Triumfettamide and Triumfettoside Ic, Two Ceramides and Other Secondary Metabolites from the Stems of Wild *Triumfetta cordifolia* A. RICH. (Tiliaceae)

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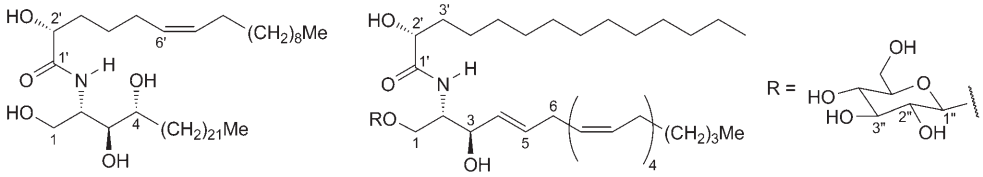
Two new ceramides, triumfettamide (**1**) and triumfettoside Ic (**2**), characterized as (2*R*,6*Z*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxyhexacosan-2-yl]heptadec-6-enamide and (2*R*)-*N*-{(1*S*,2*R*,3*E*,6*Z*,9*Z*,12*Z*,15*Z*)-1-[(β-D-glucopyranosyloxy)methyl]-2-hydroxyheneicosa-3,6,9,12,15-pentaen-1-yl}-2-hydroxytetradecanamide, respectively, were isolated from the stems of *Triumfetta cordifolia* A. RICH. besides eight known secondary metabolites identified as heptadecanoic acid, β-sitosterol glucopyranoside, friedelin, lupeol, betulin, maslinic acid, 2-hydroxyoleanolic acid and the mixture of stigmasterol and β-sitosterol. Their structures were determined on the basis of spectroscopic methods as well as HR-MALDI-FT-ICR-MS analysis, 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. Five pentacyclic triterpenoids, friedelin, lupeol, betulin, maslinic acid, and 2-hydroxyoleanolic acid, have been isolated from *Triumfetta* genus for the first time.

Introduction. – *Triumfetta cordifolia* A. RICH. is localized in the tropical Africa and it is a shrub which grows up to 5 m [1]. In Central Africa, its crushed stems are used to treat wounds [2] and aqueous decoction of the leaves is employed as laxative [3]. Aqueous extract of leaves and stems are used to treat diarrhoea and dysentery in East Africa [4]. In Cameroon, this species is a wild plant; however, some tribes grow it as foodstuff. There is no previous chemical study on *T. cordifolia* reported in the literature. Phytochemical studies of other Tiliaceae species and the importance of *T. cordifolia* in traditional medicine prompted us to investigate this plant. In the course of this work, we have isolated and characterized two new ceramides, triumfettamide (**1**) and triumfettoside Ic (**2**), besides eight known compounds. We herein report the isolation and structural elucidation of the two new ceramides based on spectroscopic methods.

Among the isolated compounds, pentacyclic triterpenoids have not been yet reported in *Triumfetta* genus. Previous biological studies showed that maslinic acid and the oxidized derivative of betulin (betulinic acid) have anti-HIV-1 properties [5].

Results and Discussion. – The methanolic extract of the stems of *T. cordifolia* was subjected to repeated column chromatography to yield ten compounds. Two new ceramides, triumfettamide (**1**) and triumfettoside Ic (**2**), and eight known compounds identified as heptadecanoic acid [6], mixture of stigmasterol and β-sitosterol [7], β-sitosterol glucopyranoside [6], friedelin [8], lupeol [9], betulin [10], maslinic acid [11], and 2-hydroxyursolic acid [12].



1 **2**

Compound **1** was obtained as a colorless powder from column chromatography with CH_2Cl_2/MeOH (19:1) as eluent. The positive HR-MALDI-FT-ICR-MS showed a *pseudo*-molecular ion at *m/z* 718.6315 ($[M+Na]^+$), corresponding to the formula $C_{43}H_{85}NO_5$, which accounted for two degrees of unsaturation. The FT-IR spectrum of **1** showed characteristic absorption bands for free OH groups (3336 cm^{-1}) and an amide CO group (1622 cm^{-1}) [13]. The ^{1}H-NMR spectral data of **1** showed the presence of an NH group at δ(H) 8.29 (*d*, $J = 8.3$) which was exchangeable with D_2O, two *doublet*s of *triplet* of two olefinic H-atoms at δ(H) 5.20 and 5.25 ($J = 8.0$, 4.0), a broad *singlet* at δ(H) 1.35 (CH_2 group), a *triplet* at δ(H) 0.95 ($J = 7$, two terminal Me groups), two carbinol H-atoms at δ(H) 4.15 (*dd*, $J = 10.3$, 5.3) and 4.05 (*dd*, $J = 10.3$, 5.0) suggesting compound **1** to be a ceramide [14]. The ^{13}C-NMR spectrum of **1** (*Table 1*) exhibited C-

Table 1. *The ^{1}H-NMR* (400 MHz) *and ^{13}C-NMR* (100 MHz) *Spectral Data of Compound* **1**. Measured in C_5D_5N; δ in ppm, *J* in Hz.

Position	δ(H)	δ(C) (APT)
1	4.15 (*dd*, $J = 10.3$, 5.3, H_a), 4.05 (*dd*, $J = 10.3$, 5.0, H_b)	61.7
2	4.70–4.90 (*m*)	52.8
3	3.95–4.01 (*m*)	76.5
4	3.85–3.95 (*m*)	72.8
5	1.50–1.70 (*m*)	33.9
6–25, 9′–16′ (CH_2 groups)	1.35 (br. *s*)	20.0, 23.0, 23.9, 30.0, 32.0
NH	8.29 (*d*, $J = 8.3$)	
1′	–	175.0
2′	4.20–4.30 (*m*)	72.2
3′	1.85–1.75 (*m*, H_a), 1.60–1.71 (*m*, H_b)	36.5
4′	1.30–1.48 (*m*)	33.5
5′	1.60–1.71 (*m*)	26.5
6′	5.20 (*dt*, $J = 8.0$, 4.0)	130.4
7′	5.25 (*dt*, $J = 8.0$, 4.0)	130.6
8′	1.75–1.85 (*m*)	26.5
17′, 26 (Me groups)	0.95 (*t*, $J = 7.2$)	14.0

atom signals at δ(C) 175.0, 52.8, and the range 23.9–33.9, further supporting the fatty acid amine nature of **1**. In addition, the NMR spectral data of triumfettamide showed resonances for three oxymethine H-atoms at δ(H)/δ(C) 3.85–3.95/72.8, 3.95–4.01/76.5, and 4.20–4.30/72.2. Compound **1** could be considered as a ceramide containing a C=C bond with *cis* configuration and three oxymethine groups in its structure. The *cis* configuration of the C=C bond was evident from the coupling constant between the olefinic H-atoms ($J = 8.0$) and the chemical shifts of the allylic C-atoms at δ(C) 26.5, which could be more than 30 ppm if the configuration was *trans* [15]. In the HMBC spectrum of **1** (*Fig. 1*), the H-atom signal of the NCH group (δ(H) 4.70–4.90) was correlated with the CH_2OH group (δ(C) 61.7) and two oxygenated CH groups (76.5 (C(3)) and 72.8 (C(4))), suggesting the location of two OH groups at C(3) and C(4), further confirmed by the COSY spectrum (*Fig. 2*), which showed correlations from the NCH group to the CH_2OH and an oxygenated CH group between 3.95–4.01 (*m*, H–C(3)); the former did also correlate to another oxygenated CH group between 3.85–3.95 (*m*, H–C(4)). The third OH group was located at C(2′), which was proved by the correlations observed in the HMBC spectrum from the O–CH group (δ(H) 4.20–4.30 (*m*, H–C(2′)) and the NH group (δ(H) 8.29) to the CO of the ester group (δ(C) 175.0). Other HMBC correlations were observed between H–C(2′) and C=O and C(3′), between H_a–C(3′) and C=O, C(4′), and C(5′), between H_b–C(3′) and C=O and C(2′), and between H–C(6′) and C(5′). Both HMBC and COSY spectra showed correlations from the oxymethine at 4.20–4.30 (*m*, H–C(2′)) to the olefinic H-atom, which suggested that the C=C bond was in the fatty acid side chain. By application of the fragmentation mechanism developed by *Hsu et al.* [16], we had confirmed this C=C bond position (*Scheme 1*). From the foregoing data, we suggested compound **1** to be a sphingolipid [17].

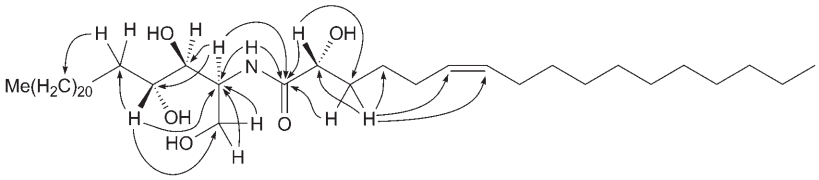


Fig. 1. *HMBC Correlations of compound* **1**

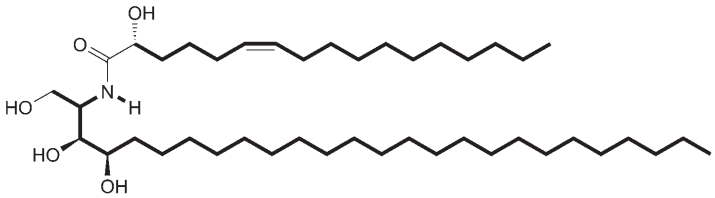
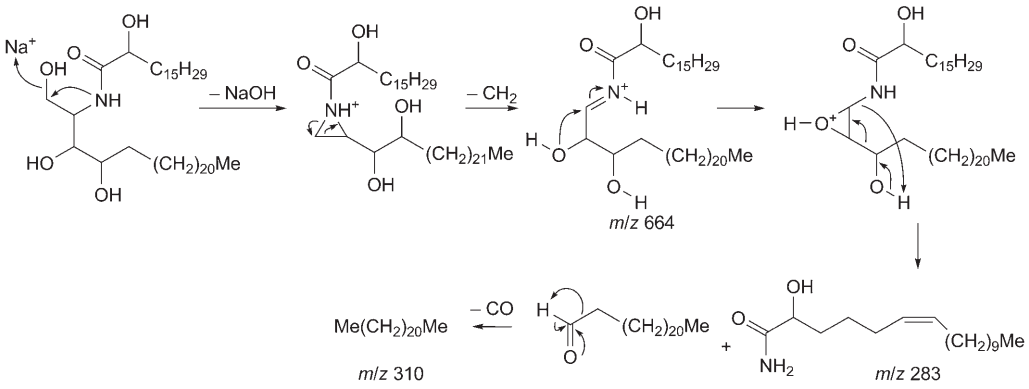


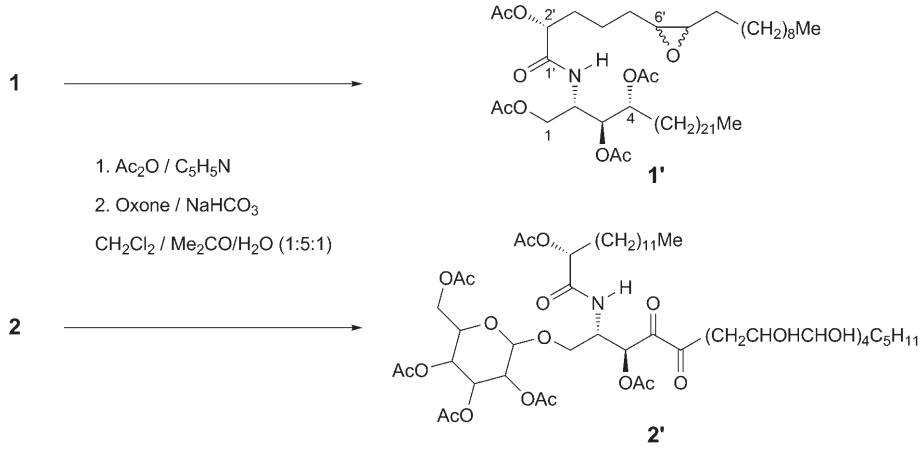
Fig. 2. *COSY Correlations of compound* **1**

Acetylation of **1** followed by oxidation with oxone [18][19] gave compound **1′** (*Scheme 2*), which was detected at *m*/*z* 902 ($[879 + Na]^+$) after a soft ionization by

Scheme 1. *SORI-CID-MALDI-FT-ICR MS/MS of the Ion Fragment* m/z *664* (compound **1**)



Scheme 2. *Acetylation and Oxidation* (with oxone) *of Compounds* **1** *and* **2**



MALDI-TOF-MS. This value supported that this sphingolipid has effectively four OH groups and one C=C bond. Products resulting from oxidation with oxone and acetone as mediator of dioxirane are epoxides. Their formation depends on the pH of the reaction mixture. The pH is controlled by addition of $NaHCO_3$; when it is near of neutral, an epoxide is formed, which is converted to *trans* diols, if the mixture is acidic. In the last case, the reaction will continue yielding a diketone [18][19].

Methanolysis (0.9N HCl/MeOH, at 70° during 18 h) [20] of compound **1** gave the fatty acid methyl ester and the long chain base which were carefully characterized on the base of mass spectrometry as methyl (2*R*,6*Z*)-2-hydroxyheptadec-6-enoate and 2-aminohexacosane-1,3,4-triol. The long chain base (LCB) was observed at *m*/*z* 452 corresponding to $[LCB + Na]^+$.

The absolute configuration at C(2), C(3), C(4), and C(2′) was determined to be (*S*), (*S*), (*R*), and (*R*), respectively, by comparison of ^{1}H- and ^{13}C-NMR data with

those obtained from the literature. In fact, compound **1** exhibited the characteristic signals of phytosphingosine-type ceramides possessing mainly (2*R*)-hydroxyfatty acid [15]. The above information was confirmed by the biosynthesis of phytoceramide [21]. The analyses of the NMR and mass spectroscopy data led to the unambiguous assignment of structure **1** as (2*R*,6*Z*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxyhexacosan-2-yl]heptadec-6-enamide, which was trivially named as triumfettamide.

Compound **2** was obtained as a colorless solid from the mixture of AcOEt/MeOH 9:1. Its HR-MALDI-FT-ICR-MS exhibited a *pseudo*-molecular ion peak at *m*/*z* 736.5327 ($[M+H]^+$), corresponding to a molecular formula $C_{42}H_{73}NO_9$, which indicated seven degrees of unsaturation. The FT-IR spectrum of **2** showed a broad absorption band for OH groups (3350 cm^{-1}) [17], and strong absorptions for a secondary amide (1637 and 1542 cm^{-1}) [17]. These data were supported by the presence of a C–N C-atom signal at δ(C) 53.5 and a CO signal at 175.8 in the ^{13}C-NMR spectrum. Compound **2** gave a positive reaction in the *Molish* test, suggesting the presence of a sugar moiety. The NMR spectra of **2** (*Table 2*) showed the anomeric

Table 2. *The ^{1}H-NMR* (400 MHz) *and ^{13}C-NMR* (100 MHz) *Spectral Data of Compound* **2**. Measured in CD_3OD; δ in ppm, *J* in Hz.

Position	δ(H)	δ(C) (APT)
1	3.80–3.85 (*m*, H_a), 3.72–3.76 (*m*, H_b)	68.5
2	3.99–4.03 (*m*)	53.5
3	4.18 (*dd*, *J* = 8.0, 12.0)	71.4
4	5.61 (*dd*, *J* = 8.0, 16.0)	130.6
5	5.87 (br. *d*, *J* = 16.0)	133.0
6	2.10 (br. *s*), 2.00 (br. *s*)	31.7
9	2.10, 2.00 (br. *s*)	317
12	2.10, 2.00 (br. *s*)	317
15	2.10, 2.00 (br. *s*)	317
18	1.99, 1.80 (br. *s*)	29.4
7, 8, 10, 11, 13, 14, 16, 17	5.49–5.58 (*m*)	130.2, 130.2, 130.0, 129.9, 129.8, 129.5, 129.4, 129.3
19, 20, 21	144 (br. *s*)	22.5–29.3
1′	–	175.8
2′	4.05 (*dd*, *J* = 4.0, 8.0)	71.6
3′	1.68–1.79 (*m*), 1.57–1.68 (*m*)	33.3
4′–13′ (CH_2 groups)	1.30–1.60 (*m*)	29.3, 29.0, 28.8, 22.5
14′, 22 (Me groups)	1.03 (*t*, *J* = 7.3)	13.0
Glucose		
1″	4.30 (*d*, *J* = 8.0)	103.2
2″	3.21 (*dd*, *J* = 7.0, 11.6)	74.1
3″	3.30 (*dd*, *J* = 7.6, 8.0)	76.4
4″	3.32 (*dd*, *J* = 8.0)	70.1
5″	3.40 (*ddd*, *J* = 4.0, 12.0, 8.0)	76.6
6″	3.91 (br. *d*, *J* = 12.0), 3.74 (*dd*, *J* = 4.0, 12.0)	61.2

center at δ(H) 4.30 (1 H, *d*, $J = 8.0$) and δ(C) 103.2, and a set of C-atom signals (δ(C) 74.1, 76.4, 70.1, 76.6, and 61.2), consistent with a β-D-glucopyranoside. Furthermore, a CH_2O group (3.80–3.85 (*m*, H_a–C(1)) and 3.72–3.76 (*m*, H_b–C(1)); δ(C) 68.5), two CH–O groups (δ(H) 4.18 and 4.05; δ(C) 71.4 and 71.6), two ethylenic H-atoms (H–C(4) and H–C(5)) in *trans*-geometry [22] at δ(H) 5.61 (*dd*, $J = 16.0, 8.0$) and δ(C) 130.6 and at δ(H) 5.87 (br. *d*, $J = 16.0$) and δ(C) 133.0, eight olefinic H-atoms in *cis*-configuration [22] between δ(H) 5.58 and 5.49 (*dt*, $J = 8.0, 4.0$, δ(C) 130.2, 130.2, 130.0, 129.9, 129.8, 129.5, 129.4, 129.3), a long aliphatic chain characterized by an intense signal between δ(H) 1.30 and 1.60 (*m*), and two terminal Me groups (δ(H) 1.03, *t*, $J =$ 7.3; δ(C) 13.0) were also observed. From the foregoing data, a polyunsaturated β-D-glucopyranosyl ceramide structure was suggested for compound **2**. Pertinent correlations (*Fig. 3*) observed in the HMBC spectrum between the two carbinol H-atoms H_a–C(1), H_b–C(1) and the anomeric C-atom C(1″) prompted us to place the sugar moiety at C(1). HMBC Correlations were also observed between H_a–C(1) and C(2″) of the sugar, H_b–C(1) and C(2) and C(3), H–C(3) and C(2), C(1), C(4), and C(5), H–C(4) and C(3) and C(6), H–C(5) and C(3) and C(6) showing the *trans* C=C bond to be in the amino polyalcohol side chain. H–C(2′) showed correlations with C=O, C(3′), and C(4′), and H_a–C(3′) with C(2′). The COSY spectrum (*Fig. 3*) showed that the anomeric H-atom H–C(1″) had a correlation with H_a–C(1), supporting the sugar position. H–C(3) correlated with H_b–C(1), H–C(2), and H–C(4), and H–C(4) with H–C(5), H_a–C(6), and H_b–C(6). H–C(5) correlated with both H_a–C(6) and H_b–C(6). All the olefinic H-atoms showed correlations with the CH_2 groups at δ(H) 2.10 and 2.00 (δ(C) 31.7, 29.4) suggesting the presence of allylic C-atoms. In the COSY spectrum, H–C(2′) correlated with H_a–C(3′) and H_b–C(3′), both H_a–C(3′) and H_b–C(3′) correlated with multiple H-atom signals between 1.30 and 1.60, which showed cross-peaks with the Me group at δ(H) 1.03. The former data suggest that the fatty acid side chain is saturated.

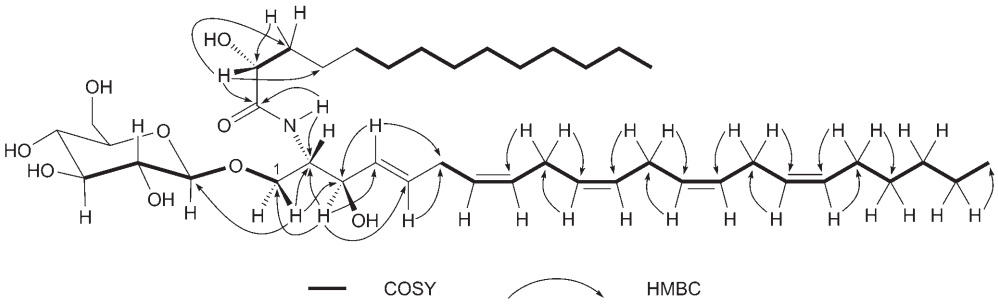


Fig. 3. *Important HMBC and COSY correlations of compound* **2**

The NOESY spectrum (*Fig. 4*) showed interactions between H–C(4) at δ(H) 5.63 and the CH H-atoms at δ(H) 2.10 and 2.00 (H_a–C(6) and H_b–C(6)), suggesting them to be in the same orientation. This NOESY spectrum further supported the C=C bonds to be in the same carbon chain and the sugar moiety to be a glucopyranosyl moiety due to interactions between the anomeric H-atom H–C(1″) and the H-atoms H–C(3″) and H–C(5″).

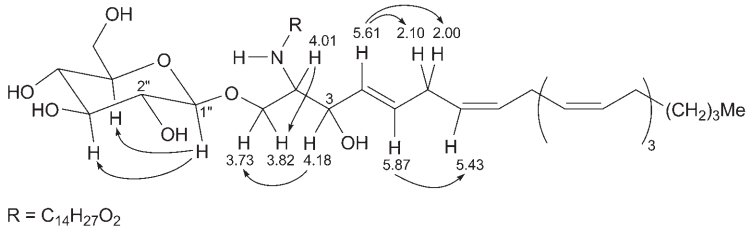


Fig. 4. *NOESY Correlations of compound* **2**

Compound **2** was subjected to acetylation (Ac_2O/pyridine), followed by oxidation [18][19] (*Scheme 2*). These reactions yielded a compound, which had a peak at *m*/*z* 1176 ($[1153 + Na]^+$) in the mass spectra indicating that **2** has six free OH groups and five olefinic functions.

The methanolysis solution (aq. 0.9N HCl/MeOH) of compound **2** [20] was neutralized with Na_2CO_3 solution and extracted with $CHCl_3$ to yield methyl (2*R*)-2-hydroxytetradecanoate and the LCB, which were evident after a detailed analysis of its mass spectrum, showing the major peak at *m*/*z* 386 ($[385 + H]^+$), corresponding to the LCB. The tandem mass spectrum of **2** (*Fig. 5*, *Scheme 3*) exhibited an important peak at *m*/*z* 156.0417 (4, $[C_{11}H_{24}]^+$), which, with the correlations described above, confirmed that the olefinic bonds were not in the fatty acid side chain. The absolute configuration at C(2), C(3), and C(2′) was determined to be (*S*), (*R*), and (*R*), respectively, by comparison of ^{1}H- and ^{13}C-NMR data with those obtained from the literature. Compound **2** exhibited, as compound **1**, the characteristic signals of phytosphingosine-type ceramides possessing mainly (2*R*)-hydroxyfatty acid [15]. From the foregoing data, triumfettoside Ic (**2**) was characterized as (2*R*)-*N*-{(1*S*,2*R*,3*E*,6*Z*,9*Z*,12*Z*,15*Z*)-1-[(*β*-D-glucopyranosyloxy)methyl]-2-hydroxyheneicosa-3,6,9,12,15-pentaen-1-yl}-2-hydroxytetradecanamide.

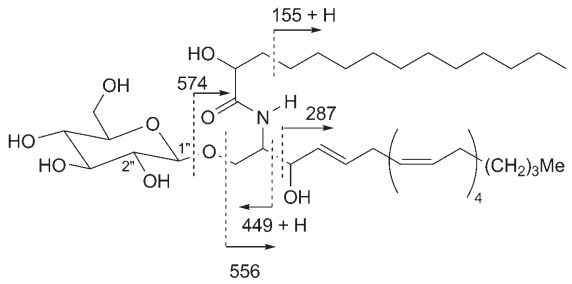
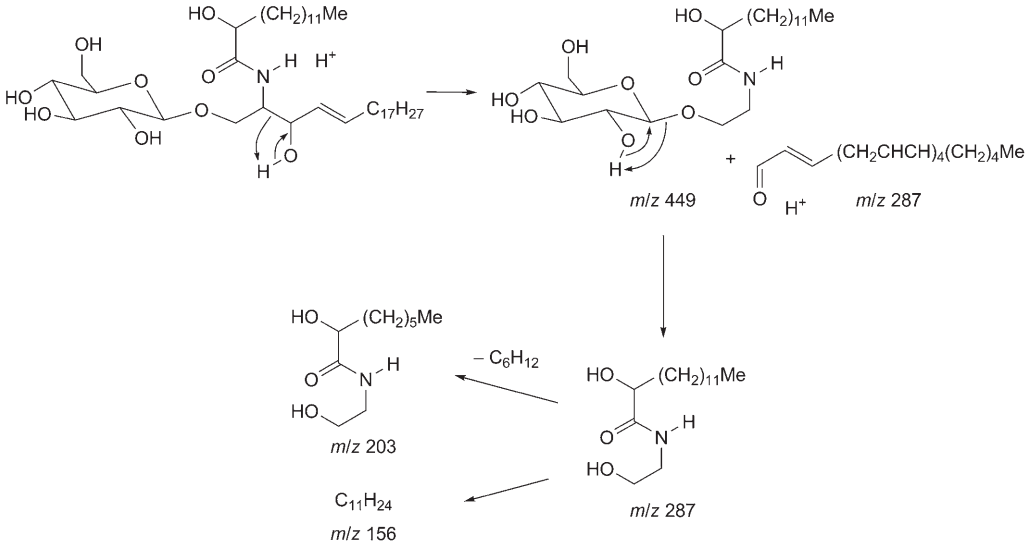


Fig. 5. *SORI-MALDI-FT-ICR-MS Fragmentation of compound* **2**

The NMR data of the known compounds were in agreement with those reported in the literature.

Scheme 3. *SORI-CID-MALDI-FT-ICR MS/MS of the Ion Fragment* m/z *736* (compound **2**)



Conclusions. – The present study provides the first report of the presence of pentacyclic triterpenoids, sphingolipids, and glycosphingolipids in *Triumfetta* species, in addition to the identification of two new ceramides, **1** and **2**. Ceramides have a wide range of biological activities, all probably dependent of their amphipathic nature [23]. The broad bioactivity spectrum of triterpenoids [5], steroids [24], and ceramides [23] explain probably the biological properties of *Triumfetta* in folk medicine.

Experimental Part

General. Vacuum column chromatography (VCC), column chromatography (CC) and thin layer chromatography (TLC) were performed over silica gel *60 H* (particle size 90% < 45 μm), or 200–300 mesh silica gel, *Silica gel GF254*, resp. 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. ^{1}H- and ^{13}C-NMR: *Bruker DRX-400* MHz for 1D- and 2D-NMR spectra. The matrix used for the MALDI-FT-ICR-MS and MALDI-TOF-MS was a saturated 2,5-dihydroxybenzoic acid (2,5-DHB) soln. in 50% MeOH, 0.1% trifluoroacetic acid (TFA), and 2,5-DHB 1M soln. in 50% MeCN/50% ultrapure H_2O, and 0.1% TFA respectively. All the depositions were made using the dried-droplet method. MALDI-FT-ICR-MS: *IonSpec Explorer* Fourier Transform Mass Spectrometer *9.4 T* equipped with a *ProMALDI* module (*IonSpec*, Lake Forest, USA) provided with an *Orion* air cooled *Nd : YAG* System (355 nm, *New wave research*, Fremont, USA). MS/MS: by Sustained Off-Resonance Irradiation Collision Induced Dissociation (SORI-CID) directly in the ICR cell. In these SORI-CID-MALDI-FT-ICR-MS experiments, the activation time was 20 ms, which means that the ions are excited and may encounter gas molecules during 20 ms, leading to fragmentations. Then, all the ions in the cell are cooled down and are therefore ready to be detected. MALDI-TOF-MS measurements were possible using a *Bruker Reflex IV* time-of-flight mass spectrometer (TOF-MS) (*Bruker-Daltonic*, Bremen, Germany) equipped with the *SCOUT 384* probe ion source, using a N_2 pulsed laser (337 nm, model *VSD-337ND*, *Laser Science Inc.*, Boston, MA) with energy output of 400 μJ/pulse.

Plant Material. T. cordifolia A. RICH. was collected from Yaoundé centre province of Cameroon and a specimen (N° 12830SRF Cam) has been deposited in the national herbarium of Yaoundé, Cameroon.

Extraction and Isolation. Dried stems of *T. cordifolia* A. RICH. (3.93 kg) were cut into small pieces and powdered. 250 g of the powder were extracted with MeOH by heating for 8 h. The MeOH extract (80 g) was concentrated under vacuum and partitioned between H_2O and AcOEt. Both phases were concentrated under vacuum to give 40 g of org. phase and 39 g of the aq. extract. The AcOEt extract was subjected to flash chromatography (SiO_2, cyclohexane, cyclohexane/AcOEt 3:1 to 1:1 to 1:3, AcOEt, in order of increasing polarity) yielding 5 fractions *A–E*. *Fr. B* was further purified by CC with different mixtures of cyclohexane/AcOEt yielding 217 fractions. The *Frs. 22–32* further eluted with different mixtures of cyclohexane/AcOEt. At a ratio of 93:7, 3-friedelanone (46.4 mg) was obtained. At 23:2, heptadecanoic acid (72.4 mg), and at 9:1, lupeol (32.0 mg) were isolated. *Frs. 40–52* were further eluted with a mixture of cyclohexane/AcOEt (17:3) yielding betulin (45 mg). *Fr. C* yielded a mixture of sterols (11.0 mg). When further chromatographed with a mixture cyclohexane/AcOEt, at a ratio of 3:1, 2-hydroxyoleanolic acid (32.5 mg) was obtained, and 2-hydroxyursolic acid (5.0 mg) was isolated at a ratio of 2.9:1.1. *Fr. D* was further eluted with a mixture of CH_2Cl_2/MeOH with increasing polarity. From the *Frs. 20–36* eluted with the mixture CH_2Cl_2/MeOH (19:1), triumfettamide was obtained (50.0 mg); the same mixture of solvents gave the β-D-glucopyranoside of β-sitosterol (11.0 mg) from *Frs. 54–59*. The dried aq. partition was extracted with acetone yielding 30.1 mg of extract. It was eluted with a mixture AcOEt/MeOH with increasing polarity to yield 114 fractions. *Frs. 74–80* were eluted with the mixture AcOEt/MeOH (9:1), yielding triumfettoside Ic (30 mg).

Triumfettamide (*=(2R,6Z)-2-Hydroxy*-N-*[(2S,3S,4R)-1,3,4-trihydroxyhexacosan-2-yl]heptadec-6-enamide*; **1**). Colorless powder. M.p. 135–137°. $[\alpha]_D^{27} = -7.36$ ($c = 0.095$, C_5H_5N). IR: 3336, 3210, 1622, 1545, 1466. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-MALDI-FT-ICR-MS: 718.6315 (14.5, $[M+Na]^+$; calc. 718.6320), 704.6156 (56.2, $[M+Na-CH_2]^+$), 690.6009 (40, $[M+Na-C_2H_4]^+$), 676.5854 (38, $[M+Na-C_3H_6]^+$), 664.6234 (100, $[M-MeO]^+$), 650.6071 (71.8, $[M-MeO-CH_2]^+$), 632.5976 (20, $[M-MeO-CH_2-H_2O]^+$). SORI-MALDI-FT-ICR-MS/MS of *m/z* 664: 415 (6, $[C_{26}H_{54}O_3+H]^+$), 384 (6.5, $[C_{25}H_{51}O_2+H]^+$), 311 (6, $[C_{22}H_{46}+H]^+$), 283 (6.5, $[C_{17}H_{33}O_2N]^+$).

Triumfettoside Ic (*=(2R)*-N-*[(1S,2R,3E,6Z,9Z,12Z,15Z)-1-{(β-D-Glucopyranosyloxy)methyl}-2-hydroxyheneicosa-3,6,9,12,15-pentaen-1-yl]-2-hydroxytetradecanamide*; **2**). Colorless powder. M.p. 172–173°. $[\alpha]_D^{30} = +35.64$ ($c = 0.27$, MeOH). IR: 3350, 1637, 1542, 1467, 1074. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-MALDI-FT-ICR-MS: 736.5327 (100, $[M+H]^+$; calc. 736.5358), 718.5753 (30, $[M+H-H_2O]^+$), 574.4991 (56, $[M+H-C_6H_{11}O_5]^+$), 556.4867 (76, $[M-C_6H_{11}O_6]^+$), 510.3301 (30, $[M-C_6H_{11}O_6-MeOH-CH_2]^+$), 482.3205 (100, $[M-C_6H_{11}O_6-MeOH-3\ CH_2]^+$), 156.0417 (4, $[C_{11}H_{24}]^+$), 287.2401 (20, $[C_{20}H_{31}O]^+$), 449.3070 (30, $[C_{22}H_{42}O_8N+H]^+$).

Acetylation and Oxidation of **1** *and* **2**. 1 mg (1.44 μmol) of compound **1** and 1.1 mg (1.5 μmol) of **2** were separately solved in 1 ml of pyridine under magnetic stirring and 5 min later, 1.5 ml Ac_2O was added. The reaction was controlled by TLC (cyclohexane/AcOEt 9:1) and acetylated products were formed after 1 h. Both solutions *a*) were concentrated under vacuum, and the obtained residues were solved in a mixture CH_2Cl_2 (2 ml)/acetone (10 ml); *b*) 1 ml of aq. $NaHCO_3$ (0.011 g, 0.13 mmol) and 1 ml of aq. oxone (0.03 g, 0.05 mmol) were prepared two times and added to the different solns. Both mixtures were magnetically stirred for 3 h at r.t. The mixtures were concentrated under vacuum, poured separately onto H_2O and extracted with AcOEt. The org. phases were concentrated under vacuum to yield compounds **1′** and **2′**, which were identified without ambiguity with soft ionization method (MALDI-TOF) in the mass spectrometry.

Methanolysis. Compounds **1** and **2** (1.2 mg) were separately refluxed (70°) for 18 h in 2.5 ml of MeOH containing 1.5 ml of 0.9N HCl under magnetic stirring. Both mixtures were neutralized with Na_2CO_3 and extracted with $CHCl_3$. The mass spectrometry analysis using soft ionization method (MALDI-TOF) showed only the peaks corresponding to the long chain base of the two compounds. $[M+H]^+$ at *m/z* 452 for **1** and 386 for **2**.

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