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Isolation and Structure Elucidation of Cerebrosides from *Euphorbia Platyphyllos* L.

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Abstract

A new cerebroside (1), together with three known ones (2–4) have been isolated from the whole plants of *Euphorbia platyphyllos* L. The structures were established by FT-IR spectroscopy, FAB-MS, advanced two-dimensional NMR, including ^{1}H-NMR, ^{1}H,^{1}H-COSY, HMQC and HMBC experiments and chemical reactions. The structures of the cerebrosides were characterized as 1-*O*-β-D-glycosides of phytosphingosines, which comprised a common long-chain base, (2*S*,3*S*,4*R*,8*Z*)-2-aminooctadec-8-ene-1,3,4-triol (**1–3**) and (2*S*,3*S*,4*E*,8*E*)-2-aminooctadeca-4,8-diene-1,3-diol (**4**) with 2-hydroxy fatty acids of varying chain lengths (C_{16}, C_{24}, $C_{26:1}$, $C_{28:1}$) linked to the amino group. The isolated compounds have been evaluated for their antifungal and antitubercular activities.

Keywords

Euphorbia platyphyllos L.• Cerebrosides • Phytosphingosines• 2-Hydroxy-fatty acids.

Introduction

Euphorbia platyphyllos L. (Family Euphorbiaceae) is a glabrous or pubescent annual plant, widely distributed in southern parts of Europe. The plant produces a white milky latex characteristic of Euphorbiaceae species. The latex and other plant

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parts of *E. platyphyllos* have been used in traditional medicine for the treatment of warts, wens and hangnails [1].

Previous chemical studies on *E. platyphyllos* L. have revealed the presence of new jatrophane diterpenoids [2], phytoecdysteroids [3], flavonoids [4] and *n*-alkanes [5] but no further data have been reported previously on the pharmacology and chemistry of the species.

As part of our current interest on bioactive substances from Euphorbiaceae [6], we have chemically examined the plant *E. platyphyllos* L.

The present paper describes the isolation and structure determination of a new cerebroside (**1**) from *E. platyphyllos* L., together with three known ones (**2**–**4**) (Figure 1). The structure elucidation was performed by FAB-MS, ^{1}H-NMR, ^{1}H, ^{1}H-COSY, HMQC, HMBC experiments and chemical reactions. The isolated compounds have shown an interesting antifungal and antitubercular activity.

Results and Discussion

The MeOH extract of the whole plants of *Euphorbia platyphyllos* L. was subjected to silica gel column chromatography (CC), using $CHCl_3$ and MeOH as eluents. The fraction eluted with MeOH was purified over a CC on silica gel, eluted with $CHCl_3$/MeOH (5:1) and $CHCl_3$/MeOH (5:2), to give four subfractions (A–D). Fraction B, eluted with $CHCl_3$/MeOH (5:1), was subjected to CC on RP-18 (MeOH), separately, yielding the pure compounds **1**–**4**.

Compound **1** was obtained as an amorphous solid, showing the presence of hydroxyl and amide NH (3500–3200 cm^{-1}) and amide carbonyl (1640 cm^{-1}) in the FT-IR spectrum. The ^{1}H- and ^{13}C-NMR spectra of **1** (Table 1) indicated the presence of a sugar moiety [δ_H 4.21, 1H, d, J = 7.5 Hz, anomeric proton; δ_C 105.0], and long chain aliphatic and olefinic functions [δ_H 0.83, t, J = 7.0 Hz, CH_3; δ_H 1.24, br s, CH_2, δ_H 5.41 and 5.43, 4H; δ_C 130.32, 130.40, 130.58]. The data were suggestive of a glycosphingolipid structure.

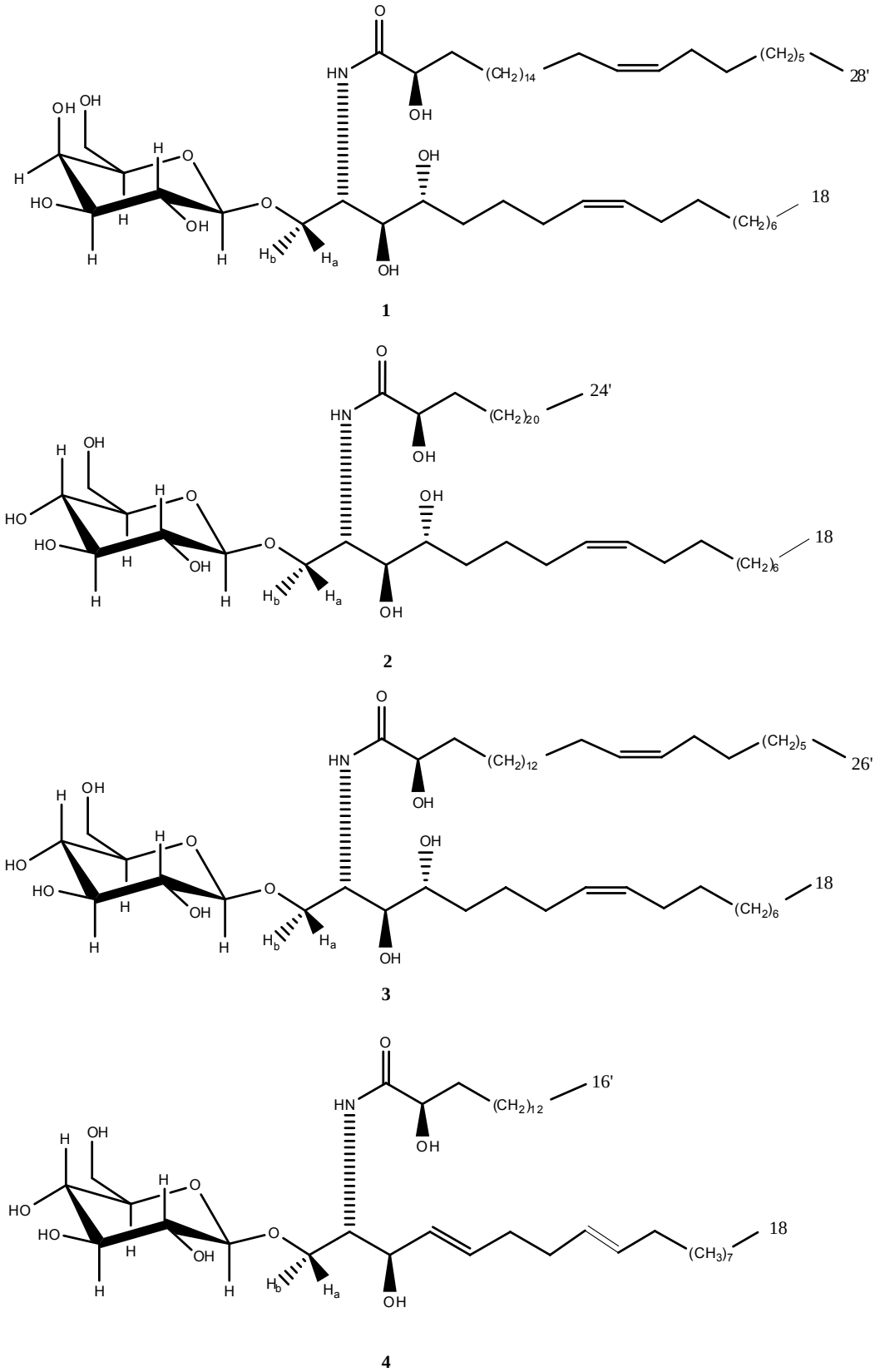

Fig. 1. Chemical structures of cerebrosides **1**–**4**.

The ^{13}C-NMR spectrum of **1** (Table 1) was assigned by a combination of distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) experiments. Important long-range correlations were observed between C-1″ and H_{ab}-1; C-1 and H-1″, H-2, H-3; C-2 and NH and C-1' and NH and H-2' (Figure 2). These results again supported the glycosphingolipid structure.

1D and 2D ^{1}H-NMR spectroscopy, DQF-COSY and HMQC indicated that the head group consists of a single galactose residue in the β configuration. The galactose configuration was determined by the characteristic chemical shifts, the spin-spin splitting and the multiplicity of the characteristic resonance of the H-4″ proton, as well as by the splittings of the other ring protons. The absolute configuration of the galactopyranose moiety was determined to be the D-form using the Hara method [7].

The stereochemistry of the ceramide moiety was determined by comparison of the ^{1}H-NMR data of the cerebrosides isolated from *Euphorbia platyphyllos* L. with that of synthetic analogs as reported in literature in terms of the signals due to H-1 to H-4 [8]. Methanolysis [9] of **1** yielded methyl galactoside, fatty acid methyl ester (FAM) and a trihydroxy long-chain base (LCB).

Methyl glycosides obtained from methanolysis of **1** were converted to trimethylsilyl derivatives using *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) for 3 h at 70°C and the silylated derivatives obtained were analyzed by gas chromatograph mass spectrometer (GC-MS) system. The monosaccharides presented two GC peaks due to the 1α- and 1β- configurations of the OH on the pyrano ring (Table 2). The mass spectra of saccharides with the pyrano ring are mainly characterized by the *m/z* 204 fragment ion [e.g. galactopyranose as trimethylsilyl (TMS)]; in good agreement with the data reported in literature [10]. In fact, this fragment is normally used as key ion to identify sugar compounds (especially monosaccharides as TMS) in complex extracts. Identification of galactose was carried out by study of the MS spectra,

comparison with members of the NBS library and with retention times of methyl α and β-D-galactopyranoside and methyl α- and β-D-glucopyranoside standard TMS derivatives.

This is the first example of the presence of a galactose unity as part of a cerebroside isolated from Euphorbiaceae.

The fatty acid methyl ester was identified by GC/MS as methyl 2-hydroxyoctacosenoate. The MS spectrum of **1** showed a molecular ion $[M + H]^+$ peak at m/z 898 and a fragment ion at m/z 736 $[898\text{-Gal}]^+$. The absolute configuration at C(2) of the 2-hydroxy fatty acid was presumed to be R from the specific rotation of the fatty acid methyl ester ($[\alpha]_D$ −11.0 ºC) [11–13].

On the other hand, on the basis of mass spectrometry analysis the LCB component of **1** was suggested to be 2-amino-1,3,4-trihydroxy-8-octadecene (LCB-1). In order to establish the position of the double bond, the FAM-1 and LCB-1 were treated with dimethyl disulfide (DMDS) and I_2 and the products subjected to electron impact EI-MS analysis [14]. The EI-MS spectrum of the DMDS derivative showed a molecular ion at m/z 409 and significant fragments ions at m/z 221 and 187 arising from selective fragmentation at C(8)=C(9) position of C18 chain, thus confirming the position of the double bond at C(8)=C(9) in the long-chain base (Figure 3).

The Δ^8 double bond in **1** was determined to be *cis* (Z) by the upfield shifted carbon chemical shifts of C-7 (δ 27.74) and C-10 (δ 27.62) [13] and the relative small coupling constant of H-8 at δ 5.43 (dt, J = 10.8, 7.2 Hz) and H-9 at δ 5.46 (dt, J = 10.8, 6.7 Hz). The FAM obtained from the methanolysis of the cerebroside **1** exhibited ^{13}C-NMR signals at about 130.1 and 130.2 expected for monounsaturated fatty acid methyl ester. The resonance at about 27.8, 27.9 confirms the Z geometry of the double bond in the long-chain fatty acid (Table 1). The position of the double bond in the monounsaturated fatty acid methyl ester was determined by EI-MS analysis of the corresponding dimethyl disulfide (DMDS) derivative [14]. The

characteristic fragments at m/z 373 and 173, obtained from the cleavage between the sulphide carbons, indicated the position of the double bond in **1**.

Therefore **1** was characterized as (2*R*,19*Z*)-*N*-{(1*S*,2*S*,3*R*,7*Z*)-1-[(β-D-galactopyranosyloxy)methyl]-2,3-dihydroxyheptadec-7-en-1-yl}-2-hydroxyoctacos-19-enamide (**1**).

Compounds **2**–**4** were found to be identical in all of their characteristics, with the cerebrosides previously isolated from *Euphorbia nicaeensis* All. [15] and *Euphorbia peplis* L. [16]. The extract containing the complex mixture of cerebrosides **2**–**4** exhibited cytotoxic activity against KB cells and moderate antifungal and antitubercular activities.

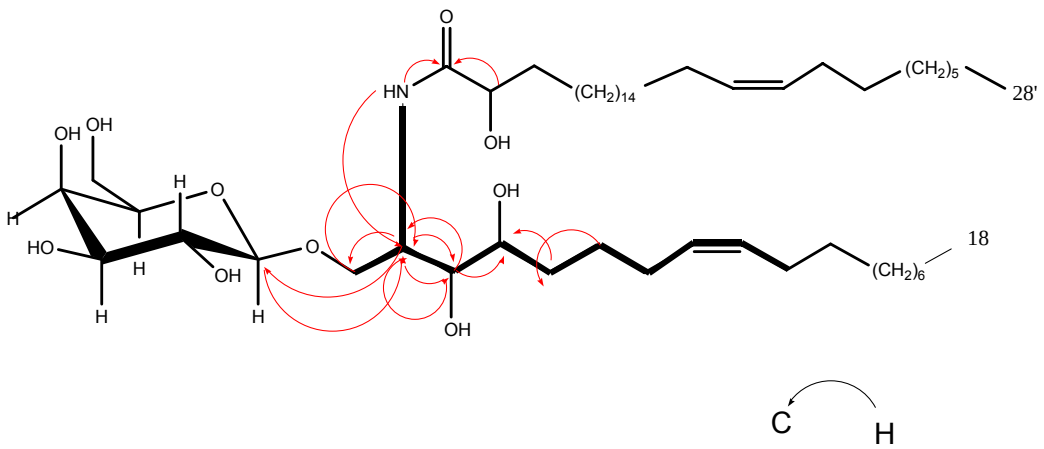

Fig. 2. Selected ^{1}H-^{1}H COSY (Bold Lines) and Selected HMBC (Full-Line Arrows) Correlations of **1**.

Tab. 1. NMR Spectral Data of **1–4** in C_5D_5N (δ in ppm, multiplicities, J in Hz)

Position	**1**		**2**		**3**		**4**	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
Ceramide								
NH	8.5 (d, 8)	–	8.5 (d, 7.8)	–	8.5 (d, 7.8)	–	8.4 (d, 8.9)	–
1a	4.7 (dd, 10, 5.4)	70.5	4.7 (dd)	70.5	4.7 (dd)	70.5	4.7 (dd)	70.3
1b	4.5 (m)		4.5 (m)		4.5 (m)		4.5 (m)	
2	5.3 (m)	51.8	5.2 (m)	51.8	5.2 (m)	51.8	4.8 (m)	54.7
3	4.2 (dd, 4, 3.4)	76.0	4.3 (dd)	76.0	4.3 (dd)	76.0	4.8 (dd)	72.4
4	4.2 (obs)	72.6	4.2 (obs)	72.6	4.2 (obs)	72.6	6.0 (dd)	132.1
5a	2.4 (m)	34.2	1.9 (m)	33.7	1.9 (m)	34.1	5.9 (dt)	132.2
5b	1.9 (m)		2.4 (m)		2.4 (m)			
6a	1.7 (m)	25.8	1.6 (m)	26.3	1.6 (m)	26.0	2.1 (m)	33.1
6b	1.6 (m)		1.7 (m)		1.7 (m)			
7	2.2 (m)	27.7	2.2 (m)	27.8	2.2 (m)	27.8	2.1 (m)	32.9
8	5.4 (dt, 10.8, 7.2)	130.6	5.4 (m)	130.2	5.4 (m)	130.6	5.4 (t)	130.0
9	5.4 (dt, 10.8, 6.7)	130.4	5.4 (m)	129.5	5.4 (m)	130.4	5.4 (t)	131.2
10	2.2 (m)	27.6	2.2 (m)	27.7	2.2 (m)	27.7	1.8 (m)	33.1
11–15	1.3 (m)	29.6–30.2	1.31 (m)	29.7–30.3	1.3 (m)	29.6–30.3	1.3 (m)	29.7–30.3
16	1.2 (m)	32.3	1.2 (m)	32.3	1.3 (m)	32.3	1.2 (m)	32.4
17	1.2 (m)	23.2	1.2 (m)	23.1	1.3 (m)	23.3	1.2 (m)	23.2
18	0.8 (t, 7)	14.5	0.8 (t, 7)	14.5	0.8 (t, 7)	14.6	0.9 (t, 7)	14.5
1'	–	175.7	–	175.8	–	175.6	–	175.8
2'	4.6 (dd, 8.2, 4.0)	72.5	4.6 (dd, 8.1, 4.1)	72.5	4.6 (dd, 8.0, 4.0)	72.6	4.5 (dd, 8.1, 4.1)	72.6
3'a	2.2 (m)	35.6	2.0 (m)	35.7	2.0 (m)	35.7	1.9 (m)	35.8
3'b	2.1 (m)		2.2 (m)		2.2 (m)			
4'a	1.9 (m)	26.7	1.7 (m)	26.7	1.7 (m)	26.8	1.7 (m)	26.1
4'b	1.7 (m)		1.9 (m)		1.9 (m)			
5'–17'	1.30 (m)	19.6–30.2						

Tab. 1. (Cont.)

Position	1		2		3		4	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
CH_2 aliph.			1.3 (m)	23.2–32.3	1.2–1.3 (m)	23.1–32.3	1.3–1.4 (m)	23.1–32.3
$CH_2CH=$					2.2 (m)	27.9		
$CH=CH$					5.4 (m)	131.3		
CH_3			0.8 (m)	14.5	0.8 (m)	14.4	0.9 (t)	14.5
18', 21'	2.2 (m)	27.8, 27.9						
19', 20'	5.4 (m)	130.1, 130.3						
22'–25'	1.3 (m)	29.6–30.2						
26'	1.2 (m)	32.3						
27'	1.2 (m)	23.2						
28'	0.8 (t, 7)	14.4						
OH-2'	7.6 (br s)	–	7.6 (br s)	–	7.6 (br s)	–	7.7 (br s)	–
OH-3	6.8 (br s)	–	6.8 (br s)	–	6.8 (br s)	–	6.9 (br s)	–
OH-4	6.0 (br s)	–	6.0 (br s)	–	6.0 (br s)	–	–	–
Sugar moiety								
1"	4.2 (d, 7.5)	105.0	4.9 (d, 7.6)	105.6	4.9 (d, 7.6)	105.6	4.9 (d, 7.7)	105.7
2"	3.5 (dd, 9.7, 7.5)	73.0	4.1 (t)	75.3	4.0 (t)	75.3	4.0 (t)	75.2
3"	3.5 (dd, 9.7, 3.32)	73.0	4.2 (obs)	78.5	4.2 (obs)	78.6	4.2 (obs)	78.5
4"	3.8 (dd, 3.3, 0.9)	70.0	4.2 (obs)	71.6	4.2 (obs)	71.6	4.2 (obs)	71.6
5"	3.5 (m)	70.0	3.9 (m)	78.7	3.9 (m)	78.6	3.9 (m)	78.7
6"a	3.7 (dd, 11.4, 5.2)	62.0	4.5 (dd, 11.9, 5.3)	62.7	4.5 (dd, 11.9, 5.3)	62.7	4.5 (dd, 11.8, 5.4)	62.7
6"b	3.8 (dd, 11.4, 6.9)		4.4 (dd, 11.9, 2.0)		4.4 (dd, 11.9, 2.0)		4.4 (dd, 11.8, 2.1)	
OH-6"	6.4 (br s)		6.4 (br s)		6.3 (br s)		6.6 (m)	

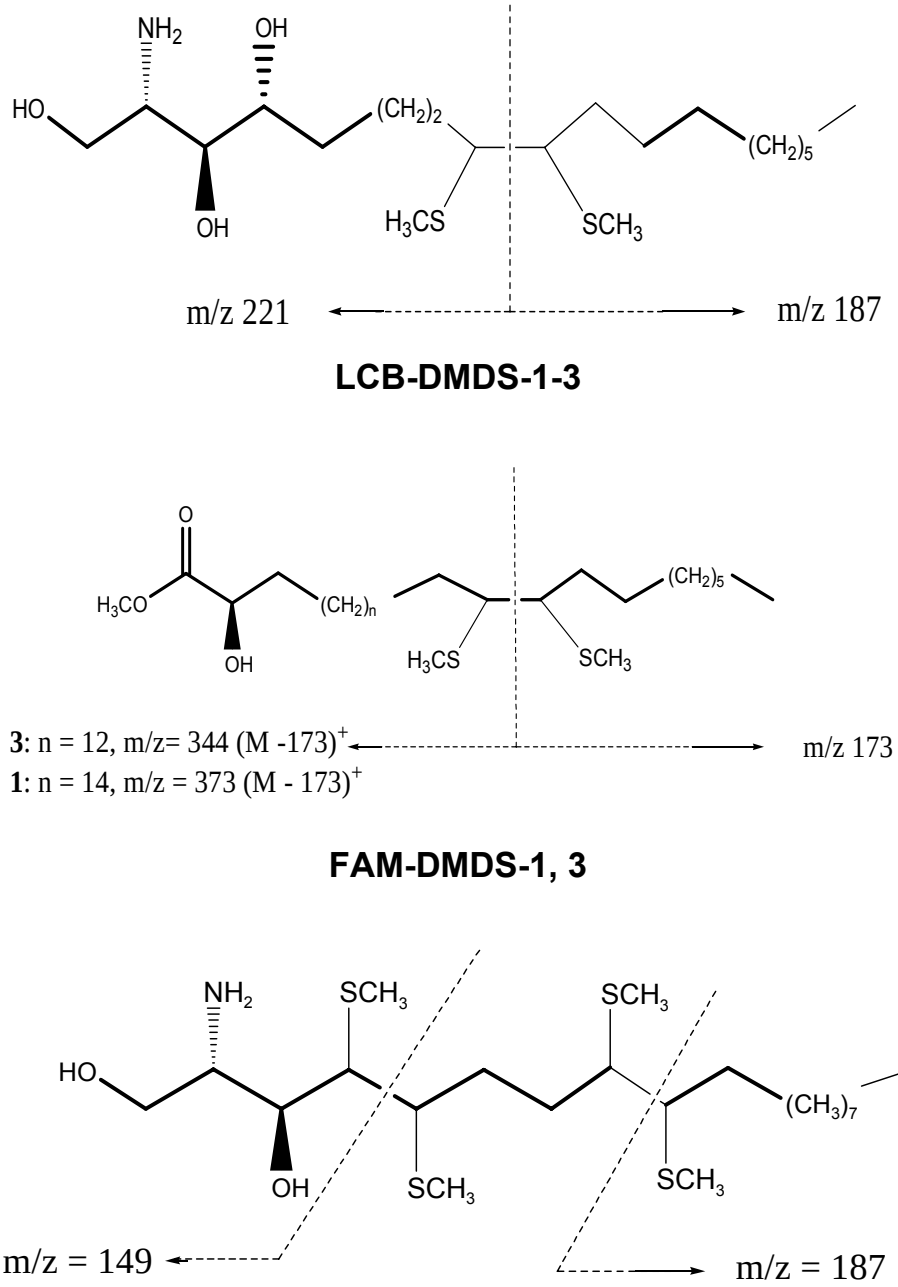

Fig. 3. LCB-DMDS Derivatives of **1**–**4** and FAM-DMDS Derivative of **1**, **3**.

Tab. 2. Characteristics of methyl glycosides analyzed by GC-MS as TMS derivatives.

Compounds	Molecular formula	Molecular mass	Molecular mass-TMS	Retention time (min)	m/z
Methyl α-D-galacto-Pyranoside	$C_7H_{14}O_6$	194	482	13.188	204, 191, 217
Methyl β-D-galacto-Pyranoside	$C_7H_{14}O_6$	194	482	14.307	204, 191, 217
Methyl α-D-gluco-Pyranoside	$C_7H_{14}O_6$	194	482	12.770	204, 191, 217
Methyl β-D-gluco-pyranoside	$C_7H_{14}O_6$	194	482	14.008	204, 191, 217

Biological Activity Evaluation

The fraction B containing the complex mixture of cerebrosides and the individual isolated compound **1** were tested for the antimicrobial, antitubercular and antifungal activities (Tables 3, 4). The biological activity evaluation of compounds **2**-**4** has been reported in a previous paper [16]. Reference drugs are reported.

No activity was exhibited against Gram positive nor Gram negative reference strains. We registered an interesting antitubercular activity of the fraction B containing the complex mixture of cerebrosides **1**-**4** with a MIC of 40 μg/mL against a human clinical *M. tuberculosis* strain and MICs of 80 μg/mL against the reference strain and two additional clinical human isolates. Compound **1** has shown an interesting antitubercular activity with MICs of 40 μg/mL on reference strain and on three clinical isolates and MICs of 80 μg/mL against clinical strains H331, H242, H172; all strains being sensitive to ciprofloxacin with MICs ranging from 0.125 to 0.25 μg/mL (Table 3).

As concerning antifungal activity, the complex mixture of cerebrosides had a MIC of 8 μg/mL on *C. neoformans* clinical strain after 24 h incubation and a MIC of 32 μg/mL after 48 h incubation; the cerebrosides mixture showed an interesting

inhibiting activity against the three different species of *Candida* studied, having a MIC of 64 μg/mL after 24 h incubation and a MIC of 128 μg/mL after 48 h incubation. No pure cerebroside showed any activity against *Candida* spp.; *C. neoformans* was slightly sensitive after 24 h incubation time with MIC of 64 μg/mL for compound **1** (Table 4).

Tab. 3. Antimicrobial activity of cerebrosides against Gram-positive and Gram-negative and tubercular bacteria.

MIC (μg/mL)	*S. aureus* ATCC 25923	*E. coli* ATCC 25922	*M. tuberculosis* H37Rv	*M. tuberculosis* H331	*M. tuberculosis* H242	*M. tuberculosis* H172
Fraction B	> 160	> 160	80	40	80	80
1	> 160	> 160	40	80	40	80
Ciprofloxacin	0.5	0.06	0.25	0.125	0.125	0.25

Tab. 4. Antifungal activity of cerebrosides against *Candida* species and *Cryptococcus neoformans*.

MIC (μg/mL)	*C. albicans*		*C. parapsilosis*		*C. glabrata*		*C. neoformans*	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Fraction B	64	128	64	128	64	128	8	32
1	>128	>128	>128	>128	>128	>128	64	128
Miconazole	2.5	40	0.625	1.25	5	10	0.156	0.156

Conclusions

Euphorbia platyphyllos accumulates complex sphingoglycolipids in which dihydroxy (**4**) and trihydroxy (**1**–**3**) long-chain bases (LCBs) occur as part of the cerebroside moiety.

Compound **1** is new, since it has been isolated for the first time in Euphorbiaceae; an analogous cerebroside with a glucose moiety as head group, instead of galactose, has been isolated previously from *Euphorbia wulfenii* Hoppe ex Koch and *Euphorbia characias* L. [6]. Compounds **2**–**4** have been found to be identical to the cerebrosides isolated from *Euphorbia nicaeensis* All. and *Euphorbia peplis* L. Besides, the pure isolated cerebrosides show a synergistic antifungal activity when present in mixture. As concerning antitubercolar activity, only compounds **1** and **3** are responsible for this effect.

Experimental

General

NMR spectra were recorded on a Varian Unity spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C), respectively. The chemical shift values were reported in parts per million units and the coupling constants were in Hz. FAB-MS was recorded on Kratos MS 80 RFA mass spectrometer using a beam of Argon/Xenon (2-8 Kv), methanol as solvent and glycerol as the matrix. IR spectra were recorded on a Jasco IR-700 infrared spectrophotometer. Silica gel column chromatography was carried out using silica gel Kieselgel 60 (230-400 Mesh, 60Å Merck), reversed-phase chromatography was carried out using Lichroprep RP-18 (40-63 μm, Merck). GC-MS experiments were carried out on Fisons GC 8000 (Carlo Erba, 20090 Milan, Italy) instrument, equipped with a split/splitless injector (1:20); the capillary gas chromatograph system was coupled directly to a MD 800 mass spectrometer (Carlo Erba, 20090 Milan, Italy). A SPB 5 fused silica capillary column, 30 m x 0.32

mm I.D., 0.25 µm film thickness (Supelco, Inc., Bellefonte, PA) was employed. Sugar standards were purchased from Fluka/Aldrich/Sigma (Milano, Italy).

All solvents were distilled before use. TLC were carried out using Kieselgel 60 F254 (20 x 20 cm; 0.2 mm, Merck) and RP-18 F254 (10 x 10 cm, Merck); spots were detected by spraying with 10% aq. H_2SO_4 followed by heating.

Plant Material

E. platyphyllos was collected from wild stock growing in Carso Triestino (Trieste), Italy in May 2006. A voucher specimen was deposited at the Herbarium of the Department of Biology (TSB) of the University of Trieste (Italy).

Extraction and Isolation

Dried, ground whole plants of *E. platyphyllos* (300 g) were extracted with MeOH and evaporated to a crude residue (15.6 g), which was fractionated on a silica gel column with $CHCl_3$ and MeOH, respectively. The fraction eluted with MeOH was subjected to silica-gel column chromatography using mixtures of $CHCl_3$/MeOH 5:1 and 5:2, respectively, yielding four subfractions (A–D). Subfraction B, eluted with $CHCl_3$/MeOH 5:1, was further chromatographed on RP-18 (MeOH) affording **1** (36.1 mg), **2** (9.4 mg), **3** (7.8 mg) and **4** (11.4 mg).

(2R,19Z)-N-{(1S,2S,3R,7Z)-1-[(β-D-galactopyranosyloxy)methyl]-2,3-dihydroxyheptadec-7-en-1-yl}-2-hydroxyoctacos-19-enamide (1)

A colorless solid. FT-IR ν_{max}^{Nujol} cm^{-1}: 3315 (broad), 2921, 2851, 1588, 1075, 1036. Positive-ion FAB MS: m/z = 920 $[M + Na, 74\%]^+$, 898 $[M + H, 94\%]^+$, 880 $[M + H - 18, 51\%]^+$, 759 $[M + Na- C_6H_{11}O_5 + H, 21\%]^+$, 736 $[M + H - C_6H_{11}O_5, 62\%]^+$, 718 $[M + H - Gal, 26\%]^+$, 500 $[M + Na - C_{28}H_{53}O_2, 100\%]^+$, 476 $[M + H - C_{28}H_{52}O_2, 18\%]^+$. Negative-ion FAB MS: m/z = 896 $[M - H, 100\%]^-$, 734 $[M - H - C_6H_{11}O_5, 42\%]^-$, 717 $[M - H - Gal, 16\%]^-$. ^{1}H- and ^{13}C-NMR data are reported in Table 1.

(2R)-N-{(1S,2S,3R,7Z)-1-[(β-D-glucopyranosyloxy)methyl]-2,3-dihydroxyheptadec-7-en-1-yl}-2-hydroxytetracosanamide (2)

Amorphous powder. FT-IR ν_{max}^{Nujol} cm^{-1}: 3415, 1646, 1542. Positive-ion FAB MS: m/z = 866 $[M + Na, 90\%]^+$, 844 $[M + H, 21\%]^+$, 826 $[M + H - H_2O, 29\%]^+$, 682 $[M + H - C_6H_{11}O_5, 78\%]^+$, 664 $[M + H - Glc, 53\%]^+$, 500 $[M + Na - C_{24}H_{47}O_2, 10\%]^+$. Negative-ion FAB MS: m/z = 842 $[M - H, 96\%]^-$, 680 $[M - H - C_6H_{11}O_5, 33\%]^-$, 663 $[M - H - Glc, 19\%]^-$. ^{1}H- and ^{13}C-NMR data are reported in Table 1.

(2R,17Z)-N-{(1S,2S,3R,7Z)-1-[(β-D-glucopyranosyloxy)methyl]-2,3-dihydroxyheptadec-7-en-1-yl}-2-hydroxyhexacos-17-enamide (3)

Amorphous powder. FT-IR ν_{max}^{Nujol} cm^{-1}: 3413, 1646, 1540. Positive-ion FAB MS: m/z = 892 $[M + Na, 63\%]^+$, 870 $[M + H, 84\%]^+$, 852 $[M + H - H_2O, 51\%]^+$, 730 $[M + Na - C_6H_{11}O_5, 17\%]^+$, 708 $[M + H - C_6H_{11}O_5, 55\%]^+$, 690 $[M + H - Glc, 20\%]^+$, 476 $[M + H - C_{26}H_{50}O_2, 16\%]^+$. Negative-ion FAB MS: m/z = 868 $[M - H, 100\%]^-$, 706 $[M - H - C_6H_{11}O_5, 31\%]^-$. ^{1}H- and ^{13}C-NMR data are reported in Table 1.

(2R)-N-{(1S,2S,3E,7E)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxyheptadeca-3,7-dien-1-yl}-2-hydroxyhexadecanamide (4)

Amorphous powder. FT-IR ν_{max}^{Nujol} cm^{-1}: 3415, 1645, 1543. Positive-ion FAB MS: m/z = 736 $[M + Na, 46\%]^+$, 714 $[M + H, 7\%]^+$, 696 $[M + H - H_2O, 52\%]^+$, 534 $[M + H - Glc, 100\%]^+$, 516 $[M + H - Glc - H_2O, 28\%]^+$, 482 $[M + Na - C_{16}H_{31}O_2, 15\%]^+$, 336 $[M + Na - C_6H_{11}O_5 - C_{16}H_{29}O, 25\%]^+$, 319 $[M + Na - Glc - C_{16}H_{29}O, 20\%]^+$. Negative-ion FAB MS: m/z = 712 $[M - H, 30\%]^-$, 533 $[M - H - Glc, 25\%]^-$. ^{1}H- and ^{13}C-NMR data are reported in Table 1.

Methanolysis of 1

Compound **1** (35 mg) was refluxed with 0.9 M HCl in 82% aq. MeOH (10 ml) for 18h. The mixture was extracted with *n*-hexane and the combined organic phase was washed with water and dried over Na_2SO_4. Removal of the solvent gave a

colorless wax (18.4 mg) which was chromatographed on silica gel [hexane/EtOAc (20:1, 5:1)] to yield fatty acid methyl ester as a colorless wax (13 mg).

The compounds **2**–**4** were methanolyzed using the same method described above. The esters were analyzed by GC-MS. The results were as follows: FAM-1 (2-hydroxyoctacosenoic acid methyl ester), EI-MS: m/z = 452 $[M]^+$, 420 $[M - CH_3OH]^+$, 393 $[M - CH_3COO]^+$; FAM-2 (2-hydroxytetracosanoic acid methyl ester), EI-MS: m/z = 398 $[M]^+$, 366 $[M - CH_3OH]^+$, 339 $[M - CH_3COO]^+$; FAM-3 (2-hydroxyhexacosenoic acid methyl ester), EI-MS: m/z = 424 $[M]^+$, 392 $[M - CH_3OH]^+$, 365 $[M - CH_3COO]^+$; FAM-4 (2-hydroxyhexadecanoic acid methyl ester), EI-MS: m/z = 286 $[M]^+$, 254 $[M - CH_3OH]^+$, 227 $[M - CH_3COO]^+$.

The aq. MeOH layer was neutralized with NH_4OH and extracted with EtOAc. The combined EtOAc extract was washed with H_2O, dried over Na_2SO_4 and evaporated to give the long-chain base (LCB) as a slightly yellow wax (17 mg). The aq. MeOH layer was then evaporated to dryness and chromatographed on silica gel [CH_2Cl_2/MeOH/H_2O (lower layer) (20:3:1, 10:3:1, 7:3:1)] to give methyl galactopyranoside (α- and β- anomer) (1) and methyl glucopyranoside (α- and β-anomer) (**2**-**4**) as colorless solids. TLC [silica gel, CH_2Cl_2/MeOH/H_2O (lower layer) (10:3:1)] of the resulting methyl galactopyranoside (α- and β- anomer) (**1**) and methyl glucopyranoside (α- and β- anomer) (**2**-**4**) were identical to thats of the standard methyl α-D-galactopyranoside, methyl β-D-galactopyranoside, methyl α-D-glucopyranoside and methyl β-D-glucopyranoside, respectively.

GC-MS Analysis of TMS Ethers of Methyl Glycoside from 1

The mixture of methyl glycosides obtained by column chromatography of the aq. MeOH layer derived from methanolysis of **1** was converted to their trimethylsilyl derivatives using BSTFA containing 1% TMCS for 3 h at 70°C. 0.1 μl of silylated mixture was analyzed by gas chromatograph mass spectrometer (GC-MS) system. The chromatographic conditions were: column temperature was programmed from 100°C to 260°C at 8°C/min, with 15 min of final isotherm, injector temperature 280°C, carrier gas (helium) flow rate 1.5 ml/min. Transfer line temperature was kept

at 270°C. The mass spectrometer scanned from *m*/*z* 100 to *m*/*z* 600 at 1.0 second cycle time. The ion source was set at 180°C and spectra were obtained by electron impact (70 eV).

Methyl glycoside (GC-MS): methyl α- and β-galactopyranoside were detected (Table 2).

GC-MS Analysis of TMS Ethers of Methyl Glycosides from 2–4

The mixture of methyl glycosides obtained by column chromatography of the aq. MeOH layer derived from methanolysis of **2**-**4** was trimethylsilylated and the reaction mixture was analyzed by GC-MS in the same manner as described for **1**.

Methyl glycoside (GC-MS): methyl α- and β-glucopyranoside were detected (Table 2).

Dimethyl Disulfide Derivative of FAMs from cerebrosides 1,3

FAM-1 (6 mg) was dissolved in carbon disulfide (0.5 ml) and dimethyl disulfide (0.5 ml) and iodine (10 mg) were added. The reaction mixture was then kept at 60°C for 48h in a small sealed vial. The reaction was subsequently quenched with 5% aq. $Na_2S_2O_3$ and the mixture was extracted with n-hexane. The n-hexane layer was dried over Na_2SO_4, filtered and concentrated to give the dimethyl disulfide derivative of FAM-1 as a light yellow solid (6 mg). EI-MS: m/z (rel. int. %): 546 $[M]^+$ (7%), 492 $[M\text{-}SCH_3]^+$(10), 452 $[M\text{-}CH_3S\text{-}CH_3S]^+$ (8), 373 $[M - C_{10}H_{21}S]^+$ (15), 173 $[C_{10}H_{21}S]^+$ (100). The compound FAM-3 was subjected to the procedure described above. FAM-3 DMDS derivative; EI-MS: m/z (rel. int. %): 517 $[M]^+$ (6), 470 $[M - CH_3S]^+$ (18), 423 $[M - CH_3S - CH_3S]^+$ (9), 344 $[M - C_{10}H_{21}S]^+$ (20), 173 $[C_{10}H_{21}S]^+$ (100). (Figure 3)

MS analysis of LCBs from cerebrosides 1–4

The LCBs of cerebrosides **1**–**4** were subjected to ESI-MS analysis. LCB-1 (2-amino-1,3,4-trihydroxy-octadeca-8-ene), ESI-MS: m/z = 339 $[M + Na]^+$, LCB-2 (2-amino-1,3,4-trihydroxy-octadeca-8-ene), ESI-MS: m/z = 339 $[M + Na]^+$, LCB-3 (2-

amino-1,3,4-trihydroxy-octadeca-8-ene), LCB-4 (2-amino-1,3-dihydroxy-octadeca-4,8-diene), ESI-MS: m/z = 320 $[M + Na]^+$.

Dimethyl Disulfide Derivatives of LCB from cerebrosides 1–4

LCBs DMDS derivatives from cerebrosides **1**–**4** were synthesized according to the procedure described above. The LCBs DMDS derivatives of cerebrosides **1**–**4** were subjected to MS analysis. The results were as follows: LCB-1 DMDS derivative, EI-MS: m/z = 221, 187. LCB-2 DMDS derivative, EI-MS: m/z = 221, 187. LCB-3 DMDS derivative, EI-MS: m/z = 221, 187. LCB-4 DMDS derivative, EI-MS: m/z = 149, 187.

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