Ceramide and Cerebroside from the Stem Bark of *Ficus mucuso* (Moraceae)

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Two new sphingolipids mucusamide (1) and mucusoside (2) have been isolated from methanol soluble part of the stem bark of *Ficus mucuso* Welw., together with fifteen known secondary metabolites including cellobiosylsterol (3), β-sitosterol (4), stigmasterol (5), β-sitosterol 3-*O*-β-D-glucopyranoside (6), lupeol acetate (7), ursolic acid (8), procatechuic acid (9), 2-methyl-5,7-dihydroxychromone 8-*C*-β-D-glucoside (10), apigenin (11), (−)-epicatechin (12), (+)-catechin (13), *N*-benzoyl-L-phenylalanilol (14), α-acetylamino-phenylpropyl α-benzoylamino-phenylpropionate (15), asperphenamate (16) and bejaminamide (17). Structures of compounds 1 and 2 were elucidated by spectroscopic analysis and chemical methods.

Key words *Ficus mucuso*; Moraceae; sphingolipid; mucusamide; mucusoside

Ficus is a genus of the family Moraceae that comprises about 150 species distributed worldwide.[1)] Most of the members of the family are very high trees, shrubs and rarely herbs often with milky juice.[1)] A number of *Ficus* species are used as food and medicines in Traditional Chinese Medicine (TCM). Their uses, however, originated and are most widely found in the Middle East.[2)] *Ficus* species are widely used in traditional medicine in Cameroon too. A mixture of powdered leaves of *F. mucuso* and palm oil are used in the treatment of epilepsy in Fongo Tongo, a village of the Western Region of Cameroon.[3)] A decoction of stem bark is used in the East Region of Cameroon by Baka pygmies to treat jaundice.[4)] Plants of the genus *Ficus* are known as sources of biologically active compounds such as 3-keto-urs-12-ene, α-amyrin, α-amyrin acetate, β-amiryn, β-amiryn acetate, boswellonate, β-sitosterol, β-sitosterone, stigmasterol, lupeol and lupenone.[5,6)]In the previous study on the figs of *F. mucuso*, we reported the isolation of isoderrone and alpinumisoflavone as its major constituents.[7)] In continuation of our investigation for the search of bioactive constituents of *F. mucuso*, we examined the methanolic extract of the stem bark.

In this paper we report the isolation and the characterization of two new sphingolipids mucusamide (**1**) and mucusoside (**2**).

Results and Discussion

Repeated column chromatography of the MeOH extract of the stem bark of *F. mucuso*, followed by purification on silica and sephadex column, afforded seventeen compounds, including cellobiosylsterol (**3**),[8,9)] β-sitosterol (**4**),[10)] stigmasterol (**5**),[11)] β-sitosterol 3-*O*-β-D-glucopyranoside (**6**),[12)] lupeol acetate (**7**),[13)] ursolic acid (**8**),[14)] procatechuic acid (**9**),[15)] 2-methyl-5,7-dihydroxychromone 8-*C*-β-D-glucoside (**10**),[16)] apigenin (**11**),[17)] (−)-epicatechin (**12**),[18)] (+)-catechin (**13**),[18)] *N*-benzoyl-L-phenylalanilol (**14**),[19)] α-acetylamino-phenylpropyl α-benzoylamino-phenylpropionate (**15**),[10)] asperphenamate (**16**)[19)] and benjaminamide (**17**)[20)] (Fig. 1).

Mucusamide (**1**) was isolated as a colourless powder mp 125—126 °C. Its molecular formula was assigned as $C_{41}H_{79}NO_6$ with the aid of its High Resolution (HR)-FAB-MS which showed the pseudo-molecular ion peak $[M+H]^+$ at *m/z* 682.5931 (Calcd, 682.5986 for $C_{41}H_{80}NO_6$), containing three degrees of unsaturation. The IR spectrum showed absorption bands for hydroxyl and amide functions (3200—3500 cm^{-1}) and an amide carbonyl group (1660 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table 1) showed signals for an amide proton at δ 8.57 (d, *J*=9 Hz), two terminal methyls at δ 0.84 (6H, t, *J*=7.2 Hz) and an oxygenated methylene at δ 4.49 (1H, dd, *J*=4.8, 11.1 Hz, H-1a) and 4.41 (1H, dd, *J*=4.8, 11.1 Hz, H-1b), four carbinylic protons at δ 4.27 (1H, m), 4.29 (1H, overlapped dd), 4.33 (1H, overlapped dd) and 4.61 (1H, br dd), and a downfield signal at δ 5.18 (1H, m) assigned to H-2 of sphingosine.[21)] Also, the ^{1}H-NMR

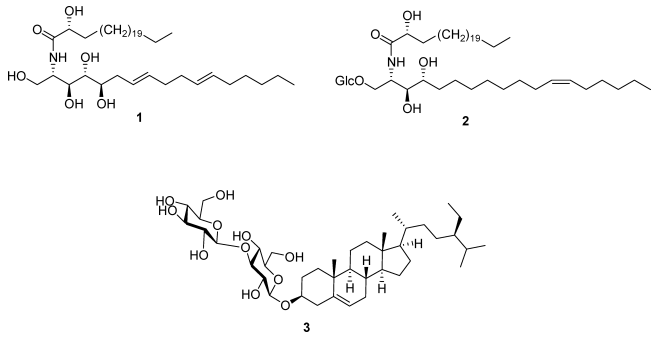

Fig. 1. Structures of Compounds **1**—**3**

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Table 1. ^{1}H- and ^{13}C-NMR Data of **1** and **2** in Pyridine-d_5 (δ in ppm, J in Hz)

Position	**1**		**2**	
	δ_C	δ_H (multi., J Hz)	δ_C	δ_H (multi., J in Hz)
NH	—	8.57 (d, 9)	—	8.55 (d, 9)
1a	62.0 (CH_2)	4.49(dd, 11.1, 4.8)	70.3 (CH_2)	4.69 (dd, 9.9, 6.6)
1b		4.41(dd, 11.1, 4.8)		4.49 (dd, 9.9, 6.6)
2	53.0 (CH)	5.18 (m)	51.7 (CH)	5.27 (m)
3	76.9 (CH)	4.33 (overlapped dd)	75.9 (CH)	4.44 (overlapped dd)
4	73.0 (CH)	4.29 (overlapped dd)	72.5 (CH)	4.18 (overlapped dd)
5a	72.9 (CH)	4.27 (m)	33.9 (CH_2)	2.27 (m)
5b	—	—		1.90 (m)
6a	34.0 (CH_2)	2.19 (m)	26.6	1.90 (m)
6b		2.02 (m)	—	—
–CH_2 (7—10)	—	—	29.6—33.9	1.29 (m)
7	130.2	5.51 (m)	—	—
8	130.8	5.48 (m)	—	—
9	32.1	2.00 (m)	—	—
10	32.1	2.00 (m)	—	—
11	130.4	5.48 (m)	27.5	2.04 (m)
12	130.7	5.51 (m)	130.2	5.45 (m)
13	33.3	2.22 (m)	130.4	5.48 (m)
14	—	—	27.9	2.20 (m)
14—16	22.9—33.0	1.22—1.29 (m)	—	—
15—17	—	—	22.9—33.0	1.22—1.29 (m)
17	14.3	0.84 (t, 7.2)		
18	—	—	14.3	0.84 (t, 7.2)
1′	175.2	—	175.7	
2′	72.5	4.61 (br dd)	72.4	4.55 (br dd)
3′a	35.7	2.20 (m)	35.5	2.17 (m)
3′b		1.99 (m)		1.98 (m)
4′	26.8	1.29 (m)	26.7	1.90 (m)
5′—23′	22.9—33.0	1.22—1.29 (m)	22.9—33.0	1.22—1.29 (m)
24′	14.3	0.84 (t, 7.2)	14.2	0.84 (t, 7.2)
OH-2′	—	7.61 (br s)	—	7.65 (br s)
OH-3	—	6.71 (br s)	—	6.84 (br s)
OH-4	—	6.28 (br s)	—	6.08 (br s)
OH-5	—	6.71 (br s)	—	—
Glucose	—	—	—	—
1″	—	—	105.4	4.92 (d, 7.8)
2″	—	—	75.1	3.98 (dd, 7.8, 7.5)
3″	—	—	78.4	4.15 (overlapped dd)
4″	—	—	71.5	4.18 (overlapped dd)
5″	—	—	78.5	3.85 (m)
6″a	—	—	62.6	4.45 (dd, 10.8, 4.8)
6″b	—	—		4.30 (dd, 11.4, 4.8)

spectrum showed four olefinic protons at δ 5.48 (2H, m) and 5.51 (2H, m), in addition to signals of two alkyl long chains appearing as multiplets at δ 1.22—1.29.[22] The ^{13}C-NMR spectrum of **1** (Table 1) showed characteristic carbon signals for an amide C=O group at δ 175.2, a methine linked to the amide N-atom at δ 53.0 and four carbinylic methines at δ 76.9, 73.0, 72.9 and 72.5. The ^{13}C-NMR spectrum also displayed four olefinic methine carbons at δ 130.8, 130.7, 130.4 and 130.2, suggesting the presence of two double bonds in **1**. A downfield signal due to an oxymethylene (C-1) appeared at δ 62.0. The spectral data and the molecular formula suggested that **1** have a sphingolipid type skeleton.[22] Analysis of the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) spectra (Fig. 2) led to the assignments of proton and carbon signals for **1**. Methylene protons at δ 4.41 (H-1b) and 4.49 (H-1a) showed connectivities with the methine at δ 53.0 (C-2) which in turn was connected to N–H and C–H protons at δ 8.57 and 4.33,

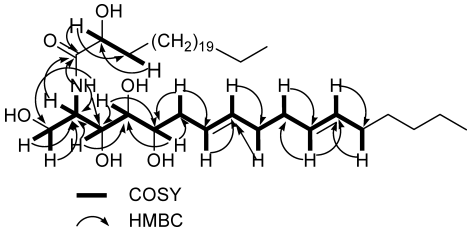

Fig. 2. Some COSY and HMBC Connectivities in **1**

respectively. The C–H proton at 4.29 also correlated with C-3 (δ 76.9) and C-5 (δ 72.9), suggesting the location of four OH groups at C-1, C-3, C-4 and C-5. This was further confirmed by the COSY spectrum (Fig. 2), which showed correlations from the N–CH group (δ 5.18) to CH_2OH (H-1a, H-1b) and an oxygenated C–H group at δ 4.33 (H-3). The former CH group (δ 4.33, H-3) also correlated to another oxygenated C–H group at δ 4.29 (H-4), which in turn correlated with the oxygenated C–H group at δ 4.27 (H-5). The fifth

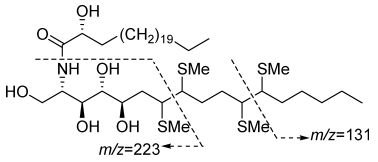

Fig. 3. Important Fragmentations of the DMDS Derivative of **1**

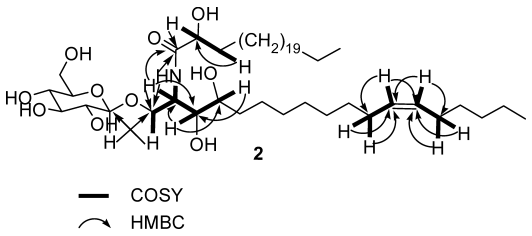

Fig. 4. Some COSY and HMBC Connectivities in **2**

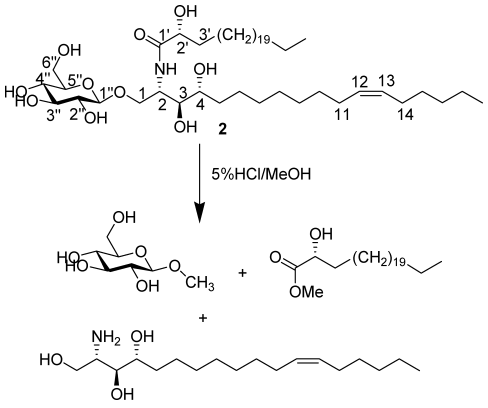

Fig. 5. Methanolysis of **2**

OH group was located at C-2′, which was proved by the HMBC correlations from the C-H group at δ 4.61 (br dd, H-2′) and the N–H group at δ 8.57 to the C=O of the amide group (δ 175.2). One of the olefinic protons at δ 5.51 (m, H-7) showed correlations for two methylene protons each at δ 2.02 (H-6b) and 2.19 (H-6a) and the other olefinic proton at δ 5.48 (H-8). These two methylene protons were inter-correlated and correlated to a proton at δ 4.27 (H-5), thus indicating the presence of –CH(OH)–CH_2–CH=CH– moiety. The fatty acid and sphingosine chain lengths of **1** were determined through methanolysis. When **1** was methanolysed with methanolic hydrochloric acid, a fatty acid methyl ester (FAME) was obtained together with a long-chain base (LCB). Gas chromatography-mass spectrometry (GC-MS) analysis of the FAME characterized methyl 2(*R*)-2-hydroxytetracosanoate {electron ionization (EI): *m*/*z* 398, $[\alpha]_D^{20}$ −3.25° (in $CHCl_3$)},[23] and confirming the existence of two double bonds on the LCB moiety. The positions of double bonds in the LCB part were determinated by the characteristic mass of dimethyldisulfide (DMDS) derivative {[EI: *m*/*z* 131, $[MeSCHC_5H_{11}]^+$ and *m*/*z* 223, $[C_8H_{17}NO_4S]^+$} (Fig. 3). The above characteristic fragments ion peaks obtained after the cleavage of the disulfide bond, indicated that the two double bonds in **1** were located at C-7 and C-11 (Fig. 4). The geometry of the two double bonds was determined to be *trans* as evidenced by the chemical shift of C-6 (34.0), C-9 (32.1), C-10 (32.1) and C-13 (33.3). Typically, the signals of a carbon next to a *trans* double bond appear at $\delta \approx 32$, while those of a *cis* double bond appear at $\delta \approx 27$.[24] The relative and absolute stereochemistry at the chiral centers C-2 to C-5 has already been established in similar molecules.[25] Based on the biogenetic considerations, the absolute stereochemistry of C-2, C-3, C-4 and C-5 in **1** was then proposed as 2*S*, 3*S*, 4*R*, 5*R*. This was confirmed by ^{13}C-NMR data of C-2 (53.0), C-3 (76.9), C-4 (73.0) and C-5 (72.9) which were in agreement with those of nubenamide reported in the literature.[25] Based on these evidences, mucusamide (**1**) could be assigned the structure (2*S*,3*S*,4*R*,5*R*,7*E*,11*E*)-2-{[(2*R*)-2-hydroxytetracosanoyl]amino}heptadeca-7,11-diene-1,3,4,5-tetrol.

Mucusoside (**2**) was isolated as a colourless powder, mp 209—210 °C. The molecular formula $C_{48}H_{93}NO_{10}$ was established by the fast-atom-bombardment mass spectrometry (HR-FAB-MS), showing $[M+H]^+$ ion peak at *m*/*z* 844.6849 (Calcd 844.6877 for $C_{48}H_{94}NO_{10}$) with three degrees of unsaturation. The IR spectrum showed absorption bands for hydroxyl functions (3437 cm^{-1}), an amide group (1670 cm^{-1}) and an olefinic moiety (1643 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra were typical for a sphingosine type skeleton like in **1** with a sugar unit, and possessing 2-hydroxy fatty acid and a β-glucopyranosyl moiety (Table 1).

Assignments of all protons and carbons in **2** could be made by ^{1}H–^{1}H COSY, HMBC and HSQC experiments (Fig. 4). The long range correlations allowed in clearly deducing the presence of a sphingosine-type containing a sugar unit. The fatty acid linked to C-2 (δ 51.7) of the sphingosine moiety was confirmed by the correlation between the NH proton and C-2. HMBC correlations of the carbonyl at δ 175.7 with the proton at δ 4.55 (H-2′) which in turn correlated with C-3′ (δ 35.5), confirmed the presence of an α-hydroxy fatty acid side chain.

When cerebroside **2** was treated with methanolic hydrochloric acid, a FAME was obtained together with LCB and methylglucopyranoside (Fig. 5). Gas chromatography-mass spectrometry (GC-MS) analysis of the FAME showed methyl 2(*R*)-2-hydroxytetracosanoate {EI: *m*/*z* 398, $[\alpha]_D^{20}$ −3.25° (in $CHCl_3$)},[23] and confirming the existence of a double bond in the LCB moiety. The EI-MS of the dimethyldisulfide (DMDS) derivative of **2** showed a characteristic fragment ion peak at *m*/*z* 131 $[MeSCHC_5H_{11}]^+$ due to the cleavage between the carbons bearing the methylthio groups. Therefore, the double bond in LCB moiety of **2** was located at C-12. Moreover, the *cis* geometry (*Z*) of the double bond was determined from δ values 27.5 (C-11) and 27.9 (C-14) of the allylic carbons.

The absolute configuration of the glucopyranosyl moiety was determined as β-D-form by comparing the sign of the optical rotation and spectral data of methyl-β-D-glucopyranoside obtained from **2** with those of an authentic sample.[26,27] The relative stereochemistry of **2** at C-2, C-3, C-4 was proposed as 2*S*, 3*S*, 4*R*, identical to that of D-sphingosine on the basis of ^{13}C-NMR spectral data, since the chemical shifts of C-2 (51.7), C-3 (75.9) and C-4 (72.5) were in agreement with those reported in the literature.[28] Therefore, the structure of **2** was elucidated as 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*,12*Z*)-2-{[(2*R*)-2-hydroxytetracosanoyl]amino}octadec-12-ene-1,3,4-triol.

Compound **3**, sitosterol 3-*O*-[β-D-glucopyranosyl-1(1→3)-

Table 2. ^{1}H- and ^{13}C-NMR Data of **3** in $CD_3OD+CDCl_3$

Position	**3**	
	δ_C	δ_H (multi., J Hz)
1	38.6 (CH_2)	1.97 (m)
2	30.7 (CH_2)	1.28 (m)
3	79.8 (CH)	3.60 (m)
4a	39.7 (CH_2)	2.24 (m)
4b		2.42 (m)
5	141.9 (C)	—
6	122.9 (CH)	5.36 (m)
7a	33.1 (CH_2)	1.47 (m)
7b		1.98 (m)
8	33.3 (CH)	1.51 (m)
9	51.7 (CH)	0.95 (m)
10	37.9 (C)	—
11	22.2 (CH_2)	1.50 (m)
12	41.1 (CH_2)	2.01 (m)
13	43.5 (C)	—
14	58.2 (CH)	1.02 (m)
15	25.3 (CH_2)	1.61 (m)
16	29.4 (CH_2)	1.97 (m)
17	57.4 (CH)	1.12 (m)
18	12.3 (CH_3)	0.87 (s)
19	19.9 (CH_3)	0.99 (s)
20	37.5 (CH)	1.37 (m)
21	19.3 (CH_3)	0.78 (d, 8)
22a	35.1(CH_2)	1.03 (m)
22b		1.38 (m)
23	27.1 (CH_2)	1.19 (m)
24	47.3 (CH)	0.94 (m)
25	30.3 (CH)	1.68 (m)
26	20.2 (CH_3)	0.77 (d, 8)
27	19.4 (CH_3)	0.88 (d, 6)
28	24.1 (CH_2)	1.30 (m)
29	12.4 (CH_3)	0.72 (d, 8)
Glucose-I	—	—
1′	102.0 (CH)	4.42 (d, 8)
2′	73.8 (CH)	3.55 (m)
3′	88.0 (CH)	3.53 (m)
4′	70.0 (CH)	3.38 (m)
5′	78.2 (CH)	3.30 (m)
6′a	64.7 (CH_2)	3.58 (m)
6′b		3.72 (m)
Glucose-II	—	—
1″	105.3 (CH)	4.54 (d, 7.2)
2″	74.5 (CH)	3.33 (m)
3″	75.5 (CH)	3.24 (m)
4″	71.6 (CH)	3.25 (m)
5″	77.5 (CH)	3.26 (m)
6″a	62.6 (CH_2)	3.64 (m)
6″b		3.86 (m)

β-D-glucopyranoside] (cellobiosylsterol), was previously isolated from the rice bran as a colourless amorphous powder. Previous determination of its structure was based on chemical transformations and mass spectrometry. We reported here for the first time its ^{1}H- and ^{13}C-NMR data, and signal assignments of **3** based on the COSY, HSQC and HMBC connectivities (Table 2).

Experimental

General Experimental Procedure Melting points were obtained on a Gallenkamp melting point apparatus. Optical rotations were measured with a JASCO DIP-360 polarimeter. UV spectra were recorded on a Hitachi UV 3200 spectrophotometer. A JASCO 320-A spectrophotometer was used for scanning IR spectroscopy using KBr pellets. 1D- and 2D-NMR spectra were run on Bruker spectrometer operating 75, 100, 125, 150, 300, 400, 500 and 600 MHz where chemical shifts (δ) were expressed in ppm with reference to the solvent signals (in the case of mixture of $CD_3OD+CDCl_3$, some drops of $CDCl_3$ were added to complete the dissolution of the sample and the reference was CD_3OD signal). EI-MS spectra were obtained on Varian MAT 311A mass spectrometer. FAB-MS spectra were measured on a JOEL-HX 110 mass spectrometer. Column chromatography was performed on silica gel. Fractions were monitored by TLC using Merck pre-coated silica gel sheets (60 F_{254}), and spots were visualized by heating silica gel plates sprayed with ceric sulfate reagent solution.

Plant Material The stem bark of *F. mucuso* was collected in March, 2005 from Ngoa-Ekelle, Yaounde, Central Region of Cameroon and identified by Mr. Nana Victor, botanist at the National Herbarium, where the voucher specimen is deposited (HN no. 41204).

Extraction and Isolation The air-dried and powdered stem bark (5.5 kg) of *F. mucuso* was extracted with MeOH (15 l) (1 week, repeated three times) at room temperature. The extract was then concentrated to dryness under vacuum at 40 °C to give 290 g of dark brownish crude extract. The extract was subjected to medium pressure liquid column chromatography over silica gel eluting with *n*-hexane, *n*-hexane/EtOAc, EtOAc and EtOAc/MeOH, in increasing order of polarity. One hundred and fifty subfractions, each containing 500 ml, were collected and combined according to their TLC profiles on pre-coated silica gel 60 F_{254} plates developed with *n*-hexane/EtOAc and $CHCl_3$/MeOH mixture to give 6 fractions (F1—6). Fraction F1 was subjected to CC over silica gel, eluted with *n*-hexane/EtOAc mixture starting from 100% *n*-hexane to 50% of the mixture. This resulted in the isolation of lupeol acetate (**7**) (2.05 g), β-sitosterol (**4**) (115 mg), stigmasterol (**5**) (8 mg) and ursolic acid (**8**) (10 mg). Fraction 2 was also subjected to successive CC using the same quality of silica and eluted with a mixture of EtOAc and *n*-hexane (1 : 9—100% EtOAc) to give: asperphenamate (**16**) (14 mg) and α-acetylamino-phenylpropyl α-benzoylamino-phenylpropionate (**15**) (3 mg), *N*-benzoyl-L-phenylalanilol (**14**) (5 mg).

Fraction 3 was eluted with the same mixture with different polarity (3 : 7 to 100% EtOAc) to give apigenin (**11**) (4.5 mg). Fraction 4 was eluted with $CHCl_3$/MeOH of increasing polarity (100% $CHCl_3$ to 1 : 9) to yield sitosterol 3-*O*-β-D-glucopyranoside (**6**) (120 mg), (−)-epicatechin (**12**) (10 mg), (+)-catechin (**13**) (3 mg), benjaminamide (**17**) (10 mg) and mucusamide (**1**) (21 mg). Fraction 5 was also successively eluted with $CHCl_3$/MeOH mixtures to yield procatechuic acid (**9**) (31 mg), 2-methyl-5,7-dihydroxychromone 8-*C*-β-D-glucoside (**10**) (22 mg) and mucusoside (**2**) (17 mg), respectively. Fraction 6 was also eluted with $CHCl_3$/MeOH mixtures to yield sitosterol 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside] (**3**) (5 mg).

Mucusamide, (2*S*,3*S*,4*R*,5*R*,7*E*,11*E*)-2-{[(2*R*)-2-Hydroxytetracosanoyl]-amino}heptadeca-7,11-diene-1,3,4,5-tetrol (**1**): Colourless powder, mp 125—126 °C, $[\alpha]_D^{30}$ +29° (c=0.11, pyridine), IR (KBr) ν_{max} cm^{-1}: 3200—3500 (OH and NH), 1660 (NH–C=O), *m/z*: 682.5931 (Calcd for $C_{41}H_{80}NO_6$: 682.5986, $[M+H]^+$), ^{1}H- (300 MHz, pyridine-d_5) and ^{13}C- (100 MHz, pyridine-d_5) NMR data: see Table 1.

Mucusoside, 1-*O*-(β-D-Glucopyranosyl)-(2*S*,3*S*,4*R*,12*Z*)-2-{[(2*R*)-2-hydroxytetracosanoyl]amino}octadec-12-ene-1,3,4-triol (**2**): Colourless powder, mp 209—210 °C, $[\alpha]_D^{30}$ +34° (c=0.08, pyridine), IR (KBr) ν_{max} cm^{-1}: 3437 (OH), 1670 (NH–C=O), 1643 (C=C), *m/z*: 844.6849 (Calcd for $C_{48}H_{94}NO_{10}$: 844.6878, $[M+H]^+$), ^{1}H- (300 MHz, pyridine-d_5) and ^{13}C- (125 MHz, pyridine-d_5) NMR data: see Table 1.

Sitosterol 3-*O*-[β-D-Glucopyranosyl-1(1→3)-β-D-glucopyranoside] (**3**): Colourless amorphous powder, $[\alpha]_D^{30}$ +63° (c=0.03, pyridine), IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 1480 (C=C) and 1084 (C–O–C), *m/z*: 738.4920 (Calcd for $C_{41}H_{70}O_{11}$: 738.4918), ^{1}H- (400 MHz, $CD_3OD+CDCl_3$) and ^{13}C- (150 MHz, $CD_3OD+CDCl_3$) NMR data: see Table 2.

Methanolysis of 1 and 2 Compounds **1** and **2** (1 mg each) were heated separately with 5% HCl in MeOH (1 ml) at 70 °C for 12 h in a sealed small-volume vial. The reaction mixture of each compound was extracted with *n*-hexane and the hexane layer was concentrated to give FAME for GC-MS analysis. The MeOH layer was concentrated *in vacuo* to give LCB-1 part for **1** and the mixture LCB-2 part and methyl glycoside for **2**.

To determine the nature of LCB and the methyl glycoside, the process was repeated from 10 mg of **1** and **2**. The MeOH layer from the methanolysis of **1** was suspended in H_2O and LCB-1 (3.4 mg, 73.5%) was extracted with EtOAc. LCB-1: EI-MS *m/z*: 315 $[M]^+$, $[\alpha]_D^{30}$ +28.7° (c=0.05, MeOH), ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 7.81 (2H, d, J=9 Hz, NH_2), 5.44 (4H, m, H-7, H-8, H-11, H-12), 5.16 (1H, m, H-2), 4.46 (1H, dd, J=10.2, 3.9 Hz, H-1b), 4.42 (1H, dd, J=10.2, 4.3 Hz, H-1a), 4.35 (1H, m, H-3), 4.31 (1H, m, H-4), 4.25 (1H, m, H-5), 2.20 (2H, m, H-13), 2.17 (1H, m, H-6a), 2.00 (1H, m, H-6b), 1.90 (4H, m, H-9, H-10), 1.25 (6H, br s), 0.86 (3H, t, J=8.4 Hz, CH_3-17).

The aqueous methanolic layer from **2** was evaporated to dryness and the

residue separated by TLC as sphingosine base and methylated sugar. LCB-2 (2.9 mg, 77.6%) EI-MS *m/z*: 315 $[M]^+$, $[\alpha]_D^{30}$ +11.5° (*c*=0.09, $CHCl_3$/CH_3OH); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.02 (2H, d, *J*=9 Hz, NH_2), 5.32 (2H, m, H-12, H-13), 5.10 (1H, overlapped m, H-2), 4.18 (1H, dd, *J*=4.3, 7.4 Hz, H-1a), 3.78 (3H, m, H-1b, H-3, H-4), 2.31—2.04 (2H, m), 1.80 (1H, m), 1.64 (4H, m), 1.25 (16H, br s), 0.88 (3H, t, *J*=7.0 Hz, CH_3-18). The presence of methyl-β-D-glucopyranoside (1.8 mg, 78.2%) was established by comparison of its NMR data with those reported in the literature[26,27] and with an authentic sample, $[\alpha]_D^{28}$ +74° (*c*=0.1, MeOH), *Rf* 0.46 (EtOAc/MeOH/H_2O, 5 : 2 : 0.5). Authentic sample $[\alpha]_D^{28}$ +77° (*c*=0.1, MeOH), *Rf* 0.46.

GC-MS Analysis of FAME from Compounds 1 and 2 The FAME from **1** and **2** were subjected to GC-MS [column temp. 100—250 °C (rate of temp. increases 5 °C/min)]. The results were as follows: FAME-1 from **1** (methyl 2-hydroxytetracosanoate), t_R=40.62 (29), EI-MS *m/z*: 398 $[M]^+$, 339 $[M-59]^+$. The FAME-2 from **2** gave the same fragments like the previous one.

DMDS Derivatives of 1 and 2 Compounds **1** and **2** (1.0 mg each) were dissolved separately in carbon disulfide (0.2 ml) and iodine (1 mg) was added to the solution of each. The resulting mixtures were stored at 60 °C for 40 h in two separate small-volume sealed vials. The reaction was subsequently quenched with aqueous $Na_2S_2O_3$ (5%), and mixtures were extracted with *n*-hexane (0.3 ml). The extracts were concentrated to give the **1** and **2** DMDS derivatives. EI-MS *m/z*: 131 (3.2) $[MeSCHC_5H_{11}]^+$, 223 (4.0) $[C_8H_{17}NO_4S]^+$, 279 (7.9) $[C_{13}H_{27}S_3]^+$, 384 (8.1) $[C_{24}H_{50}O_2N]^+$, 522 (48.3) $[C_{31}H_{56}O_3NS]^+$ and 540 (38.2) $[C_{31}H_{58}O_4NS]^+$ for DMDS derivatives of **1** and fragments at *m/z*: 131 (9) $[MeSCHC_5H_{11}]^+$, 163 (3) $[C_6H_{11}O_5]^+$ and 384 (8.07) $[C_{24}H_{50}O_2N]^+$ for DMDS derivatives of **2** as characteristic fragments.

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