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Abstract

A new cerebroside **1** characterized as (2*R*,9*Z*)-2-hydroxy-*N*-{(1*S*,2*S*,3*R*,4*S*)-1-[(β-D-glucopyranosyloxy)methyl]-2,3,4-trihydroxyoctacosan-1-yl}-9-pentadecenamide was isolated from the stem bark of *Ficus polita* Vahl (Moraceae) together with four known compounds identified as sitosterol 3-*O*-β-D-glucopyranoside **2**, betulinic acid **3**, stigmasterol **4** and lupeol **5**. Their structures were determined on the basis of spectroscopic methods as well as HR-ESI-MS, NMR analyses, chemical transformation, and by comparison of their physical and spectral data with those reported in the literature and with authentic specimens for some known compounds.

Keywords: Ficus polita, moraceae, cerebroside, cytoxicity

Introduction

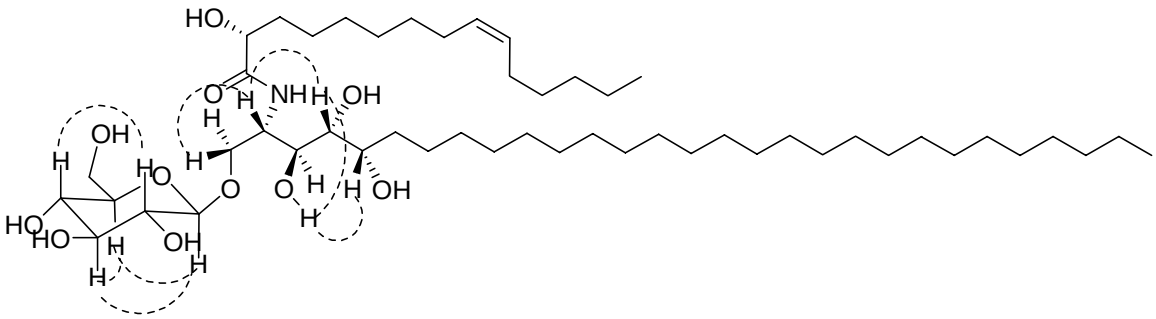
The plants are always a great source of drugs discovery. It is the case of *Ficus* which is a genus endemic in west, central, north and east Africa.[1,2] The leaves of some species are used in Ivory Coast folk medicine to treat worms and abdominal pains.[3] Some previous biological studies have shown that some species have anti-HIV[4] and antimicrobial properties.[5] That is why our attention is focused on *Ficus polita* (Moraceae) which is a small tree about 10 to 15 meters high.[1] The discovery of bioactive compounds prompted us to investigate this plant. This paper deals with isolation, structural elucidation of a new cerebroside based on the spectrometric methods.

Results and Discussion

The methanolic crude extract was subjected to repeated columns chromatography yielding a new cerebroside **1**, 3-*O*-β-D-glucopyranoside sitosterol **2**,[6] betulinic acid **3**,[7] stigmasterol **4**[8] and lupeol **5**.[9]

Compound **1** was obtained as a dark amorphous solid from mixture CH_2Cl_2/MeOH (9:1). The positive test with Molisch reagent suggested that, **1** is a glycosylated compound. Its positive mode HRESI-MS spectrum showed a peak at *m*/*z* 910.6869 (calcd 910.6954) corresponding to the formula $[C_{50}H_{97}O_{11}N+Na]^+$ requiring 3 double bond equivalents. The FT-IR spectrum exhibited a broad absorption band for OH groups (3368 cm^{-1}), two strong absorption bands for olefinic functions (1649 cm^{-1}) and for secondary amide (1637 and 1542 cm^{-1}).[10] The NMR spectra of **1** displayed signals corresponding to those of a phytoceramide.[11] In fact, a triplet of 6H was observed at δ_H/δ_C 0.83 (J = 5.9 Hz)/13.9 and assigned to the two terminal CH_3 groups. A broad singlet observed between δ_H/δ_C 1.22-1.75/(24.2-29.0) corresponded to the sequence of CH_2 groups. A proton signal linked to a nitrogen (**H**-N) appeared as doublet at δ_H 7.50 (J = 9.2 Hz) and the other linked to azomethine at δ_H/δ_C 4.11 (brd, J = 8.8 Hz)/49.8. Furthermore this information, a set of signals of β-D-glucopyranose was observed on both spectra which showed an anomeric proton at δ_H/δ_C 4.12 (d, J = 8.0 Hz)/103.4, four oxymethines at δ_H/δ_C 3.35 (m)/73.4, 3.05 (m)/76.4, 3.10 (m)/70.0, 3.16 (m)/76.8 and the oxymethylene at δ_H/δ_C [3.65 (dd, J = 3.0, 11.8 Hz), 3.42 (dd, J = 5.8, 11.8 Hz)]/61.[12] In addition, the chemical shifts of a double bond having *cis* configuration[11] were also observed at δ_H 5.30 (brd, J = 6.2 Hz)/129.4 and 5.33 (brd, J = 6.2 Hz)/130.1. The foregoing data suggested to a glucophytoceramide derivative with an olefinic bond. The sugar moiety was located using long range correlations exhibited by the HMBC spectrum. This showed correlations from diastereotopic protons H-1a [3.65 (dd, J = 5.8, 10.6 Hz)]/69.5 and H-1b [3.90 (m)]/69.5 to the anomeric carbon at δ_C 103.4. Additional correlations were observed between the proton H-N at δ_H 7.50 and the carbonyl at δ_C 173.6 as well as other interactions from proton H-2' at δ_H/δ_C 3.85 (m)/70.8 to carbonyl function (173.6) and both successive CH_2 groups [C-3' (δ_C 34.3), C-4' (δ_C 24.2)]. Fatty acid moiety is α-hydroxylated since the protons of α-OH group [δ_H 5.59 (d, J = 6.2 Hz)] and the one of the oxymethine C-2' [δ_H 3.85 (m)/70.8] correlated with the carbonyl (173.6).

The spatial location of hydroxyl groups in sphingosine was evident by exploitation of NOESY spectrum (Scheme 1) which showed interactions between the proton at δ_H 4.11 and those at δ_H 3.90, 3.35; both protons at δ_H 3.35 and 2.92 interacted with the one at δ_H 4.90 (HO-C-3) supporting that all the five protons were in the same spatial orientation.



Scheme 1. Spatial interactions (NOESY) of compound **1.**

The absolute configurations of carbons C-2, C-3, C-4, and C-2' were determined to be (*S*), (*S*), (*R*) and (*R*) respectively since the NMR data were close to those of phytoceramide.[11] So the carbon C-5 is in the (*S*) configuration considering its spatial position.

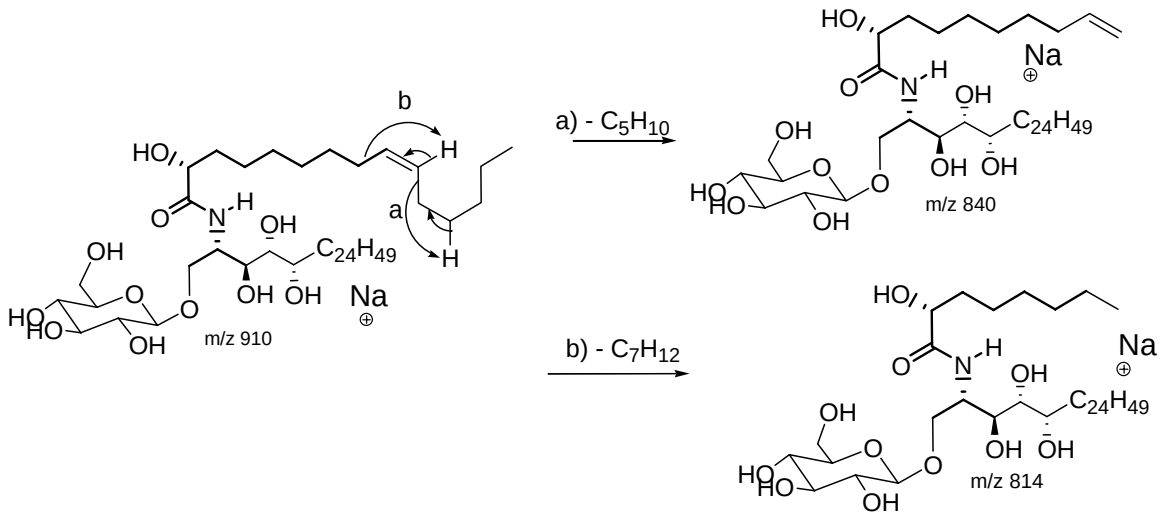
Table 1. The ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (100 MHz) spectral data of compound **1**

Position	δH (J Hz)	δC (APT)	Cosy	HMBC
ceramide				
NH	7.50 (d, 9.2)	-	4.12	49.8, 173.6
1	3.65 (dd, 5.8, 10.6, Ha) 3.90 (m, Hb)	69.5	4.12, 4.11	49.8, 103.4
2	4.11 (brd, 8.8)	49.8	3.90, 7.50	69.5
3	3.10 (dd, 8.8, 11.2)	76.8	4.90	49.8, 69.5
4	3.35 (m)	69.9	4.75	
5	2.92 (dt, 5.9, 8.8, 11.2)	73.4	3.35, 3.10	69.9, 76.8
6	1.95 (m, Ha), 2.10 (m, Hb)	31.9	1.22-1.75, 3.35	24.2-29.0, 73.4
7-25, 4'-7',12'-14'	1.22-1.75 (brs)	24.2-29.0	0.83, 1.90, 2.10	13.9
29, 15'	0.83 (t, 6.0, CH3)	13.9	1.22-1.75	24.2-29.0
1' (C=O)	-	173.6	-	-
2'	3.85 (m)	70.9	1.90, 5.59	24.2, 34.1, 173.6
3'	1.61 (m, Ha), 1.90 (m, Hb)	34.1	1.22, 2.10	24.2, 70.9
9'	5.30 (brd, 6.2)	129.4	2.00, 5.33	26.7, 130.1

Table 1. Continued

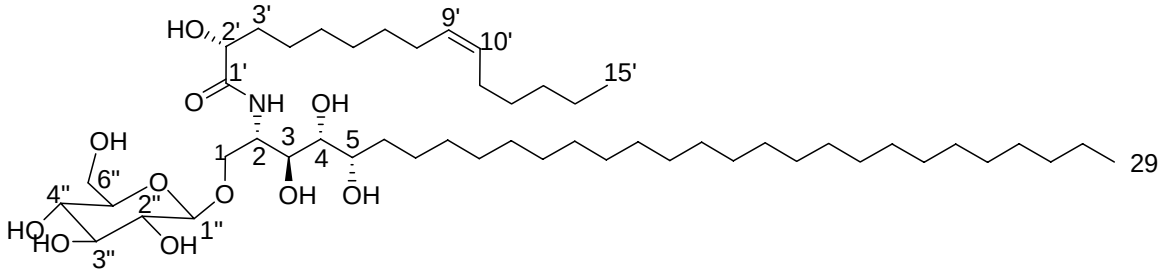
Position	δH (J Hz)	δC (APT)	Cosy	HMBC
10’	5.33 (brd, 6.2)	130.1	1.90, 5.30	129.4
8’,11’	1.90 (m, Ha), 2.00 (m, Hb)	26.7	5.30, 5.33	129.4, 130.1
OH (C3)	4.90 (d, 6.2)	-	3.10	69.5, 76.8
OH (C4)	4.75 (d, 6.2)	-	3.35	49.8, 73.4
OH (C2’)	5.59 (d, 6.2)	-	3.85	34.1, 70.9, 173.6
Glucose				
1’’	4.12 (d, 8.0)	103.4		69.5
2’’	3.35 (m)	73.4	4.95	103.4
3’’	3.05 (m)	76.4		
4’’	3.10 (m)	70.0	4.90	
5’’	3.16 (m)	76.8		
6’’	3.42 (dd, 5.8, 11.8) 3.65 (dd, 3.0, 11.8)	61.0	4.55	76.8
OH (C2’’)	5.01 (brd, 6.2)	-	3.35, 3.05	73.4, 76.4, 103.4
OH (C4’’)	4.90 (brd, 6.2)	-	3.10	70.0, 76.4, 76.8
OH (C6’’)	4.55 (dd, 3.0, 6.2)	-	3.42, 3.65	61.0, 76.8

Methanolysis (0.9N HCl/MeOH, at 70 °C during 18 h) of compound **1** gave the fatty acid methyl ester and the long chain base which were characterized by LC-ESI-MS analysis. The peak at *m*/*z* 270 (Retention time: 8.87 min) corresponded to a fatty acid methyl ester having two double bond equivalents (carbonyl function and C=C double bond). The position of olefin function was determined by fragment ions obtained on the FAB-MS spectrum (Scheme 2).



Scheme 2. Proposal of fragmentation mechanism justifying olefin bond position in fatty acid side.

From information above-mentioned, **1** was identified as (2*R*,9*Z*)-2-hydroxy-*N*-{(1*S*,2*S*,3*R*,4*S*)-1-[(β-D-glucopyranosyloxy)methyl]-2,3,4-trihydroxyoctacosan-1-yl}-9-pentadecenamide.



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Experimental Section

General. Vacuum column chromatography (VCC), column chromatography (CC) and thin layer chromatography (TLC) were performed over silica gel 60H (particle size 90 % < 45 mm), or 200 – 300 mesh silica gel silica gel GF254, respectively. Melting points (m.p.): Stuart Scientific Melting Point apparatus SMP3; uncorrected. Optical rotations: Perkin Elmer polarimeter model 341 at 589 nm. IR Spectra: Perkin-Elmer FT-IR system spectrum BX spectrometer, KBr disks. HR-ESI-MS and FAB-MS were recorded by micro-TOF-Q 98 (Bruker-Daltonics, Germany) and JEOL JMS-700 (Japan) instruments respectively. ^{1}H- and ^{13}C-NMR: Bruker DRX-400 MHz for 1 D- and 2D-NMR spectrum. LC-MS analyses were performed on an HPLC system (LC pump P4000 and autosampler AS3000 from Thermo Separation Products) coupled to a LCQ Duo on

Trap detector (Thermo Electron, Zellik, Belgium) equipped with an ESI interface run in the positive ion mode. The separation of sample components was achieved on an X-Terra MS C18 (5 μm particle size, 3.9 x 150 mm) (Waters, Overijse, Belgium), equipped with an X-Terra MS C18 pre-column (5 μm particle size, 3.9 x 10 mm) and operated at 37 °C. Injection volume was 15 μL. The mobile phase consisted of a mixture of 5 mM ammonium formate buffer at pH 3.8 (A) and acetonitrile (B). Separation conditions for all compounds were as follows: 0.0-0.50 min, A/B hold at 50/50, v/v; 0.50–9.0 min, eluant B increase to 97%; 9.0–12.0 min, eluant B hold at 97%, 12.0–12.5 min, eluant decrease to 50%, 12.5–13.0 min, A/B hold at 50/50, Before each run the column was equilibrated for 6 min at A/B 50/50. The flow rate for column equilibration and analytical runs was 0.4 ml/min. Ionization of the analytes was carried out as follows: sheath gas flow rate (nitrogen), 47 arbitrary units; auxiliary gas flow rate (helium), 18 arbitrary units; spray voltage, 5.0 kV; capillary temperature, 200 °C; capillary voltage, 36 V; Data acquisition was performed in a time segment between 0.2 and 11.5 min after injection. The full MS-MS spectrum of $[M+H]^+$ ions was monitored for all compounds, isolation width was 2.5 *m/z*, normalized collision energy was 28.0%.

Extraction and isolation

Ficus polita (Moraceae) was collected From Yaoundé central region of Cameroon in May 2007. A sample (N° 39955/HNC) has been deposited in the National Herbarium of Yaoundé, Cameroon.

Dried stem bark of *F. polita* (3.4 kg) was cut into small pieces, powdered and extracted 2 times by maceration with 10 L of mixture CH_2Cl_2/MeOH (1:4) during 72 h (each time lasted 72 hours). Organic extract was concentrated to yield 204 g of crude extract which were subjected to the VCC (SiO_2, hexane, hexane/ EtOAc 3:1 to 1:1, EtOAc in order of increasing polarity and MeOH) yielding 5 fractions A–E. A was purified by CC with different mixtures of hexane/EtOAc yielding 157 fractions. The ones (36-40) eluted in the ratio 9:1 yielded lupeol (29.5 mg). From the fraction B, 90 fractions were obtained and stigmasterol (240 mg) was isolated from the fractions 30-40 eluted in the ratio 17:3. From the same fraction B, 5 mg of betulinic acid were isolated from the fractions 42-44 eluted with the mixture Hexane/EtOAc (4:1). EtOAc fraction (D) was further eluted with a mixture of CH_2Cl_2/MeOH with increasing polarity. 104 fractions was obtained and from the ones 20-36 eluted with the mixture CH_2Cl_2/MeOH (19:1), the 3-*O*-β-D-glucopyranoside of β-sitosterol (11.0 mg) was isolated. From the same fraction D, politamide (15 mg) was obtained from the fractions 94-104 eluted in the ration 9:1.

Politamide (1). Dark amorphous solid; M.p 168.5-170.5; $[\alpha]_D^{20}$+0.009 (*c* 0.075, DMSO). FT-IR: 3368, 1649, 1637, 1542, and 1468. ^{1}H- and ^{13}C-NMR: Table 1. HR-ESI-MS: 910.6869 $[C_{50}H_{97}O_{11}N+Na]^+$, 926.6825 $[C_{50}H_{97}O_{11}N+K]^+$, 896.6749 $[C_{50}H_{97}O_{11}N-CH_2+Na]^+$, 882.6594 $[C_{50}H_{97}O_{11}N-C_2H_4+Na]^+$, 770.5387 $[C_{50}H_{97}O_{11}N-C_{10}H_{20}+Na]^+$; ESI-MS-MS of the peak at m/z 910: 684 $[C_{50}H_{97}O_{11}N$-glucose-$H_2O-C_2H_4+Na]^+$, 668 $[C_{50}H_{97}O_{11}N-CH_3OH-C_{15}H_{30}+Na]^+$, 624 $[C_{50}H_{97}O_{11}N -CH_3OH-C_{18}H_{36}+Na]^+$; FAB-MS 814 $[M-C_7H_{12}+Na]^+$, 840 $[M-C_5H_{10}+Na]^+$.

Methanolysis and LC-ESI-MS analysis. Compound **1** (1.2 mg) was refluxed (70 °C) for 18 h in 2.5 mL of MeOH containing 1.5 mL of 0.9N HCl under magnetic stirring. The mixture was neutralized with aqueous solution of Na_2CO_3 and extracted with $CHCl_3$. The fatty acid methyl ester was carefully characterized by LC-ESI-MS at *m*/*z* 270 (Retention time: 8.87 min).

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