and δ 5.38 (1H, dt, J = 6.0, 15.0 Hz, H-4') attributable to the presence of one double bond. The configuration of the double bond was deduced from the coupling constants of the olefinic protons. (*trans*-protons J > 13.0 Hz, *cis*-protons J < 13.0 Hz). The geometry of a double bond in a long-chain alkene can also be determined on the basis of the ^{13}C NMR chemical shifts of the methylene carbon adjacent to the olefinic carbon. These are observed at $\delta_c \approx 32$ in (*E*) isomers and at $\delta_c \approx 27$ in (*Z*) isomers.[13-15] Accordingly, the *E*-geometry of the double bond in this compound was established from the coupling constant between the olefinic protons (J = 15.0 Hz) and the chemical shifts (δ_c 32.9 and 32.4) assigned to the allylic carbons.

The ^{13}C NMR signals (Table 1) at δ 85.2 (C-5), 75.3 (C-4), 72.4 (C-2"), 70.3 (C-2) suggested the presence of four oxygenated carbons. This was confirmed by the following HSQC correlations: δ 3.73 (1H, dt, J = 5.0, 6.5 Hz, H-5)/85.2 (C-5); 3.99 (1H, dd, J = 4.5, 6.5 Hz, H-4)/75.3 (C-4); 4.14 (1H, t, J = 6.5 Hz, H-2")/72.4 (C-2"); 4.18 (1H, dd, J = 7.0, 9.0 Hz, H-2), 3.55 (1H, dd, J = 7.5, 10.5 Hz, H-2)/70.3 (C-2). The ^{13}C NMR signal (Table 1) at δ 51.8 was assigned to the carbon linked to a nitrogen atom. This was supported by a HSQC correlation at δ 4.37 (1H, m, H-3)/51.8 (C-3), HMBC correlations of δ 4.37 (1H, m, H-3)/174.6 (C-1") and ^1H–





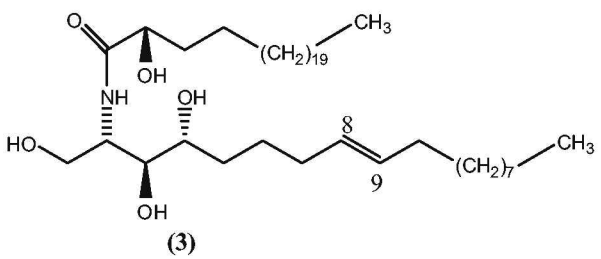


Fig. 1 Chemical structures of compounds **1–3**.

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Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of angelicamide A in $CDCl_3$ (δ, ppm; *J*, Hz)

Positions	δ_H (m, *J*)	δ_C (m)	^{1}H–^{1}H COSY	HMBC (H–C)
2	4.18 (1H, dd, *J* = 7.0, 9.0 Hz)			
	3.55 (1H,dd, *J* = 7.5, 10.5 Hz)	70.3	H-3	C-3, C-4, C-5
3	4.37 (1H, m)	51.8	NH, H-2, H-4	C-1"
4	3.99 (1H, dd, *J* = 4.5, 6.5 Hz)	75.3	H-3, H-5	C-2, C-1'
5	3.73 (1H, dt, *J* = 5.0, 6.5 Hz)	85.2	H-4, H-1'	C-4
1'	1.58 (2H, m)	32.9	H-5, H-2'	C-4, C-5, C-3'
2'	1.47 (2H, m)	29.2–29.7	H-1', H -3'	C-5, C-4'
3'	2.02 (2H, q, *J* = 7.0 Hz)	32.4	H-2', H-4'	C-1', C-4', C-5'
4'	5.38 (1H, dt, *J* = 6.0, 15.0 Hz)	129.5	H-3', H-5'	C-3', C-6'
5'	5.40 (1H, dt, *J* = 6.0, 15.0 Hz)	131.2	H-4', H-6'	C-3', C-6'
6'	1.96 (2H, q, *J* = 6.5 Hz)	32.6	H-5', H-7'	C-4', C-5'
7'-13' and	1.26-1.31 (about 66H, m)	22.9, 31.9,		
6"-23"		29.2–29.7		
14', 24"	0.88 (6H, t, *J* = 7.0 Hz)	14.1		
NH	6.86 (1H, d, *J* = 7.0 Hz)		H-3	C-1"
1"		174.6		
2"	4.14 (1H, t, *J* = 6.5 Hz)	72.4	H-3"	C-1", C-5"
3"	1.65 (1H, m), 1.84 (1H, m)	35.1	H-2", H-4"	C-1", C-2", C-4"
4"	1.43 (1H, m), 1.26 (1H, m)	25.0	H-3", H-5"	C-3", C-5"
5"	1.53 (1H, m), 1.56 (1H, m)	25.8	H-4"	C-4"

^{1}H COSY correlations of δ 4.37 (1H, m, H-3)/6.86 (1H, d, *J* = 7.0 Hz, NH). The presence of methyl groups and a long aliphatic chain was concluded from the ^{1}H NMR signals (Table 1) at δ 0.88 (6H, t, *J* = 7.0 Hz) and 1.26–1.31 (about 66H, m), and the ^{13}C NMR signals at δ 14.1 and 29.2–29.7, respectively. All of these data suggested that angelicamide A was a ceramide possessing a 2"-hydroxy fatty acyl moiety.

The structure of angelicamide A was further deduced from the ^{1}H–^{1}H COSY, HSQC, and HMBC correlations (Table 1). C-2 and C-5 were linked via an ether bond because there were key HMBC correlation between δ 4.18 (1H, dd, *J* = 7.0, 9.0 Hz, H-2), 3.55 (1H, dd, *J* = 7.5, 10.5 Hz, H-2) and 85.2 (C-5) and the HSQC correlative signal at δ 3.73 (1H, dt, *J* = 5.0, 6.5 Hz, H-5)/85.2 (C-5) (Table1). This suggested that angelicamide A was a 1,4-dehydrated ceramide. The position of the double bond was assigned at C-4' and C-5' by 2D NMR (^{1}H–^{1}H COSY, HSQC, HMBC) and the typical ion peak at *m/z* 551 which was formed by elimination of octene from that at *m/z* 664 through McLafferty rearrangement (Table 1, Fig. 2).[16-18] The sphingoid base, 3-amino-4-hydroxy-5-[(4'*E*)-tetradecane-4'-ene]-2,3,4,5-tetrahydrofuran was postulated from the characteristic fragment ion peak at *m/z* 298, 280 (Fig. 2).

The relative configurations of C-3, C-4, C-5 were presumed as *S*, *S* and *S*, respectively. This was confirmed by the coupling constants for those *cis* -protons (*J* < 7.2 Hz) on the tetrhhydrofuran (for *trans*-protons, *J* > 7.2 Hz) (Table 1).[19,20] The relative configuration of C-2" was determined to be *R* by comparing the chemical shift and coupling constants of H-2" with those reported in the literature (Table 1).[21,22]

Thus, the structure of angelicamide A (**1**) was elucidated as (3*S*, 4*S*, 5*S*)-3-[(2"*R*)-2"-hydroxytetracosanoylamino]-4-hydroxy-5-[(4'*E*)-tetradecane-4'-ene]-2,3,4,5-tetrahydrofuran (Fig. 1). This compound is a regioisomer of (3S, 4S, 5S)-3-[(2R)-2-hydroxytetracosanoylamino]-4-hydroxy-5-[(4*Z*)-tetradecane-4-ene]-2,3,4,5-tetrahydrofuran, whose geometry of the double bond was confirmed from the coupling constants of the corresponding H-4/H-5 protons in the 1H NMR spectrum (*J* = 5.5, 2.5 Hz*)*.[19] The two isomers differ in the stereochemistry of the double bond.

Angelicamide B (**2**) was obtained as a white amorphous powder, m.p. 142.7–143.0°C. The molecular formula of angelicamide B was found to be $C_{34}H_{67}NO_5$ on the basis of HR–APCI–MS (*m/z* 570.5091 $[M + H]^+$, calcd. 570.5092) as well as ^{1}H and ^{13}C NMR spectral interpretations (Table 2). Again, the IR and NMR spectral were found to be similar to angelicamide A except for the position of double bond and the chemical shift of the header group of the lipid base. This confirmed that compound **2** was also a ceramide.

The ^{13}C NMR signals (Table 2) at δ 74.6 (C-3), 71.1 (C-2'), 71.0 (C-4), 60.5 (C-1) suggested the presence of four oxygenated carbons. This was confirmed by the following HSQC correlations: δ 3.38 (1H, m, H-3)/74.6 (C-3); 3.83 (1H, m, H-2')/71.1 (C-2'); 3.35 (1H, m, H-4)/71.0 (C-4); 3.53 (2H, d, *J* = 5.4 Hz, H-1)/60.5 (C-1), and the ^{1}H–^{1}H COSY correlations: 3.83 (1H, m, H-2')/5.51 (1H, d, *J* = 4.8 Hz, 2'-OH); 3.38 (1H, m, H-3)/4.63 (1H, d, *J* = 5.5 Hz, 3-OH); 3.53 (2H, m, H-1)/4.56 (1H, t, *J* = 5.5 Hz, 1-OH); 3.35 (1H, m, H-4)/4.32 (1H, d, *J* = 5.7 Hz, 4-OH) (Table 2). The ^{1}H NMR spectrum also showed a pair of olefinic proton signals at

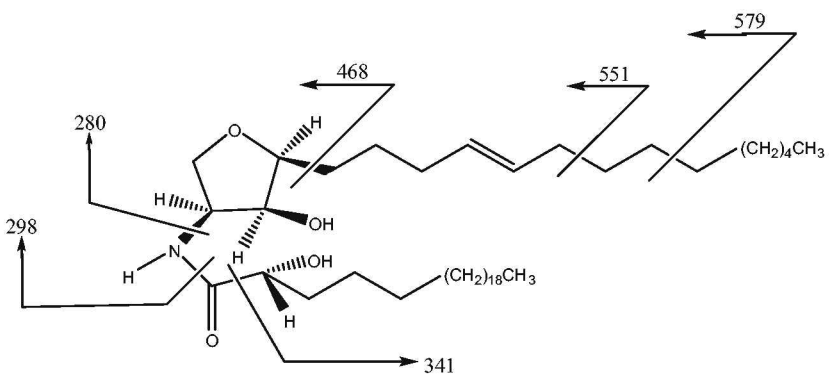

Fig. 2 APCI–MS/MS fragment analysis of angelicamide A (**1**).

Table 2 ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of angelicamide B in DMSO-d_6 (δ, ppm; *J*, Hz)

Positions	δ_H (m, *J*)	δ_C (m)	^{1}H–^{1}H COSY	HMBC (H–C)
1	3.53 (2H, d, *J* = 5.4 Hz)	60.5	1-OH, H-2	C-2, C-3
2	3.90 (1H, m)	51.4	H-1, H-3, NH	C-3
3	3.38 (1H, m)	74.6	3-OH, H-2	C-1, C-2, C-4, C-5
4	3.35 (1H, m)	71.0	4-OH	C-5
5	1.48 (2H, m)	31.7	H-6	C-4
6, 9	1.92 (4H, m)	32.0, 32.3	H-5, H-7, H-8, H-10	C-7, C-8
7	5.34 (1H, dt, *J* = 5.0, 15.0 Hz)	129.7	H-6	C-6, C-8
8	5.38 (1H, dt, *J* = 5.0, 15.0 Hz)	130.3	H-9	C-6, C-10
10–17 and 5'–15'	1.23 (about 44H, m)	22.1–31.3		
18,16'	0.82 (6H, t, *J* = 6.6 Hz)	13.9		
NH	7.35 (1H, d, *J* = 9.0 Hz)		H-2	C-2, C-1'
1'		173.5		
2'	3.83 (1H, m)	71.1	2'-OH	C-1', C-3', C-4'
3'	1.48 (2H, m)	34.5	H-2', H-4'	C-1', C-2', C-4'
4'	1.23 (2H, m)	24.5	H-3'	C-3'
1-OH	4.56 (1H, t, *J* = 5.5 Hz)		H-1	C-1, C-2
3-OH	4.63 (1H, d, *J* = 5.5 Hz)		H-3	C-2, C-3, C-4
4-OH	4.32 (1H, d, *J* = 5.7 Hz)		H-4	C-3, C-4, C-5
2'-OH	5.51 (1H, d, *J* = 4.8 Hz)		H-2'	C-1', C-3'

δ 5.34 (1H, dt, *J* = 5.0, 15.0 Hz, H-7), and 5.38 (1H, dt, *J* = 5.0, 15.0 Hz, H-8), attributable to the presence of one double bond. The position and geometry of the double bond were confirmed by the NMR spectrum including the coupling constants between the olefinic protons (*J* = 15.0 Hz) and APCI–MS/MS fragment analysis (Mclafferty rearrangment) (Table 2, Fig. 3). Meanwhile according to the MS data, the numbers of carbon in the lipid base and lipid amide were determined to be 18 and 16, respectively (Fig. 3).

It is reported that the absolute configurations of C-2, C-3 and C-4 in all sphingolipids isolated from natural plants are 2*S*, 3*S* and 4*R*.[13] The chemical shifts (δ) of C-1 (60.5), C-2 (51.4), C-3 (74.6), C-4 (71.0), C-1' (173.5) and C-2' (71.1) were very similar to those sphingolipids which have the same configuration.[14, 23]

The linkages of the two components units of angelicamide B were deduced from the HMBC spectrum (Table 2). Based on the above evidence, the structure of angelicamide B was established as: (2*S*, 3*S*, 4*R*, 7*E*)-2-[(2'*R*)-2'-hydroxypalmitoylamino]-7-octadecene-1,3,4-triol (Table 2, Fig. 1). This compound is an isomer of (2'-hydroxyhexacosanoyl)-1,3,4-trihydroxy-2-amino-octadeca-5-ene.[24]

Compounds **3** were identified by comparison of the NMR, MS spectroscopic data with those reported in literatures as (2*S*, 3*S*, 4*R*, 8*E*)-2-[(2'*R*)-2'-hydroxytetracosanoyl]-8-octadecene-1,3,4-triol (**3**) (Fig. 1).[25]

Experimental

Melting points were determined with a WRS-1B melting point apparatus (Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, People's Republic of China) and are uncorrected. Optical

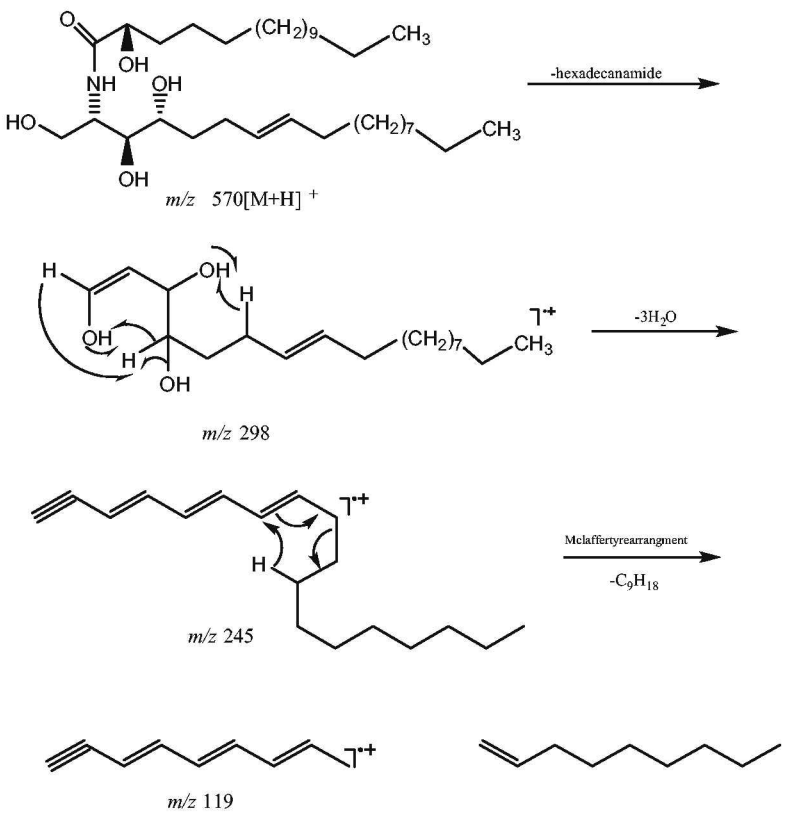

Fig. 3 APCI–MS/MS fragment analysis of angelicamide B (**2**).

rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were taken on a Nicolet IR-100 FT-IR spectrometer. NMR spectra were measured on a Bruker Avance-500/300 with TMS as internal standard. HR–APCI–MS spectra were obtained on a Shimadzu LC_IT_TOF_MS. All solvents used were of analytical grade (Nanjing Chemical Plant, Nanjing, People's Republic of China). Silica gel (200–300 mesh) was used for column chromatography and silica gel GF_{254} for TLC were obtained from the Qing-dao Marine Chemical Factory of China.

Plant material

The radix of *Angelica sinensis* (Oliv) Diels. was collected in July 2006 from Min Xian (Gansu, China), and authenticated by Prof. Jin-ao Duan, Jiangsu Key laboratory for TCM Formulae Research, Nanjing University of Chinese Medicine. A voucher specimen (GS-20060715) is kept in the Herbarium of Nanjing University of Chinese Medicine, Nanjing, P.R. China.

Extraction and purification

The powder of the dried radix of *A. sinensis* (20 kg) was extracted with hot 80% (v/v) ethanol (3 × 160 l) for 2 h. After removal of the solvent under reduced pressure at 60 °C, the residue was partitioned sequentially with petroleum ether, EtOAc and *n*-BuOH (five times), respectively. The petroleum ether extract was subjected to silica gel colunm chromatography and eluted with petroleum ether-EtOAc (100:0–1:1) to give 22 fractions (P1-P22). Fraction P18 was chromatographed on a silica gel column eluting with petroleum ether–EtOAc (10:1) to give compound **1** (20 mg). The ethyl acetate extract was chromatographed on a silica gel column and eluted with petroleum ether–EtOAc (2:1–0:100) and EtOAc–MeOH (100:0–0:100) to afford 18 fractions (E1-E18). Fraction E14 was subjected to silica gel column chromatography and eluted with CH_2Cl_2–MeOH (100:1) to afford compound **3** (15 mg). The *n*-BuOH extract was chromatographed on a silica gel column eluting with solvents EtOAc–MeOH (100:0–0:100) and MeOH–H_2O (100:0–0:100) to afford sixteen fractions (N1–N16). Compound **2** (20 mg) was afforded in fraction N5 on silica gel column chromatography eluting with CH_2Cl_2-MeOH (50:1).

Angelicamide A (**1**): white amorphous powder, m.p. 100.6–102.1 °C, $[\alpha]^{23}_D$ + 8.8 °[c 0.21, $CH_3OH/CHCl_3$ (1:1)]; IR (KBr): 3397, 2916, 2848, 1662, 1526, 1470, 1260, 1101 cm^{-1}; HR–APCI–MS *m/z* 664.6237 (calc. for $C_{42}H_{81}NO_4$ + H 664.6238); APCI-MS m/z: see Fig. 2. For ^1H and ^{13}C NMR data see Table 1.

Angelicamide B (**2**): white amorphous powder, m.p. 142.7–143.0 °C, $[\alpha]^{23}_D$ + 6.4° [c 0.12, $CH_3OH/CHCl_3$ (1:1)]; IR (KBr): 3331, 2917, 2848, 1622, 1544, 1464, 1357, 1277 cm^{-1}; HR–APCI–MS *m/z* 570.5091 (calc. for $C_{34}H_{67}NO_5$ + H 570.5092). APCI-MS *m/z*: see Fig. 3. For ^1H and ^{13}C NMR data see Table 2.

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