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ORIGINAL PAPER

# Two New Fatty Acid Derivatives from the Stem Bark of *Alchornea laxiflora* (Euphorbiaceae)

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**Abstract** Euphorbiaceae is a family of plants used in traditional remedies in central Africa to treat selected diseases. Some of the phytochemical components in the stem bark of *Alchornea laxiflora* that have biochemical activity were identified. A number of novel compounds were isolated, including a new fatty acid ester, (**1**) a new ceramide, (**2**) some triterpenoids, (**3**–**5**), ellagic acid (**6**) and its derivatives (**7**, **8**) were isolated. The structures of these compounds were determined on the basis of spectroscopic methods as well as HR-ESI-TOF-MS analysis, chemical transformation and by comparison of their physical and spectral data with those reported in the literature. The cytotoxicity of some isolated compounds was investigated against human promyelocytic leukaemia (HL60) cell line by using the MTT method. Compounds **1**, **4** and **5** showed a cytotoxic activity with $IC_{50}$ at 58.7, 6.6 and 6.8 μM, respectively.

**Keywords** Euphorbiaceae · *Alchornea laxiflora* · Isolation · Fatty derivatives · Cytotoxicity

**Abbreviations**

| | |
|---|---|
| CC | Column chromatography |
| COSY | Correlation spectroscopy |
| ESI-MS | Electrospray mass spectrometry |
| FT-IR | Fourier transformed infra-red |
| HMBC | Heteronuclear multiple bond correlation |
| HSQC | Heteronuclear single quantum correlation |
| HR-ESI-MS | Higher resolution electrospray mass spectrometry |
| m.p. | Melting point |
| NMR | Nuclear magnetic resonance |
| NOESY | Nuclear overhauser effect spectroscopy |
| TLC | Thin layer chromatography |
| VCC | Vacuum column chromatography |

## Introduction

*Alchornea laxiflora* is a shrub of the Euphorbiaceae family which is common throughout central, eastern and southern Africa. Plant material used in this investigation was harvested in Cameroon. The stems, especially the small branches are used in Nigeria as chew-sticks and its leaves are also used in this country to preserve kola nuts [1]. The decoctions of the leaves of *A. laxiflora* are also

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employed to treat inflammation and some infections [1]. Previous phytochemical studies carried out on this species by Ogundipe et al. [2] showed the presence of alkaloids, cardiac glycosides, saponins and phenolic compounds with a higher concentration in the leaves than in the roots. It seems that the biological effect usually observed in folk medicine are induced by these secondary metabolites. So, the previous works [2, 3] and the traditional uses have stimulated our interest in chemically evaluating *A. laxiflora* and in the biology of the compounds obtained.

We hereby report the isolation and structural elucidation of the new compounds based on the spectrometric methods and chemical transformation. Their cytotoxic activities will be also presented.

## Experimental Part

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. The melting point (m.p.) was measured by a 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. HR-ESI-MS and ESI-MS were recorded with a JEOL JMS-700 instrument.

### Plant Material

*A. laxiflora* (Euphorbiaceae) was collected From Yaoundé central region of Cameroon in August 2006. A sample (No 45363HNC) has been deposited in the National Herbarium of Yaoundé, Cameroon.

### Extraction and Isolation

The dried stem bark of *A. laxiflora* (2.7 kg) was cut into small pieces, powdered and extracted by maceration with 10 L of a mixture of methylene chloride/methanol (1:4, v/v) for 72 h. The organic extract was concentrated to yield 87 g of crude extract which was subjected to flash chromatography [$SiO_2$, hexane/ethyl acetate (3:1, v/v), ethyl acetate and ethyl acetate/methanol (9:1, v/v)] yielding three fractions A, B and C. A was purified by CC with different mixtures of hexane/ethyl acetate in order of increasing polarity yielding 150 fractions. The ones (36–39) eluted with hexane/ethyl acetate in the ratio 19:1, (v/v) yielded 3-*O*-acetyl of oleanolic acid **4** (9.5 mg); from the same ratio of hexane/ethyl acetate 3 mg of 3-*O*-acetyl of ursolic acid **5** were obtained. The fractions 61–68 eluted with hexane/ethyl acetate in the ratio 4:1 (v/v) afforded 8 mg of compound **1**. From the fraction B eluted with methylene chloride/methanol in gradient conditions, 165 fractions were obtained and 7 mg of compound **2** were isolated from the fractions 30–40 eluted in the ratio 97.5:2.5 (v/v); 4 mg of 3-*O*-methylellagic acid **7** were further obtained from the fractions 45–51 in the ratio 95:5 (v/v); from the same ration 2.3 mg of 3-*O*-$\beta$-D-glucopyranoside of $\beta$-sitosterol **3** were filtered. From C eluted with methylene chloride/methanol in the gradient condition, 2 mg of ellagic acid **6** were isolated from the fractions 32–40 eluted in the ratio 92.5:7.5 (v/v) and from the fractions 50–58, 4.5 mg of 3-*O*-methyl-3′-*O*-$\alpha$-rhamnopyranosylellagic acid **8** were collected from the ratio 9:1 (v/v).

## Methods

### Methanolysis

Methanolysis of compound **2**—(1 mg) was refluxed (70 °C) for 18 h in 2.5 mL of MeOH containing 1.5 mL of 0.9 N HCl under magnetic stirring. The mixture was neutralized with an aqueous solution of $Na_2CO_3$ and extracted with $CHCl_3$. The fatty acid methyl ester was carefully characterized by ESI-MS at *m/z* 441 $[C_{28}H_{56}O_3+H]^+$.

### Acetylation and Oxidative Cleavage of the Double Bond

This was carries out by taking 1 mg (3.18 μmol) of **1** and 1 mg (1.078 μmol) of **2** and these were separately dissolved in 1 mL of pyridine and 1.5 mL of acetic anhydride. The reaction under magnetic stirring at room temperature was stopped after 1 h and the usual work up gave the acetylated products. **1′** and **2′** were separately dissolved in 4 mL of a mixture of dioxane/water (3:1); two equivalents of pyridine, two drops of 4% by weight of an aqueous solution of $OsO_4$ and four equivalents of $NaIO_4$ were added. The reaction was left under magnetic stirring at room temperature for 3 h. Quenching was done with 10 mL of an aqueous solution of sodium thiosulfate and left under magnetic stirring during about 20 min. The organic parts were extracted three times with 20 mL of $CH_2Cl_2$ for each reaction and concentrated separately under vacuum. The separate analyses by ESI-MS of different organic residual phases gave important peaks at *m/z* 316 $[C_{15}H_{24}O_7+H]^+$ and 249 $[C_{15}H_{30}O+Na]^+$ from **1** and **2**, respectively.

## Chemical Properties of Compounds

*(10Z)-Tetradec-10-enoic acid-(2S)-2-carboxy-2-hydroxyethyl ester (**1**)*

Yellow viscous oil; [α] + 4.6 (*c* 0.06, DMSO); IR 3,430, 2,930, 2,863, 1,686 and 1,644 $cm^{-1}$; HRESIMS *m/z*: 313.2018 $[C_{17}H_{30}O_5–H]^-$, 239.1651 $[C_{17}H_{30}O_5–CO_2–C_2H_6–H]^-$, 183.1388 $[C_{17}H_{30}O_5–C_3H_4O_3–C_3H_6–H]^-$; $^1H$ and $^{13}C$ NMR: Table 1.

*(2R)-2-Hydroxy-N-[(2S,3S,4R,15Z)-1,3,4-trihydroxy-15-triaconten-2-yl]octacosamide (**2**)*

White solid; m.p. 139.5–140.5; [α] − 8.05 (*c* 0.090, $C_5H_5N$); IR: 3,335, 3,201, 1,649, 1,637 and 1,542 $cm^{-1}$; HRESIMS *m/z*: 928.8678 $[C_{58}H_{115}NO_5+Na]^+$, 878.8289 $[M–CH_3OH–H_2O+Na]^+$, 860.8191 $[M–CH_3OH–2H_2O+Na]^+$, 850.4157 $[M–CH_3OH–H_2O–C_2H_4+Na]^+$, 822.7675 $[M–CH_3OH–H_2O–2C_2H_4+Na]^+$; $^1H$ and $^{13}C$ NMR: Table 2.

*3-O-β-D-Glucopyranoside of β-Sitosterol (**3**)*

White powder; Positive response in the Liebermann Burchard and Molish tests characteristic of glycoside of steroid; m.p. [290–291] °C (Ref. [4]); ESI-MS *m/z* 577.44 $[C_{35}H_{60}O_6+H]^+$; Some Characteristic $^1H$- and $^{13}C$-NMR data ($C_5D_5N$) $\delta_H/\delta_C$ 5.35 (H-6, br*s*)/121.7 (C-6), 140.9 (C-5)]; *β*-D-glucopyranosyl: 101.2, 70.5, 73.9, 77.2, 77.2, and 61.5.

*3-O-Acetyl of Oleanolic Acid (**4**)*

White powder; Positive response in the Liebermann Burchard test characteristic of triterpenes; m.p. [257–259] °C (Ref. [5]); ESI-MS *m/z* 499.37 $[C_{31}H_{50}O_4+H]^+$; Some characteristic $^1H$- and $^{13}C$-NMR data ($CDCl_3$) $\delta_H/\delta_C$ 4.72 (H-3, *dd*, 2.5, 9.5 Hz)/80.9, 5.45 (H-12, br*s*)/122.6 (C-12), 143.6 (C-13), Acetyl group [α-$CH_3$, 2.50 (s)/22.4; C=O (171.0)],

*3-O-Acetyl of Ursolic Acid (**5**)*

White powder; positive response in the Liebermann Burchard test characteristic of triterpenes; m.p. [275–277] °C (Ref. [4]); ESI-MS *m/z* 577.44 $[C_{35}H_{60}O_6+H]^+$; Some Characteristic $^1H$- and $^{13}C$-NMR data ($CDCl_3$) 4.49 (H-3, *dd*, 2.1, 10.1 Hz)/80.3, 5.25 (H-12, br*s*)/124.4 (C-12), 138.2 (C-13), acetyl group [α-$CH_3$, 2.11 (s)/22.1; C=O (170.8)].

*Ellagic Acid (**6**)*

Yellowish powder; dark green color with $FeCl_3$, m.p. 358 °C (after decomposition) (Ref. [6]); *m/z* 303.01 $[C_{14}H_6O_8+H]^+$; $^1H$- and $^{13}C$-NMR data (DMSO-$d_6$) $\delta_H$ 7.40 (2H, *s*, H-5 and H-5′); $\delta_C$ 106.4 (C-1 and C-1′), 108.9 (C-5 and C-5′), 112.4 (C-6 and C-6′), 135.8 (C-2 and C-2′), 140.9 (C-3 and C-3′), 148.3 (C-4 and C-4′), 159.2 (C-7 and C-7′).

*3-O-Methylellagic Acid (**7**)*

Yellowish powder; dark green color with $FeCl_3$, m.p. [302–303] °C (Ref. [7]); *m/z* 317.03 $[C_{15}H_8O_8+H]^+$; $^1H$- and $^{13}C$-NMR data (DMSO-$d_6$) $\delta_H$ 7.50 (1H, s, H-5), 7.44 (1H, s, H-5′), 4.02 (3H, s, 3-OMe). $\delta_C$ 158.9 (C-7), 158.8 (C-7′), 152.1 (C-4), 148.3 (C-4′), 141.5 (C-2), 140.1 (C-3), 139.9 (C-3′), 136.1 (C-2′), 112.5 (C-1′), 112.2 (C-6), 111.9

**Table 1** The $^1H$-NMR (400 MHz) and $^{13}C$-NMR (100 MHz) spectral data of compound **1** measured in $CDCl_3$; δ in ppm, *J* in Hz

| Position | $\delta_H$ | $\delta_C$ (APT) | Cosy | HMBC |
|---|---|---|---|---|
| 1 | – | 172.8 | – | – |
| 2 | 2.30 (*t*, 6.3) | 34.2 | 1.60 | 24.8, 29.0–29.7, 172.8 |
| 3 | 1.60 (br *s*) | 24.8 | 1.30, 2.30 | 29.0–29.7, 172.8 |
| 4–8, 13 | 1.30 (br *s*) | 22.5, 24.8, 27.1, 29.0–29.7 | 0.83 | 27.2, 24.8, 22.5, 29.0–29.7 |
| 9 | 2.00 (*m*) | 27.2 | 1.30, 5.36, 5.37 | 29.0–29.7, 129.6, 130.1 |
| 10 | 5.36 (*m*) | 129.6 | 2.00 | 27.2 |
| 11 | 5.37 (*m*) | 130.1 | 2.00 | 27.2 |
| 12 | 2.00 (*m*) | 31.9 | 1.30 | 29.0–29.7, 129.6, 130.1 |
| 14 | 0.83 (*t*, 6.3) | 14.1 | 1.30 | 22.5, 29.0–29.7, 31.9 |
| 1′ | – | 173.2 | – | – |
| 2′ | 5.22 (br *t*, 6.3) | 68.8 | 4.15, 4.25 | 62.1, 173.2 |
| 3′ | 4.15 (*dd*, 2.3, 11.4) | 62.1 | 4.25, 5.22 | 68.8, 172.8, 173.2 |
| | 4.25 (*dd*, 6.4, 11.4) | | 4.15, 5.22 | 68.8, 172.8, 173.2 |

**Table 2** The $^{1}$H-NMR (400 MHz) and $^{13}$C-NMR (100 MHz) spectral data of compound **2** measured in $C_5D_5N$; $\delta$ in ppm, $J$ in Hz

| Position | $\delta_H$ | $\delta_C$ (APT) | Cosy | HMBC |
|---|---|---|---|---|
| N**H** | 8.62 (*d*, $J = 8.0$ Hz) | – | 5.16 | 53.2, 175.5 |
| 1 | 4.53 (*m*, $H_a$) 4.47 (*m*, $H_b$) | 62.2 | 4.47, 4.53, 5.16 | 53.2, 77.1 |
| 2 | 5.16 (*m*) | 53.2 | 4.47, 4.53, 4.37, 8.62 | 62.2, 77.1, 175.5 |
| 3 | 4.37 (br *d*, 8.0 Hz) | 77.1 | – | 53.2 |
| 4 | 4.32 (*m*) | 73.1 | 2.01, 2.02, 4.37 | 33.5, 53.2, 62.2 |
| 5 | 2.01 (*m*, $H_a$), 2.02 (*m*, $H_b$) | 33.5 | 1.27–1.33, 4.32 | 29.7–30.2, 73.1 |
| 6–13, 18–30, 5′–28′ | 1.27–1.33 (br *s*) | 22.5, 23.9–30.2, 31.9 | 0.87, 2.02, 2.25 | 14.5, 23.2 |
| 14 | 2.02 (*m*) | 27.2 | 5.55 | 131.0 |
| 15 | 5.54 (*d*, 4.0 Hz) | 130.9 | 2.02, 2.20 | 32.0 |
| 16 | 5.55 (*d*, 4.0 Hz) | 131.0 | 2.02, 2.20 | 32.0 |
| 17 | 2.20 (*m*) | 32.0 | 5.54 | 130.9 |
| 30, 28′ ($CH_3$) | 0.87 (*t*, 8.0 Hz) | 14.5 | 1.27–1.33 | 23.2, 29.7–30.2 |
| 1′ (C=O) | – | 175.5 | – | – |
| 2′ | 4.65 (*m*) | 72.7 | 2.03, 2.25 | 27.2, 35.9, 175.5 |
| 3′ | 2.03 (*m*, $H_a$), 2.25 (*m*, $H_b$) | 35.9 | 1.27–1.33, 1.80 | 27.2, 72.7 |
| 4′ | 1.80 (*m*, $H_a$) | 24.8 | 2.01 | 35.9 |
| | 2.01 (*m*, $H_b$) | | 1.80 | |

(C-1), 111.3 (C-5), 110.2 (C-5′), 107.2 (C-6′),60.9 (3-OMe).

*3-O-Methyl-3'-O-α-rhamnopyranosylellagic acid (**8**)*

Yellow gum, dark green color with $FeCl_3$ and gave positive response with Molish test characteristic of glycoside of phenolic compound, *m/z* 463.09 $[C_{21}H_{18}0_{12}+H]^+$; $^{1}$H- and $^{13}$C-NMR data ($CD_3OD$) Aglycon: $\delta_H$ 7.46 (H-5, *s*), 7.48 (H-5′, *s*), 4.11 ($CH_3$–O), rhamnopyranosyl: 5.72 (1H, *d*, 1.6), 4.34 (1H, *dd*, 1.5, 3.4), 4.04, 3.44 (1H, *t*, 9.6), 4.45 (1H, *m*), 1.23 (3H, *d*, 6.2); Aglycon: $\delta_C$ 112.2, 142.4, 143.5, 154.4, 114.1, 113.3, 161.8, 110.6, 143.6, 140.3, 153.7, 115.2, 114.6, 161.8, 61.3. Rhamnopyranosyl: 102.7, 72.0, 72.2, 73.9, 71.7, 17.7

Cell Viability

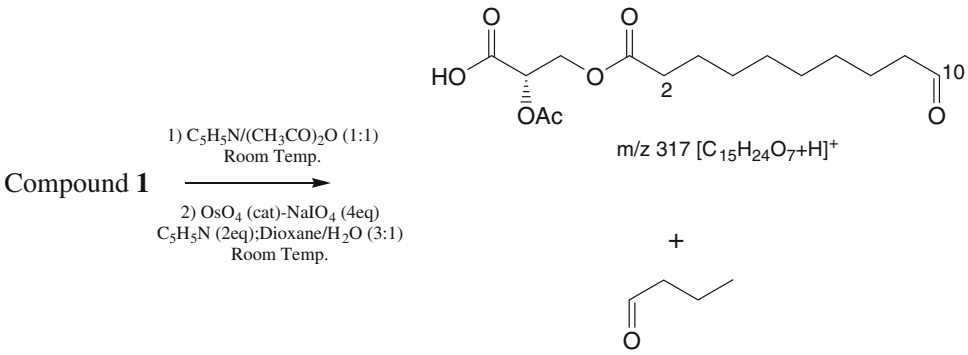
Human promyelocytic leukemia HL-60 (RCB-0041, $4 \times 10^4$ cells/mL) cells were treated with the compounds at various concentrations at 37 °C under a humidified, 5% $CO_2$ atmosphere for 4 days in RPMI 1640 medium supplemented with 10% fetal bovine serum, 50 units/mL of penicillin and 50 μg/mL of streptomycin and cytotoxicity was determined using a 96 plates with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide] assay. The percentages of viable cells were calculated as ratio of the $A_{570}$ values of treated and control cells (treated with 2% MeOH). The values are the average of two independent experiments.

## Results and Discussion

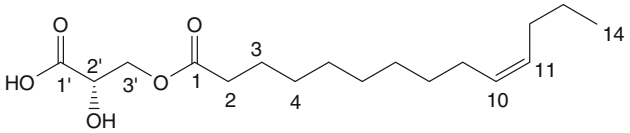
The crude methanol extract was subjected to repeated column chromatographic separations identifying a new fatty acid ester **1**, a new ceramide 2,3-*O*-*β*-D-glucopyranoside of *β*-sitosterol **3** [8], 3-*O*-acetyl of oleanolic acid **4** [9], 3-*O*-acetyl of ursolic acid **5** [9], ellagic acid **6**, 3-*O*-methylellagic acid **7**, and 3-*O*-methyl-3′-*O*-α-rhamnopyranosylellagic acid **8** [10].

Compound **1** was isolated as a yellow viscous oil from hexane/ethyl acetate (3:1, v/v) and had been detected at *m/z* 313.2018 in the negative mode HR-ESI-TOF-MS. This mass calculated at *m/z* 313.2020 corresponded to the molecular formula $[C_{17}H_{30}O_5–H]^-$ accounting for three double bond equivalents. The analysis suggested a fat which was supported by the $^{1}$H- and $^{13}$C-NMR spectra [11]. A triplet of 3H at $\delta_H/\delta_C$ 0.83/14.1 ($J = 6.3$ Hz), a broad singlet of 6H at $\delta_H/\delta_C$ 1.30/29.0–29.7, a multiplet of 2H at $\delta_H/\delta_C$ 1.60/24.8 which is $CH_2$ group *β* position of C=O function, a $CH_2$ group in α position C=O function appearing as a triplet at $\delta_H/\delta_C$ 2.30/34.2 and the carbonyl of ester function at $\delta_C$ 172.8 were displayed. Besides these resonances, four appeared downfield that consisted of one carbinol at $\delta_H/\delta_C$ 4.15 (*dd*, $J = 2.3$, 11.4 Hz), 4.25 (*dd*, $J = 6.4$, 11.4 Hz)/62.1, an oxymethine at $\delta_H/\delta_C$ 5.22 (br *t*, $J = 6.3$ Hz)/68.8 and two olefinic protons at $\delta_H/\delta_C$ 5.36 (*m*)/129.6 and at $\delta_H/\delta_C$ 5.37 (*m*)/130.1. Its configuration is *Z* since the chemical shift of allylic protons were revealed at $\delta_C$ 27.2 [12] and its position $\Delta^{10}$ was determined after acetylation and oxidative cleavage reactions. The product obtained was analysed by ESI-MS which produced a

**Scheme 1** Oxidative cleavage confirming the double bond position in the fatty acyl moiety of compound **1**

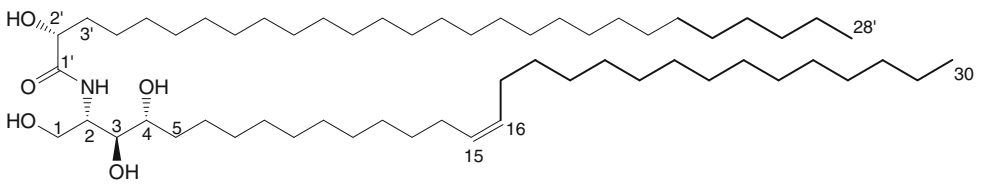



peak at *m/z* 317 corresponding to $[C_{15}H_{24}O_7+H]^+$ (Scheme 1). The HMBC and COSY spectra showed correlations from the *gem*-protons at $\delta_H$ 4.15 and 4.25 and the oxymethine signal at $\delta_H$ 5.22 to the carbonyl function at $\delta_C$ 173.2 and these information indicated that **1** is a glyceric acid derivative. The downfield *gem*-proton signals at $\delta_H$ 4.15 and 4.25 corresponded to esterification with a fatty acyl moiety and this was evident by the correlation further observed on the HMBC spectrum between this carbinol and another carbonyl function at $\delta_C$ 172.8. The absolute configuration at C-2′ was established from the positive optical rotation, which is a general feature of long-chain 1-*O*-alkyl-*sn*-glycerols [13].




From the foregoing data, compound **1** was identified as (2*S*)-3-[(10*Z*)-tetradec-10-enoyloxy]-2-hydroxypropanoic acid.

Compound **2** was obtained as a white solid from methylene chloride/methanol (39:1, v/v). Its positive mode HR-ESI-TOF-MS spectrum showed a pseudo molecular ion at *m/z* 928.8678 (calc. 928.8667) corresponding to the molecular formula $[C_{58}H_{115}O_5N+Na]^+$ accounting for two double bond equivalents. The IR spectrum exhibited an absorption band for a free OH group (3,335 $cm^{-1}$) [14], two strong absorption bands for olefinic function (1,649 $cm^{-1}$) and for secondary amide (3,201, 1,637 and 1,542 $cm^{-1}$). The NMR spectra of **2** showed signals close to those of phytoceramides [14]. A triplet of 6H at $\delta_H/\delta_C$ 0.87, $J = 8.0$ Hz/14.5 assigned to the two terminal $CH_3$ groups was observed. A broad singlet at $\delta_H/\delta_C$ 1.30/(29.7–30.2) corresponding to the sequence of $CH_2$ group was further observed as well as signals of **H**–N group at $\delta_H$ 8.62 (*d*, $J = 8.0$ Hz) and azamethine proton (**H**C–N) at $\delta_H/\delta_C$ 5.16 (*m*)/53.2. Furthermore, the chemical shifts of a double bond having *cis* configuration [10] were also observed at $\delta_H/\delta_C$ 5.54 (*d*, $J = 4.0$ Hz)/130.9 and 5.55 (*d*, $J = 4.0$ Hz)/131.0 since the allylic methylene groups appeared between 26.5–32.0 ppm instead of a resonance more than 32.0 ppm in the case of *trans* configuration. The foregoing data allowed us to suggest that, **2** is a ceramide flanked with an olefinic function. Some correlations ascertaining the amide function were observed between the proton **H**–N at $\delta_H$ 8.62 and the carbonyl at $\delta_C$ 175.5 as well as from the proton $H_{2'}$ at $\delta_H/\delta_C$ 4.65 (br *s*)/72.7 to the carbonyl function (175.5) and the successive $CH_2$ groups [$C_{3'}$($\delta_C$ 35.9), $C_{4'}$ ($\delta_C$ 24.8)]. The last observation confirmed that the fatty acid moiety is α-hydroxylated. The methanolysis (1N hydrochloride acid:methanol, at 70 °C for 18 h) of compound **2** gave the fatty acid methyl ester and the long chain base which were carefully characterized on the base of HRESI-MS. The peak at *m/z* 441.4315 corresponding to the molecular formula $[C_{28}H_{56}O_3+H]^+$ showed that the fatty acid methyl ester has one unsaturation attributed to the carbonyl function. The position of the olefin function was evident by oxidative cleavage after acetylation and the fatty aldehyde was further identified by the mass spectrometry analysis which showed the peak at *m/z* 249 $[C_{15}H_{30}O+Na]^+$ (Scheme 2). The relative configuration was defined with NOESY spectrum which presented interactions between the signals at $\delta_H$ 5.16 (H-2), 4.32 (H-4) and the one at $\delta_H$ 4.53 (H-1a) while H-1b at $\delta_H$ 4.47 interacted with H-3 ($\delta_H$ 4.37). 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 [15].



**Scheme 2** Oxidative cleavage confirming the double bond position in the amino alcohol side of compound **2**

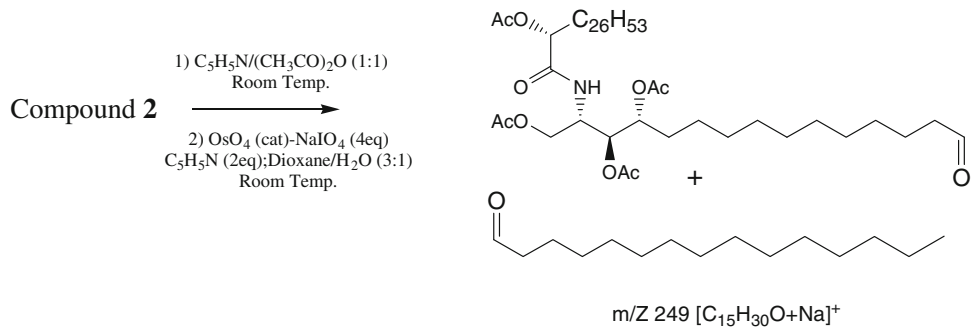



**Table 3** Cytotoxic activity against human promyelocytic leukaemia (HL60) cell line

| Compounds | $IC_{50}$ (μM) |
|---|---|
| Compound **1** | 58.7 |
| Compound **2** | >100 |
| 3-Acetyloleanolic acid **4** | 6.6 |
| 3-Acetoxyursolic acid **5** | 6.8 |
| 3-*O*-Methylellagic acid **7** | >100 |
| 3-*O*-Methylellagic acid-3′-*O*-α-rhamnopyranoside **8** | >100 |

Camptothecin = 0.02 μM was used as positive control

All the information above-mentioned led us to identify **2** as being (2*R*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*,15*Z*)-1,3,4-trihydroxy-15-triaconten-2-yl]octacosamide.

### Cytotoxic Assay

The cytotoxicity of compounds **1**, **2**, **4**, **5**, **7** and **8** were tested against HL-60 (human promyelocytic leukemia) under in vitro conditions. Compounds **1**, **4** and **5** showed a cytotoxic activity and the $IC_{50}$ are in Table 3. The pentacyclic triterpenoids (**4** and **5**) were more active than the fatty acyl ester (**1**). However, only compounds having less hydrophilic functions had cytotoxic activity. The lack of $\Delta^{4,5}$ double bond in the ceramide structure could explain its inactivity since the olefinic bond at this position is crucial for apoptosis inducing activity. [16].

## Conclusion

*A. laxiflora* has been investigated chemically and biologically and reports indicated that it contains several components with antimicrobial and antioxidant activities [2, 17] which are probably related to its ethnopharmacologic uses [17, 18]. The work presented provides additional characterization of this plant by isolating selected bark components metabolites and that may help explain its wide use in the Cameroonian traditional pharmacopeia. [19, 20] The chemical study of these species led to the identification of two new fatty acid derivatives together with two pentacyclic triterpenes, one steroid glucoside, ellagic acid and some ellagic acid derivatives. Beside the antimicrobial and antioxidant activities [17, 18] reported in this plant, some of isolated compounds have cytotoxic activity against a human promyelocytic leukaemia (HL60) cell line. The above-mentioned data indicate that *A. laxiflora* should be submitted to a large biological screening since the results could provide crucial information about the appropriate dosage. This could also be helpful for traditional healers working with phytomedicines.

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