Three new lipids from the seeds of *Trogopterus xanthipes*

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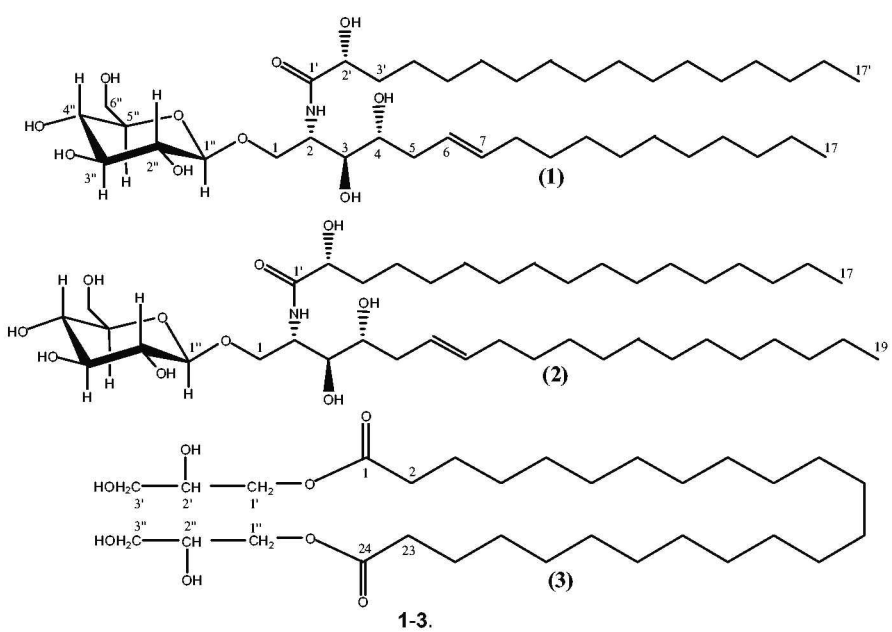
Chemical investigation of Feces Trogopterus (*Trogopterus xanthipes*) has led to the isolation of three new lipids, including two new cerebrosides, 1-*O*-(β-D-glucopyranosyloxy)-(2*S*,3*S*,4*R*,6*E*)-2-[(2'*R*)-2'-hydroxyheptadecanoylamino]-8-heptadecene-3,4-diol (**1**) and 1-*O*-(β-D- glucopyranosyloxy)-(2*S*,3*S*,4*R*,6*E*)-2-[(2'*R*)-2'-hydroxy-heptadecanoylamino]-8-nonadecene-3,4-diol (**2**), and one new fatty acid ester, bis(2,3-dihydoxypropyl)-tetracosanedioate (**3**). Their structures were elucidated by chemical and spectral means. This is the first report on the occurrence of cerebroside and fatty diacid diester in Feces Trogopterus.

Keywords: Feces Trogopterorus, cerebroside, fatty acid ester

Feces Trogopterus, called "Wulingzhi", are the dry seeds of *Trogopterus xanthipes* Milne-Edwards (Petauristidae). Feces Trogopterus has the function of increasing blood flow and relieving pain. It is often used in Chinese medicine in the treatment of amenorrhea, menses pain and postpartum abdominal pain. Modern studies have indicated that Feces Trogopterus contains terpenoids, organic acids and flavonoids, and inhibits platelet aggregation, enhances immunity and has anti-inflammatory activity.[1] Different solvent extracts of Feces Trogopterus were screened in our present study. This showed that the ethyl acetate extract was the active part. The ethyl acetate extract was investigated chemically and three new lipids, including two cerebrosides and one fatty acid ester, were isolated. Cerebrosides and fatty acid esters are two important types of lipid. They have been reported to have biological and pharmacological functions of regulating cell growth and variation, participating in immunological processes, anticoagulation and anti-platelet aggregation.[2-4] Here we deal with the isolation and structural elucidation of two new cerebrosides, 1-*O*-(β-D-glucopyranosyloxy)-(2*S*,3*S*,4*R*,6*E*)-2-[(2'*R*)-2'-hydroxyheptadecanoylamino]-8-heptadecene-3,4-diol (**1**) and 1-*O*-(β-D-glucopyranosyloxy)-(2*S*,3*S*,4*R*,6*E*)-2-[(2'*R*)-2'-hydroxyheptadecanoylamino]-8-nonadecene-3,4-diol (**2**), and one new fatty acid ester, bis(2,3-dihydoxypropyl)-tetracosanedioate (**3**) (Fig. 1). Their structures were elucidated by means of chemical and extensive spectroscopic analysis.

Compound **1** was isolated as a white powder. The molecular formula of **1** was established as $C_{40}H_{77}NO_{10}$ by HRESI-MS (*m/z* 754.5471 $[M + Na]^+$). Its positive ESI-MS showed two quasi-molecular ion peaks at *m/z* 732 $[M + H]^+$ and 754 $[M + Na]^+$, and MS/MS showed characteristic peaks at *m/z* 605 (an ion formed by McLafferty rearrangement of the olefinic bond), 569 (an ion from the *O*-glycoside bond cleavage), 534 (an ion derived from β-fission of the OH group), and 284 (an ion derived from α-fission of the NH group) (Fig. 2). The ^1H and ^{13}C NMR spectra (Table 1) were typical of a cerebroside possessing a 2-hydroxy-fatty acid. Assignments of all protons and carbons in **1** can be made by ^1H–^1H COSY, HMQC and HMBC spectra (Table 1). In the ^1H–^1H COSY spectrum, two methylene protons at δ 4.06 and 3.83 correlated with the methine proton at δ 4.22, the methine proton at δ 4.22 correlated with the methine proton at δ3.60, the methine proton at δ 3.60 correlated with the methine proton at δ 3.53. This suggested that there were three hydroxy groups at C-1, C-3 and C-4. An HMBC experiment was run to support these assignments.



1-3.

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

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Table 1 ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of compound **1** in CD_3OD (δ, ppm; *J*, Hz)

	^{1}H (m, *J* Hz)	^{13}C	'H–'H COSY	HMBC (H–C)
1a	4.08(m)	70.6	H-1b, H-2	C-3, C-1''
1b	3.83(m)		H-1a, H-2	C-3, C-1''
2	4.21(m)	52.4	H-1a, H-1b, H-3	C-3, C-1'
3	3.60(m)	76.3	H-2, H-4	C-1, C-2, C-5
4	3.53(m)	72.9	H-3, H-5a, H-5b	C-2, C-6
5	2.04(m)	34.2	H-4, H-6	C-3, C-7
6	5.41(m)	131.9	H-5, H-7	C-4, C-7
7	5.41(m)	131.9	H-6, H-8	C-6, C-9
8	1.99(m)	33.5	H-7, H-9	C-6, C-7, C-9
9–15	1.26–1.37(m)	31.7–30.8		
16	1.26(m)	24.1	H-15, H-17	C-17
17	0.88(t, 7.0)	14.8	H-16	C-16
1'		177.6		
2'	4.00(m)	73.9	H-3'a, H-3'b	C-1'
3'a	1.78(m)	36.3	H-2, H-3'b, H-4	C-1', C-2', C-4'
3'b	1.60(m)		H-2, H-3'a, H-4	C-1', C-2', C-4'
4'	1.37(m)	26.7		C-3'
5'–15'	1.26–1.37(m)	31.7-30.8		
16'	1.26(m)	24.1		C-17'
17'	0.88(t, 7.0)	14.8	H-16'	C-16'
1''	4.29(d, 7.6)	105.2	H-2''	C-1, C-3'', C-5''
2''	3.19(m)	75.6	H-1'', H-3''	C-3''
3''	3.38(m)	78.6	H-2''	C-2'', C-4''
4''	a	72.4	H-3'', H-5''	C-5''
5''	3.25(m)	78.5	H-6''a, H-6''b	C-3''
6''a	3.87(bd, 12.0)	63.4	H-5'', H-6''b	C-5''
6''b	3.67(dd, 12.0, 5.5)		H-5'', H-6''a	C-5''

[a]Signal superimposed with the solvent.

The fatty acid linked to C-2 of the sphingosine has been confirmed by the correlation between H-2 and the carbonyl carbon with the signal at δ 177.6. An HMBC correlation of the carbonyl carbon with the H-2' which in turn showed correlation with C-2' and the proton OH-2' confirmed the presence of an α-hydroxy fatty acid side chain. The structure of the α-hydroxy fatty acid side chain in **1** was examined. When **1** was methanolysed with methanolic hydrochloric acid, a fatty acid methyl ester (FAME) was obtained together with a long-chain base (LCB). On the basis of ESI-MS analysis, the FAME was characterised as methyl 2-hydroxyheptadecanoate. The ^{1}H NMR spectrum also revealed a pair of olefinic protons at δ 5.41 attributable to the presence of one olefinic bond. In the HMBC spectrum, H-4 at δ 3.53 correlated with C-5 at δ 34.2 and a olefinic carbon at δ 132.1, the olefinic proton at δ 5.41 correlated with C-4 at 72.9, which indicated that the olefinic bond in the LCB residue of **1** is located at C-6. The fragment ion at *m/z* 605 due to elimination of nonene from the molecular ion also supported this conclusion. Furthermore, the carbon signals at δ 34.2 and 33.5 confirm the *E* geometry of the olefinic bond at C-6 in the LCB. Consideration of the biogenesis and steric hindrance of sphingolipids, the chemical shifts of the carbon signals of C-2 to C-4, C-1' and C-2' of sphingolipids were generally acknowledged to determine the absolute stereochemistry of these carbons. The carbon signals at δ 52.4 (C-2), 76.3 (C-3), 72.9 (C-4), 177.6 (C-1') and 73.9 (C-2') in **1** were nearly identical with those of (2*S*,3*S*,4*R*,8*E*)-2-[(2'*R*)-2'-hydroxytetracosanoylamino]-8-pentadecene-1,3,4-triol.[5-9] Thus, the structure of compound **1** was established as 1-*O*-(β-D-glucopyranosyloxy)- (2*S*,3*S*,4*R*,6*E*)-2-[(2'*R*)-2'-hydroxyheptadecanoylamino]-8-heptadecene-3,4-diol.

Compound **2** was isolated as a white powder. The molecular formula of **1** was established as $C_{42}H_{81}NO_{10}$ by HRESI-MS (*m/z* 782.5779 $[M + Na]^+$). Its positive ESI-MS showed two quasi molecular ion peaks at *m/z* 760 $[M + H]^+$ and 782 $[M + Na]^+$, and MS/MS showed characteristic peaks at *m/z* 605 (an ion formed by McLafferty rearrangement of the olefinic bond), 597 (an ion from the *O*-glycoside bond cleavage), 534 (an ion derived from β-fission of the OH group), and 284 (an ion derived from α-fission of the NH group) (Fig. 2). The ^{1}H and ^{13}C NMR spectral data (Table 2) of **2** was very similar to those of **1**. Its quasi-molecular ion peak $[M + H]^+$ at *m/z* 760 indicated an increase of 28 Da in comparison with that of **1** and suggested the presence of two additional CH_2 units in **2**. When **2** was methanolysed with methanolic hydrochloric acid, a fatty acid methyl ester was obtained and characterised as methyl 2-hydroxyheptadecanoate. This was supported by the fragment ions of *m/z* 534 and 284.The typical fragment ion of *m/z* 605 confirmed that the olefinic bond was also located at C-6. ^{1}H-^{1}H COSY, HMQC and HMBC experiments were also run to assign all the proton and carbon signals for **2** (Table 2). The structure of compound **2** was established as 1-*O*-(β-D-glucopyranosyloxy)-(2*S*,3*S*,4*R*,6*E*)-2-[(2'*R*)-2'-hydroxyheptadecanoyl-amino]-8- nonadecene-3,4-diol.

Compound **3** was isolated as a white powder. The molecular formula of **3** was established as $C_{30}H_{58}O_8$ by HRESI-MS (*m/z* 569.4051 $[M + Na]^+$). The positive ESI-MS showed two quasi molecular ion peaks at *m/z* 547 $[M + H]^+$and 569 $[M + Na]^+$. Its ^{1}H and ^{13}C NMR spectra were typical of a fatty acid glyceryl ester, but the ^{1}H and ^{13}C NMR spectra did not reveal any methyl signals, which suggested that **3** is a symmetric dibasic acid diester.[10] The proton signals at δ 4.03 (1H, dd, $J = 11.6$ Hz) and 3.90 (1H, dd, $J = 11.7$ Hz) and the carbon signals at δ 62.8, 65.6 and 69.4 indicated that **3** is a 1-*O*-subsituted glyceryl ester. The fatty acid was deduced to be tetracosandioic acid from its MS data. Assignments of all protons and carbons in **3** were also made by ^{1}H-^{1}H COSY, HMQC and HMBC spectra. Thus, the structure of compound **3** was determined as bis(2,3-dihydoxypropyl)–tetracosanedioate.

Experimental

Melting points were determined with a WRS-lB melting point apparatus (Shanghai Precision & Scientific Instrument Co., Ltd, Shanghai, P.R. China) and are uncorrected. Optical 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 AV-300 with TMS as internal standard. ESI-MS and HR-ESI-MS spectra were obtained on a Micromass Q/TOF Mass

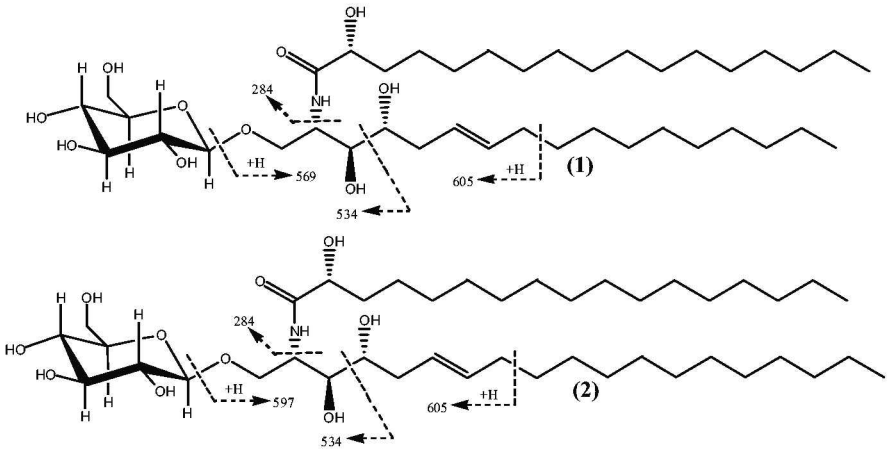

Fig. 2 ESI-MS/MS fragment analysis of **1** and **2**.

Table 2 'H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of compound **2** in CD_3OD (δ, ppm; *J*, Hz)

	^1H (m, *J* Hz)	^{13}C	'H–'H COSY	HMBC (H–C)
1a	4.09(m)	70.5	H-1b, H-2	C-3, C-1''
1b	3.84(m)		H-1a, H-2	C-3, C-1''
2	4.21(m)	52.6	H-1a, H-1b, H-3	C-3, C-1'
3	3.60(m)	76.7	H-2, H-4	C-1, C-2, C-5
4	3.53(m)	73.4	H-3, H-5a, H-5b	C-2, C-6
5	2.06(m)	34.3	H-4, H-6	C-3, C-7
6	5.42(m)	132.0	H-5, H-7	C-4, C-7
7	5.42(m)	132.0	H-6, H-8	C-6, C-9
8	2.00(m)	33.9	H-7, H-9	C-6, C-7, C-9
9–17	1.26–1.37(m)	31.4–30.8		
18	1.26(m)	24.3	H-17, H-19	C-19
19	0.87(t, 7.0)	14.0	H-18	C-18
1'		176.6		
2'	4.00(m)	73.7	H-3'a, H-3'b	C-1'
3'a	1.76(m)	36.5	H-2, H-3'b, H-4	C-1', C-2', C-4'
3'b	1.60(m)		H-2, H-3'a, H-4	C-1', C-2', C-4'
4'	1.37(m)	26.8		C-3'
5'–15'	1.26–1.37(m)	31.4–30.8		
16'	1.26(m)	24.3	H-15', H-17'	C-17'
17'	0.87(t, 7.0)	14.0	H-16'	C-16'
1''	4.29(d, 7.7)	105.3	H-2''	C-1, C-3'', C-5''
2''	3.19(m)	75.7	H-1'', H-3''	C-3''
3''	3.35(m)	78.6	H-2''	C-2'', C-4''
4''	a	72.2	H-3'', H-5''	C-5''
5''	3.25(m)	78.5	H-6''a, H-6''b	C-3''
6''a	3.86(bd, 12.0)	63.3	H-5'', H-6''b	C-5''
6''b	3.68(dd, 12.0, 5.4)		H-5'', H-6''a	C-5''

[a]Signal superimposed with the solvent.

Spectrometer. All solvents used were of analytical grade (Nanjing Chemical Plant, Nanjing, P.R. China). Silica gel (200–300 mesh) was used for column chromatography and silica gel GF_{254} for TLC was obtained from the Qingdao Marine Chemical Factory of China.

Plant material

Feces Trogopterus were collected in June from Hebei province of China, and identified as the dry seeds of *Trogopterus xanthipes* by associate professor Nianyun Yang of Nanjing University of Traditional Chinese Medicine. A voucher specimen (WLZ-20080610) is kept in the Herbarium of Nanjing University of Traditional Chinese Medicine, Nanjing, P.R. China.

Extraction and purification

Feces Trogopterus (5 kg) were extracted with 60% C_2H_5OH (2 × 50 L) for 2 h under reflux, and the combined extracts were concentrated *in vacuo*. The resulting extract (371 g) was then suspended in H_2O and extracted successively with petroleum ether, ethyl acetate, and *n*-butanol to give the respective extracts after removal of the solvent. The combined ethyl acetate layers were concentrated under *vacuum* to give a residue (278 g), which was chromatographed on silica gel (2 kg) eluting with $CHCl_3$–CH_3OH, stepwise gradient (100:0→5:1) and 5 fractions were collected. Fr. 3 (15 g) was separated by silica gel [CH_2Cl_2–CH_3OH (10:1)] to obtain compounds **1** (80 mg), **2** (35 mg) and **3** (20 mg).

1-O-(β-D-Glucopyranosyloxy)-(2S,3S,4R,6E)-2-[(2'R)-2'-hydroxyheptadecanoylamino]-8-heptadecene-3,4-diol (**1**): White powder, m.p. 186–188 °C, $[\alpha]_D^{20}$ + 10.8 °C (*c* 1.04, $CHCl_3$); IR (KBr) ν_{max} 3401, 3212, 2921, 1621, 1537, 1474 cm^{-1}; ^1H NMR and ^{13}C NMR spectra data see Table 1; ESI-MS *m/z*: 732 $[M + H]^+$, 754 $[M + Na]^+$; HRESI-MS *m/z*: *m/z* 754.5471 $[M + Na]^+$) ($C_{40}H_{77}NO_{10}Na$, Calcd 754.5445).

1-O-(β-D-Glucopyranosyloxy)-(2S,3S,4R,6E)-2-[(2'R)-2'-hydroxyheptadecanoylamino]-8-nonadecene-3,4-diol (**2**): White powder, m.p. 195–197 °C, $[\alpha]_D^{20}$ + 11.7 °C (*c* 1.04, $CHCl_3$); IR (KBr) ν_{max} 3210, 2916, 1624, 1535, 1472 cm^{-1}; ^1H NMR and ^{13}C NMR spectra data see Table 2; ESI-MS *m/z*: 760 $[M + H]^+$, 782 $[M + Na]^+$, HRESI-MS *m/z*: 782.5779 $[M + Na]^+$ ($C_{42}H_{81}NO_{10}Na$, Calcd 782.5758).

Bis(2,3-dihydoxypropyl)-tetracosanedioate (**3**): White powder, m.p. 110–112 °C, IR (KBr) ν_{max} 3209, 2911, 1626, 1534, 1470 cm^{-1}; ^1H NMR (DMSO-d_6), δ: 4.81 (2H, d, *J* = 5.2 Hz, OH-2', OH-2''), 4.57 (2H, d, *J* = 5.7 Hz, OH-3', OH-3''), 4.03 (2H, dd, *J* = 11,6 Hz,

H-1', H-1"), 3.90 (2H, dd, J = 11,7 Hz, H-1', H-1"), 3.63 (2H, m, H-2', H-2"), 3.36 (4H, m, H-3', H-3"), 2.28 (4H, t, J = 7.3 Hz, H-2, H-23), 1.51 (4H, m, H-3, H-22), 1.24 (36H, m, H-4~21); ^{13}C NMR (DMSO-d_6), δ: 173.1 (C-1, C-24), 69.4 (C-2', C-2"), 65.6 (C-1', C-1"), 62.8 (C-3', C-3"), 33.7 (C-2, C-23), 24.6 (C-3, C-22), 28.9 (C-4, C-21), 28.6 (C-5, C-20), 29.1 (C-6~19); ESI-MS *m/z*: 547 $[M + H]^+$, 569 $[M + Na]^+$, HRESI-MS *m/z*: 569.4051 $[M + Na]^+$ ($C_{30}H_{58}O_8Na$, Calcd 569.4029).

Methanolysis of **1** and **2**. Compounds **1** and **2** (*ca* 1 mg) were heated with 10% HCl in MeOH (1 mL each) at 80°C for 14 h respectively. Each reaction mixture was then extracted with *n*-hexane and concentrated *in vacuum*. The *n*-hexane extract was analysed by ESI-MS, which showed the protonated molecular ion at *m/z* 301 of methyl hydroxyheptadecanoate respectively.

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