Constituents of Euphorbiaceae, 13. Comm. [1]

Isolation and Structure Elucidation of Five Cerebrosides from *Euphorbia characias* L.

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Five cerebrosides B-1 – B-4 were isolated from the fraction B, obtained from the latex of *Euphorbia characias* L. On the basis of spectral evidences and chemical reactions they were characterized as (2S, 3S, 4R, 8Z)-1-O-(β-D-glucopyranosyl)-2N-[(2′R)-2′-hydroxytetracosenoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-1**), (2S, 3S, 4R, 8Z)-1-O-(β-D-glucopyranosyl)-2N-[(2′R)-2′-hydroxyhexacosenoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-2**), (2S, 3S, 4R, 8Z)-1-O-(β-D-glucopyranosyl)-2N-[(2′R)-2′-hydroxyoctacosenoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-3**), (2S, 3S, 4R, 8Z)-1-O-(β-D-glucopyranosyl)-2N-[(2′R)-2′-hydroxyhexacosanoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-3a**), (2S, 3S, 4R, 8Z)-1-O-(β-D-glucopyranosyl)-2N-[(2′R)-2′-hydroxyheptacosanoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-4**).

Reversed phase column flash chromatography was effective for the isolation of the cerebrosides. FAB-MS spectrometry, ^{1}H NMR, ^{13}C NMR analyses and DQF-COSY and ^{1}H-detected HMQC (single bond and multiple bond) experiments and chemical reactions were useful in providing informations for the structure elucidation.

Introduction

As described in a preceding paper [2], three complex cerebrosides mixtures, named A, B and C, were obtained from the latex of *Euphorbia characias* L. and four new cerebrosides from the fraction C have been isolated and characterized as 1-O-β-D-glucopyranosides of unsaturated 1,3,4,5-tetrahydroxysphingosine long chain base type ceramides possessing 2-hydroxy unsaturated and saturated fatty acids. In continuation of our study on the isolation and structure elucidation of cerebrosides from *Euphorbiaceae*, seven further cerebrosides of similar molecular species from *Euphorbia wulfenii* H. ex K. have been isolated and identified [3]. From our studies on the latex of *Euphorbia biglandulosa* Desf., recently, four new cerebrosides of a molecular species of glucosylceramides having 1,3,4-trihydroxy unsaturated C-18 and C-17 long chain bases and 2-hydroxy fatty acids, have been isolated and identified [1]. By extending our work to the obtained fraction B, by silica gel column chromatography with chloroform/methanol, five new cerebrosides **B-1 – B-4** were isolated and purified using reversed-phase flash-chromatography. The structures of these cerebrosides have been determined on the basis of FAB-MS, ^{1}H NMR, ^{13}C NMR spectroscopy, DQF-COSY [4] and ^{1}H-detected HMQC [5] (single bond and multiple bond) experiments and chemical reactions.

Results and Discussion

As described in a previous paper [2], a complex mixture of cerebrosides has been obtained from the latex of *Euphorbia characias* L. and subjected to a

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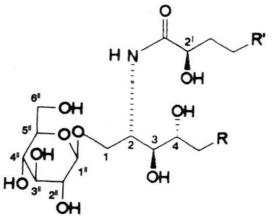


B-1: R' = $(CH_2)_{10}$–CH=CH–$(CH_2)_7$-CH_3, R = $(CH_2)_3$–CH=CH–$(CH_2)_8$-CH_3

B-2: R' = $(CH_2)_{12}$–CH=CH–$(CH_2)_7$-CH_3, R = $(CH_2)_3$–CH=CH–$(CH_2)_8$-CH_3

B-3: R' = $(CH_2)_{14}$–CH=CH–$(CH_2)_7$-CH_3, R = $(CH_2)_3$–CH=CH–$(CH_2)_8$-CH_3

B-3a: R' = $(CH_2)_{21}$-CH_3, R = $(CH_2)_3$–CH=CH–$(CH_2)_8$-CH_3

B-4: R' = $(CH_2)_{22}$-CH_3, R = $(CH_2)_3$–CH=CH–$(CH_2)_8$-CH_3

Fig. 1. Structures of the cerebrosides B-1 – B-4.

silica gel column chromatography to give three fractions named A, B and C respectively. The less polar fraction B, as the more polar fraction C, previously examined [2], showed a single spot on normal phase TLC, however, it revealed eight spots on reversed phase TLC. By reversed phase flash-chromatography, four pure major compounds **B-1 – B-4** were isolated from the fraction B and characterized (Fig. 1).

The positive FAB-MS spectra of the compounds **B-1 – B-4,** using alkali metal ions, showed molecular ion peaks at *m/z* = 865 (**B-1**), 893 (**B-2**), 921 (**B-3**), 895 (**B-3 a**) and at *m/z* = 909 (**B-4**) $(M + Na)^+$. Other fragments at *m/z* = 663 (**B-1**), 691 (**B-2**), 719 (**B-3**), 693 (**B-3 a**) and at *m/z* = 707 (**B-4**) $(M-179)^+$ were observed and attributed to the loss of glucose. The common fragment ion at *m/z* = 500, in the MS spectra of **B-1 – B-4** and attributed to the glucosphingoside moiety $(M + Na - FA)^+$, indicates the presence of a monounsaturated trihydroxy long-chain base (Fig. 2). In order to achieve structural and spectral assignments, the cerebrosides **B-1 – B-4** were subjected to DQF-COSY and ^{1}H-detected HMQC (single bond and multiple bond) analyses. The spectral assignments are reported in Table I and II. The NH resonances of **B-1 – B-4** were found at δ = 8.59 – 8.57 ppm. These signals correlate with the multiplet at δ = 5.30 – 5.27 ppm, ascribed to the proton H-2 and this again correlates with other three signals at δ = 4.70, due to the proton H_a-1, δ =

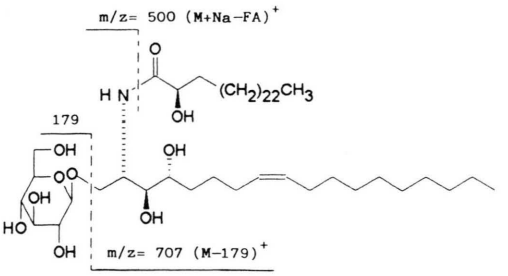


Fig. 2. FAB-MS spectrum of the cerebroside B-4.

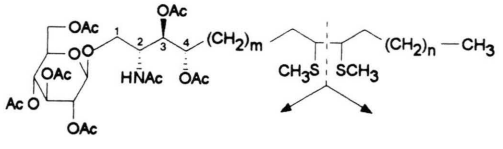
4.52 – 4.53 due to H_b-1 and at δ = 4.28 – 4.24 ppm, due to H-3 respectively. The signal of the proton H-3 at δ = 4.28 – 4.24 ppm on the other side correlates with the signal at δ = 4.23 – 4.20 ppm, suggesting so the attribution of the signal to the proton H-4, which, further, correlates with the signal at δ = 1.95 ppm ascribed to the methylen group next to the proton H-4. The signal at δ = 4.95 – 4.24 ppm, due to the anomeric proton H-1″ of the glucose, correlates with the triplet at δ = 4.00 ppm, allowing the attribution of this signal to the proton H-2″ and its correlation with the signal at δ = 4.19 – 4.14 ppm suggests the assignation of this signal to the proton H-3″. The attribution of the signals at δ = 4.48 – 4.47, due to the proton H_a-6″ and at δ = 4.34 – 4.32 ppm, due to the proton H_b-6″, has been possible on the basis of the correlations of the multiplet at δ = 3.85 – 3.84 ppm, belonging to the proton H-5″.

According to the Gover-Sweely method [6], the compounds **B-1 – B-4** were methanolyzed with methanolic hydrochloric acid to yield fatty acid methyl esters (FAMs), long chain base (LCB) and glucosphingoside together with methyl D-glucopyranoside respectively. For the determination of the structure and location of the double bond in the long chain parts, the FAMs obtained from the cerebrosides **B-1 – B-4** were analyzed by EI-MS and molecular ions at *m/z* = 396 $(M)^+$ (FAM-1), 424 $(M)^+$ (FAM-2), 452 $(M)^+$ (FAM-3), 426 $(M)^+$ (FAM-3 a) and at *m/z* = 440 (FAM-4) were observed. In the ^{13}C NMR spectra of the monounsaturated FAMs-1 – 3 characteristics signals at δ = 129.9 and 27.2 ppm have been exhibited confirming the cis (Z) geometry of the double bond (Table IV). The position of the double bond in the monounsaturated fatty acid methyl esters **B-1 – B-3** was determined by GC-MS analysis of the dimethyl disulfide (DMDS) derivatives [7, 8], which showed a common fragment at

Table I. ^{1}H NMR data of B-1 – B-4 in C_5D_5N (δ values in ppm from TMS, splitting in Hz in parentheses).

	B1	B2	B3, 3a	B4
NH	8.59 (d; 9.0)	8.57 (d; 9.0)	8.57 (d; 9.0)	8.57 (d; 9.0)
HC=	5.50 (m)	5.50 (m)	5.48 (m)	5.49 (m)
H-2	5.30 (m)	5.27 (m)	5.27 (m)	5.28 (m)
H_a-1	4.70 (dd; 10.6, 5.3)	4.70 (dd; 10.7, 5.3)	4.70 (dd; 10.6, 5.3)	4.70 (dd; 13.3, 5.3)
H-2′	4.55 (dd; 7.6, 3.8)	4.58 (dd; 8.0, 4.2)	4.57 (dd; 6.9, 4.8)	4.57 (dd; 8.0, 5.3)
H_b-1	4.52 (dd; 10.5, 4.4)	4.52 (dd; 10.6, 5.3)	4.53 (dd; 10.1, 4.8)	4.52 (dd; 11.2, 5.2)
H-3	4.28 (dd; 4.4, 3.5)	4.27 (dd; 5.4, 4.8)	4.24 (dd; 5.3, 4.2)	4.26 (dd; 3.0, 4.8)
H-4	4.23 (obs.)	4.21 (obs.)	4.20 (obs.)	4.22 (obs.)
H-3″	4.19 (obs.)	4.14 (obs.)	4.14 (obs.)	4.15 (obs.)
H-1″	4.95 (d; 8.0)	4.92 (d; 7.9)	4.93 (obs. H_2O)	4.93 (d; 8.0)
H-4″	4.20 (obs.)	4.18 (obs.)	4.17 (obs.)	4.18 (obs.)
H_a-6″	4.48 (dd; 12.2, 2.2)	4.47 (dd; 10.6, 2.2)	4.48 (dd; 11.2, 2.3)	4.48 (dd; 11.7, 2.2)
H_b-6″	4.34 (dd; 11.7, 5.4)	4.32 (dd; 10.6, 3.7)	4.32 (dd; 10.6, 3.8)	4.34 (dd; 10.6, 4.8)
H-2″	4.00 (t; 8.0)	4.00 (t; 8.0)	4.00 (t; 8.0)	4.00 (t; 8.0)
H-5″	3.85 (m)	3.84 (m)	3.84 (m)	3.85 (m)
OH-2′	7.65 (br. s)	7.63 (d; 5.3)	7.64 (d; 5.37)	7.65 (d; 5.3)
OH-3	6.82 (br. s)	6.82 (d; 5.3)	6.82 (d; 5.3)	6.85 (d; 5.3)
OH-6″	6.40 (br. s)	6.40 (m)	6.40 (m)	6.38 (m)
OH-4	6.02 (br. s)	6.04 (d; 5.3)	6.04 (d; 5.3)	6.05 (d; 6.4)

m/z = 173 $(C_{10}H_{21}S)^+$ due to the cleavage between the sulfided carbons, indicating the position of the double bond. On the basis of the above described data the FAMs-1 – 3 are methyl 2-hydroxy-(15′Z)-tetracosenoate (from **B-1**), -(17′Z)-hexacosenoate (from **B-2**), -(19′Z)-octacosenoate (from **B-3**) while the saturated FAMs-3 a, 4 are methyl 2-hydroxy-hexacosanoate (from **B-3 a**) and methyl 2-hydroxy-heptacosanoate (from **B-4**). For the determination of the location of the double bonds in the long chain bases, the glucosphingosides, derived from the methanolysis of the cerebrosides **B-1 – B-4**, have been acetilated, treated with dimethyldisulfide and subjected to EI-MS analysis. In the EI-MS spectra of the glucosphingoside-heptaacetate-DMDS derivatives 1 – 4, a common molecular ion at m/z = 865 $(M)^+$ and at m/z = 187 $(C_{11}H_{23}S)^+$ respectively have been observed, suggesting the position of the double bond at C-8, C-9 on the common long chain base (LCB) (Fig. 3). The FAB-MS data of the gluco-



GSAc$_7$DMDS-1-4: m=2, m/z = 678 (M-187), n=7, m/z = 187 $(C_{11}H_{23}S)^+$

Fig. 3. EI-MS data of the characteristic fragments, obtained from the cleavage between the sulfided carbons of the GSAc$_7$-DMDSs-1 – 4.

Table II. ^{13}C δ values of B-1 – B-4 in C_5D_5N (ppm from TMS).

C-Atom	B 1	B 2	B 3, 3 a	B 4
1	70.5	71.0	70.6	70.5
2	51.8	52.3	51.9	51.7
3	76.9	76.5	72.1	76.0
4	72.5	73.0	72.6	72.5
HC=	130.3	130.8	130.4	130.3
1′	175.7	176.2	175.8	175.7
2′	72.5	72.1	72.6	72.5
CH_3–	14.3	14.9	14.5	14.3
CH_2–C=	27.6	27.4	27.7	27.6
1″	105.6	106.1	105.7	105.6
2″	75.2	75.7	75.3	75.2
3″	78.5	79.0	78.7	78.6
4″	71.5	71.0	71.6	71.5
5″	78.6	79.1	78.6	78.5
6″	62.6	63.2	62.8	62.7

sphingoside-heptaacetates 1–4 at m/z = 778 $(M+Li)^+$ confirm the structure of the LCB respectively. On the basis of the above analytic data, the common long chain base in **B-1 – 4** is a 2-amino-1, 3, 4- (8 Z)-trihydroxyoctadecene.

Accordingly to the results obtained and on the basis of comparison of the spectroscopic data of natural and synthesized 1,3,4-trihydroxy long chain bases [9–11], the structures of the cerebrosides **B-1 – 4** were determined as (2 S, 3 S, 4 R, 8 Z)-1-O-(β-D-glucopyranosyl)-2 N-[(2′R)-2′-hydroxytetracosenoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-1**), (2 S, 3 S, 4 R, 8 Z)-1-O-(β-D-glucopyranosyl)-2 N-[(2′R)-2′-hydroxyhexacosenoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-2**), (2 S, 3 S, 4 R, 8 Z)-1-O-(β-D-glucopyranosyl)-2 N-[(2′R)-2′-hydroxyoctacosenoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-3**), (2 S, 3 S, 4 R, 8 Z)-1-O-(β-D-glucopyranosyl)-2 N-[(2′R)-2′-hydroxyhexacosanoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-3 a**), (2 S, 3 S, 4 R, 8 Z)-1-O-(β-D-glucopyranosyl)-2 N-[(2′R)-2′-hydroxyheptacosanoyl]-8 (Z)-octadecene-1,3,4-triol-2-amino (**B-4**).

Table IV. ^{13}C δ values of FAMs-1 – 4 in $CDCl_3$ (ppm from TMS).

	FAM-1	FAM-2	FAM-3, 3 a	FAM-4
C-1	175.5	175.6	175.5	175.5
HC=C	129.9	129.8	129.2	129.0
C-2	70.5	70.5	69.8	70.2
CH_3–O	52.3	52.3	51.8	52.3
CH_2–C=	27.2	27.2	26.5	–
CH_3–	14.0	14.0	13.5	13.7

The compounds **B-1** and **B-4,** thus characterized, were found to be similar with the cerebrosides 4 and 6 isolated from the latex of *Euphorbia biglandulosa* Desf. [1].

The biological activities of the compounds **B-1 – B-4** will be examined.

Experimental

^1H NMR spectra: Varian Unity 400 spectrometer and Varian Gemini 200 spectrometer. ^{13}C NMR: 100.40 MHz. NMR spectra were obtained by using pyridine-d_5 (C_5D_5N) or $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. FAB-MS, EI-MS: Kratos MS 80 RFA. FAB-MS (8 Kv, Xe; methanol as solvent and glycerol matrix + NaCl or LiCl). EI-MS: (4 Kv, 70 eV). Silica gel column chromatography: Kieselgel 60 (230–400 Mesh, 60 Å, Merck). Flash-chromatography: LiChroprep RP-18 (40–63 μm). All solvents were distilled before use. TLC: Kieselgel 60 F_{254} (20 × 20 cm; 0.2 mm, Merck) and RP-18 F_{254} S (10 × 10 cm, Merck). HPLC was performed on Waters modell 6000 A, using a R 401 detector (column RP-18, 100 Å, 10 × 0.2 cm; Waters).

	FAM-1	FAM-2	FAM-3, 3 a	FAM-4
HC=	5.32 (m)	5.35 (m)	5.35 (m)	–
H-2	4.16 (dd; 6.9, 4.2)	4.19 (dd; 7.0, 4.3)	4.19 (dd; 7.2, 4.3)	4.19 (dd; 7.1, 4.2)
CH_3– O	3.76 (s)	3.77 (s)	3.80 (s)	3.80 (s)
CH_2–	2.00–1.15 (m)	2.01–1.20 (m)	2.01–1.20 (m)	2.00–1.20 (m)
CH_3–	0.85 (t; 7.5)	0.80 (t; 7.5)	0.86 (t; 7.5)	0.90 (t; 7.5)

Table III. ^1H NMR data of FAMs-1 – 4 in $CDCl_3$ (δ values in ppm from TMS, splitting in Hz in parentheses).

Materials

The latex of *Euphorbia characias* L. (500 ml) was collected in Gibesi Country (Sicily) in May 1992; the stems of the plant cut and placed in a becker afforded the latex which was filtered and stored under nitrogen. The latex (100 ml × 5) was extracted with ether (750 ml) in an apparatus for the continuous extraction for 24 h. Ether was evaporated on a rotary evaporator and the residue taken up in $CHCl_3$:MeOH (4:1/v:v) (200 ml) to give an emulsion which was poured into a separatory funnel and allowed to stand for 8 h. The lower layer (150 ml) was separated and evaporated *in vacuo* to remove the organic solvent and chromatographed on a silica gel column (100 × 7 cm) eluted with $CHCl_3$:MeOH (4:1/v:v) to afford the less polar compounds and, then, with MeOH to provide the complex mixture of cerebrosides. The MeOH eluate was evaporated on a rotary evaporator and chromatographed on silica gel column (50 × 4 cm) eluted with $CHCl_3$:MeOH (4:1/v:v) to afford three fractions of cerebrosides named A, B and C respectively.

Separation of cerebrosides B-1 – B-4

The fraction B (0.4 g) was separated by flash-chromatography on RP-18 column (4.5 × 2 cm) eluted with methanol to afford the cerebrosides B-1 (90 mg) R_f = 0.45 [TLC:MeOH, RP-18]; B-2 (100 mg) R_f = 0.50; B-3, 3 a (80 mg) R_f = 0.51; B-4 (60 mg) R_f = 0.52, respectively.

Cerebroside B-1 [$C_{48}H_{92}NO_{10}$ (843.2)]

HPLC of the cerebroside B-1 showed one peak [column: Nova Pak C18, 60 Å, 4 μm (2 × 150 mm), solvent: MeOH, flow rate: 1 ml/min, t_r = 2.7 min]. FAB-MS: *m/z* = 865 $(M+Na)^+$, 681 $(M-162)^+$, 663 $(M-179)^+$, 500 $(M+Na-FA)^+$, 478 $(M+H-FA)^+$, 317 $(LCB)^+$, 298 $(LCB-18)^+$, 280 $(LCB-2\times18)^+$, 262 $(LCB-3\times18)^+$. [For ^1H NMR and ^{13}C NMR of the compounds **B-1 – B-4** see Tables I and II].

Methanolysis of B-1 – B-4

The cerebrosides **B-1 – B-4** were heated under reflux with 0.9 N HCl in 82% MeOH (1 ml) for 18 h respectively. The reaction mixture was extracted with *n*-hexane, the *n*-hexane layer concentrated *in vacuo* and the residue chromatographed on silica gel [*n*-hexane:ether (1:1)] to yield the fatty acid methyl esters (FAMs). The aqueous MeOH layer was neutralized with Amberlite IR-45, concentrated *in vacuo* and the residue chromatographed on Sephadex LH-20 (MeOH) to give glucosphingosides and β-methyl glucosides.

FAM-1 [methyl 2-hydroxy-(15′Z)-tetracosenoate]: EI-MS (70 eV): *m/z* = 396 (M, 42%)$^+$, 337 (M-59, 100%)$^+$. [For ^1H NMR and ^{13}C NMR of the FAMs-**B-1 – B-4** see Tables III and IV].

Preparation of Glucosphingoside heptaacetates 1–4

The glucosphingosides 1–4 were treated with Ac_2O/pyridine (1 ml) respectively and allowed to stand overnight, treated with aqueous saturated $CuSO_4$x $5H_2O$ solution, extracted three times with chloroform and the chloroform layers were washed with H_2O, dried with Na_2SO_4 and concentrated. The crude acetates were purified by silica gel column chromatography [*n*-hexane/AcOEt (3:2/v:v)] to give glucosphingosides heptaacetates 1–4.

Glucosphingoside heptaacetates 1–4: FAB-MS: *m/z* = 778 $(M+Li)^+$ respectively.

Preparation of Glucosphingoside heptaacetate-DMDS derivatives 1–4

The glucosphingoside heptaacetates 1–4 were dissolved in carbon disulfide (1 ml) and dimethyl disulfide (DMDS) (1 ml) and iodine (10 mg) were added respectively, and the mixture was kept at 60 °C for 40 h in a small volume sealed vial. The reaction was quenched with aqueous $Na_2S_2O_3$ (5%) and the reaction mixture extracted with ethyl acetate and concentrated to give the DMDS derivatives of the glucosphingoside heptaacetates 1–4.

Glucosphingoside heptaacetates-DMDSs-1–4: EI-MS (70 eV): *m/z* = 865 (M, 30.7%)$^+$, 817 (M-48, 83%)$^+$, 771 (M-94, 61%)$^+$, 711 (M-94-60, 23%)$^+$, 678 (M-187, 46%)$^+$, 618 (M-187-60, 30%)$^+$, 424 (M-94-347, 23%)$^+$, 348 (12%), 331 (100%), 169 (92%), 109 (23%) ($GluAc_4$ moiety), 187 ($C_{11}H_{23}S$, 7.7%)$^+$ respectively.

Preparation of DMDS derivatives of FAMs-1–4

The DMDS derivatives of FAMs-1–4 were prepared as described above for the glucosphingoside heptaacetates 1–4.

FAM-DMDS-1: EI-MS (70 eV): *m/z* = 490 (M, 23%)$^+$, 443 (M-47, 12%)$^+$, 383 (M-47-60, 19%)$^+$, 317 (M-173, 19%)$^+$, 269 (M-173-48, 100%)$^+$, 173 ($C_{10}H_{21}S$, 60%)$^+$.

Cerebroside B-2 [$C_{50}H_{96}NO_{10}$ (871.3)]

HPLC of the cerebroside B-2 showed one peak [t_r = 4.5 min]. FAB-MS: *m/z* = 893 $(M+Na)^+$, 709 $(M+H-162)^+$, 691 $(M-179)^+$, 500 $(M+Na-FA)^+$, 478 $(M+H-FA)^+$.

FAM-2 [methyl 2-hydroxy-(17′Z)-hexacosenoate]: EI-MS (70 eV): *m/z* = 424 (M, 46%)$^+$, 365 (M-59, 100%)$^+$.

FAM-DMDS-2: EI-MS (70 eV): *m/z* = 518 (M, 38%)$^+$, 471 (M-47, 15%)$^+$, 411 (M-47-60, 27%)$^+$, 345 (M-173, 30%)$^+$, 297 (M-173-48, 100%)$^+$, 173 ($C_{10}H_{21}S$, 69%)$^+$.

Cerebroside B-3 [$C_{52}H_{100}NO_{10}$ (899.4)]

HPLC of the cerebroside B-3 showed one peak [t_r = 5.8 min]. FAB-MS: *m/z* = 921 (M+Na)$^+$, 737 (M-162)$^+$, 719 (M-179)$^+$, 500 (M+Na−FA)$^+$, 478 (M+H−FA)$^+$.

FAM-3 [methyl 2-hydroxy-(19′Z)-octacosenoate]: EI-MS (70 eV): *m/z* = 452 (M, 54%)$^+$, 393 (M-59, 100%)$^+$.

FAM-DMDS-3: EI-MS (70 eV): *m/z* = 546 (M, 23%)$^+$, 499 (M-47, 7.7%)$^+$, 439 (M-47-60, 19%)$^+$, 373 (M-173, 18%)$^+$, 325 (M-173-48, 100%)$^+$, 173 ($C_{10}H_{21}S$, 58%)$^+$.

Cerebroside B-3 a [$C_{50}H_{98}NO_{10}$ (873.3)]

HPLC of the cerebroside B-3 a showed one peak [t_r = 5.8 min]. FAB-MS: *m/z* = 895 (M+Na)$^+$, 711 (M-162)$^+$, 693 (M-179)$^+$, 500 (M+Na−FA)$^+$, 478 (M+H−FA)$^+$.

FAM-3 a [methyl 2-hydroxyhexacosanoate]: EI-MS (70 eV): *m/z* = 426 (M, 100%)$^+$, 367 (M-59, 62%)$^+$.

Cerebroside B-4 [$C_{51}H_{100}NO_{10}$ (887.4)]

HPLC of the cerebroside B-4 showed one peak [t_r = 6.6 min]. FAB-MS: *m/z* = 909 (M+Na)$^+$, 724 (M-162)$^+$, 500 (M+Na−FA)$^+$.

FAM-4 [methyl 2-hydroxyheptacosanoate]: EI-MS (70 eV): *m/z* = 440 (M, 100%)$^+$, 381 (M-59, 43%)$^+$.

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