J. Braz. Chem. Soc., Vol. 23, No. 3, 482-487, 2012.
Printed in Brazil - ©2012 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Ceramides and Cytotoxic Constituents from *Ficus glumosa* Del. (Moraceae)

Frédéric Nana,[a,b] Louis Pergaud Sandjo,*,[a] Félix Keumedjio,[a] Pantaléon Ambassa,[a] Rizwana Malik,[b] Victor Kuete,[c] Vincent Rincheval,[d] Muhammad Iqbal Choudhary[c] and Bonaventure Tchaleu Ngadjui*,[a,e]

[a]*Department of Organic Chemistry, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon*

[b]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan*

[c]*Department of Biochemistry, University of Dschang, P.O. Box 67, Dschang, Cameroon*

[d]*Laboratoire de G*énétique et *Biologie Cellulaire Bâtiment Fermat - Maison 4, Niveau 2, 45, Avenue des Etats-Unis, 78035 Versailles Cedex, University of Versailles St Quentin-en-Yvelines, France*

[e]*Department of Pharmaceutical Sciences and Traditional Pharmacopeia, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, P.O. Box 8664, Yaoundé, Cameroon*

A investigação química da casca do caule de *Ficus glumosa* (Moraceae) deu origem a duas novas ceramidas (2*R*,7*E*)-2-hidróxi-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihidróxihexadecano-2-il]hexacos-7-enamida e (2*R*)-*N*-{(2*S*,3*S*,4*R*,9Z)-1-*O*-[(β-D-glucopiranosil]-3,4-dihidróxiheptadec-9-eno-2-il}-2-hidróxipentacosanamida, em conjunto com vinte e um compostos conhecidos. As estruturas foram estabelecidas usando-se dados de RMN, espectrometria de massas, transformação química e por comparação com dados relatados. Vinte e um compostos foram posteriormente testados contra células de câncer de próstata PC-3 e seis deles revelaram efeito citotóxico. Dongnósido E foi o composto mais ativo, com IC_{50} de 0,75 μmol L^{-1} contra células de câncer PC-3, enquanto o medicamento de referência doxorrubicina, apresentou IC_{50} de 0.91 μmol L^{-1}. Este composto também demonstrou inibir o crescimento de células do câncer de fibrossarcoma HT1080 (IC_{50} 0,7 μmol L^{-1}).

Chemical investigation of the stem bark of *Ficus glumosa* (Moraceae) yielded two new ceramides (2*R*,7*E*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxyhexadecan-2-yl]hexacos-7-enamide and (2*R*)-*N*-{(2*S*,3*S*,4*R*,9Z)-1-*O*-[(β-D-glucopyranosyl]-3,4-dihydroxyheptadec-9-en-2-yl}-2-hydroxypentacosanamide together with twenty one known compounds. The structures were established using NMR data, mass spectrometry, chemical transformation and by comparison with the reported data. Twenty one compounds were further tested against the prostate cancer PC-3 cell line and six of them revealed cytotoxic effect. Dongnoside E was the most active compound with an IC_{50} 0.75 μmol L^{-1} against the cancer cells line PC-3 while the reference drug doxorubicin displayed 0.91 μmol L^{-1}. This compound also proved to inhibit the cell growth of the fibrosarcoma cancer HT1080 (IC_{50} 0.7 μmol L^{-1}).

Keywords: *Ficus glumosa*, ceramides, glumoamide, glumoside, dongnoside E, cytotoxicity

Introduction

Ficus glumosa is a small to medium-sized tree with 5 to 10 m tall; it may become a large tree reaching 20 m with a trunk of about 2 m of girth. *Ficus* species also produce white latex like commonly see in the Moraceae.[1] In Ivory Coast, the aqueous decoction of the leaves of *F. glumosa* is prescribed in traditional pharmacopeia to relieve chest pain and to treat lung diseases such as bronchitis, pneumonia and cough.[2] The aqueous decoction of its stem bark is rather used in Central African Republic as oral solution to cure gingivitis, caries, and tooth aches.[3] Additionally, the methanolic extract of the stem bark of *F. glumosa* has demonstrated *in vivo* antidiabetic and *in vitro* antioxidant

*e-mail: plsandjo@yahoo.fr, ngadjuibt@yahoo.fr

activities.[4] The positive reactions of the diluted crude extract to $FeCl_3$, Liebermann Burchard, and Molish reagent associated to the traditional uses prompted us to look for the secondary metabolites of this plant and to evaluate their cytotoxicity.

We herein report the structure elucidation of two new ceramides and the cytotoxicity of some compounds isolated from *F. glumosa*.

Experimental

General procedure

Melting points (mp): electro thermal IA 9000 apparatus, uncorrected; optical rotation: JESCO P-2000 polarimeter ; IR (KBr disc): JASCO A-302 spectrophotometer; HR-EI-MS and LR-EI-MS: JOEL MS and FLINNIGAN MAT SSQ-700 apparatus, respectively; 1 and 2D NMR: Brüker AM-300 and DRX-400 MHz with TMS as internal reference. Preparative TLC and analytical TLC plates silica G60 type MERCK. Columns chromatography: normal phase SiO_2 0.063-0.200 mm, reverse phase silica RP18.

Plant material

The stem bark of *F. glumosa* was collected in March 2009 in Makenene, central region of Cameroon and identified by the national herbarium where a specimen was deposited under the registration 28151/SRF/Cam (Makenene 1972).

Extraction and isolation

Air-dried powder (5 kg) of the stem bark of *F. glumosa* was macerated in a CH_2Cl_2-MeOH (1:1, 6 L) mixture for 48 h. The diluted extract was concentrated under reduced pressure to afford 405 g of a dark residue which was dissolved in water and extracted successively with hexane (Hex), CH_2Cl_2 (DCM), ethyl acetate (EA) and *n*-butanol to give fractions A (12 g), B (15.3 g), C (20 g), and D (89.4 g), respectively. Fraction A was subjected to silica gel column chromatography (CC) eluted in the gradient conditions with Hex, Hex-DCM and DCM-EA, affording compounds **3** (3.5 mg), **16** (3.7 mg), **4** (4.0 mg), **5** (4.0 mg), **6** (3.2 mg), and **7** (5.3 mg), in order of elution. Compounds **1** (7.5 mg) and **2** (6.8 mg) were precipitated with acetone in the sub-fractions eluted with DCM-EA 19:5 and 9:1, respectively. Fraction B was subjected to silica gel CC and eluted in the gradient conditions with Hex-DCM, DCM and DCM-MeOH to give compounds **8** (5.4 mg), **9** (5.1 mg), **10** (6.2 mg), and **12** (4.5 mg). SiO_2 CC of fraction C, eluted with a gradient from Hex-EA to EA, afforded **15** (6 mg), **11** (5.5 mg), **13** (6.3 mg), **14** (5.7 mg), **22** (4.6 mg) and **19** (10 mg). Fraction D was loaded on reverse phase silica RP18 CC and eluted with a gradient H_2O-MeOH, yielding **20** (3.1 mg), **21** (3.8 mg), **18** (5.4 mg), **23** (12 mg), and **17** (7 mg).

(2*R*,7*E*)-2-Hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxy-hexadecan-2-yl]hexacos-7-enamide (**1**)

White powder; mp 127.0-127.3 °C; R_f (2/3, DCM-MeOH 19:1); [α] +10.5 (*c* 0.03, THF); IR (KBr disc) ν_{max}/cm^{-1}: 3330, 3203, 2918, 2850, 1620, 1544, 1263; HR-ESI-MS *m/z* 682. 6319 $[M + H]^+$, 668.6182 $[M - CH_2 + H]^+$, 654.6033 $[M - C_2H_4 + H]^+$, 640.5844 $[M - C_3H_6 + H]^+$; ^1H and ^{13}C NMR see Table 1.

Acetylation and oxidative cleavage of **1**

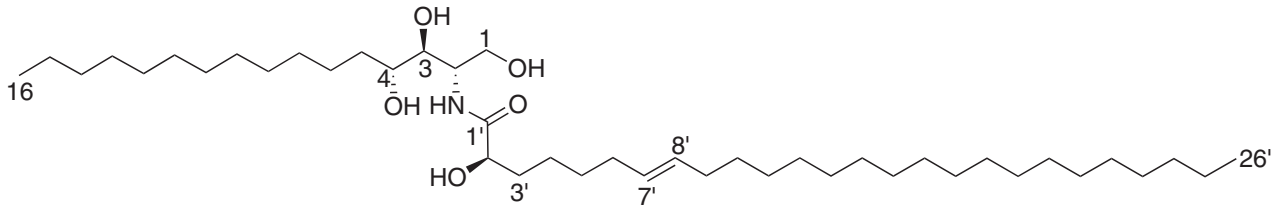
An amount of 3 mg of **1** was dissolved in 3 mL of pyridine and treated with 3 mL acetic anhydride under magnetic stirring for 6 h at room temperature. The acetylated compound was obtained by concentration of medium under vacuum. Comparative TLC revealed the formation of a non polar compound which was further dissolved in 4 mL of THF-H_2O (9:1) and treated with 5 equiv. of $KMnO_4$ and $NaIO_4$ each. The medium was stirred at room temperature overnight and poured onto water. The organic layer obtained by extraction with *n*-butanol was subjected to de-acetylation using MeONa in MeOH overnight at room temperature. The medium was concentrated under vacuum, treated with aqueous solution of 1 mol L^{-1} HCl and extracted with EA yielding compound **1a** which was characterized by ESI-MS which gave the pseudo-molecular ion at *m/z* 448.

(2*R*)-*N*-{(2*S*,3*S*,4*R*,9*Z*)-1-O-[(β-D-glucopyranosyl]-3,4-dihydroxyheptadec-9-en-2-yl}-2-hydroxypentacosanamide (**2**)

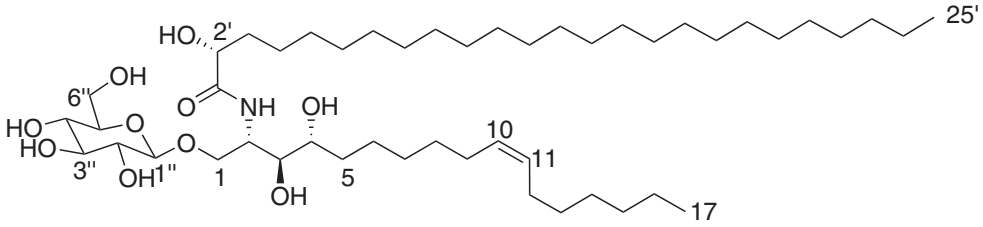
White powder; mp 199.6-199.8 °C; R_f (2.5/3, DCM-MeOH 9:1) [α] +17 (*c* 0.035, THF); IR (KBr disc) ν_{max}/cm^{-1}: 3402, 1620, 1544; HR-ESI-MS *m/z* 844.6897 $[M+H]^+$; ^1H and ^{13}C NMR see Table 1.

Methanolysis of **1** and **2**

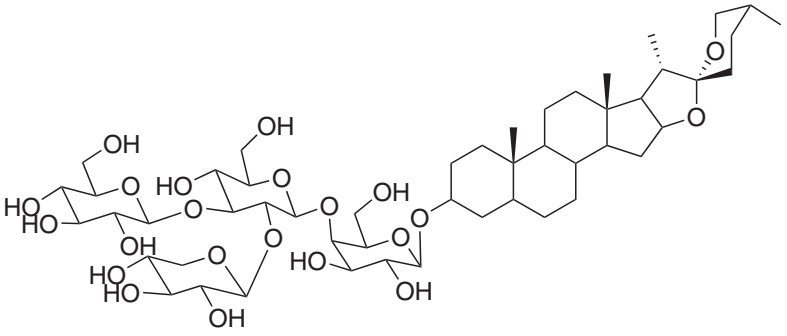
An amount of 1.5 mg of **1**, and 2 mg of **2** were separately refluxed in 2 mL of MeOH and 2 mL of aqueous solution of 1 mol L^{-1} HCl for 18 h under magnetic stirring at 70 °C. An aqueous solution of $NaHCO_3$ was used to neutralize the medium which was further extracted with DCM. The organic fractions were purified by CC on silica gel eluted



Compound **1**



Compound **2**



Compound **23**

with Hex-EA (4:1). The residual oil of both compounds were identified by EI-MS from peaks at *m/z* 424 $[C_{27}H_{52}O_3]^{+\cdot}$ and 426.3 $[C_{27}H_{54}O_3]^{+\cdot}$, corresponding to the fatty acid methyl esters (2*R*)-2-hydroxyhexacos-7-enoic acid methyl ester **1b** and (2*R*)-2-hydroxypentacos-7-enoic acid methyl ester **2a**.

Disulfenylation of **2**

To a solution of **2** (1 mg) dissolved in toluene (2 mL) were added 5 mg of $FeCl_3$ and 2 mL of dimethyl disulfide; the reaction mixture was stirred at room temperature and monitored by TLC. After 24 h, the medium was concentrated under reduced pressure and the residue was treated with a saturated aqueous solution of $NaHCO_3$ and extracted with EA. The colorless amorphous solid **2b** obtained was analyzed by EI-MS.

Cytotoxicity assays

The cytotoxicity of the compounds was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)[5] assay for the prostate cancer PC-3 cell line and flow cytometry for the fibrosarcoma cancer HT1080 cell line.[6] The compounds were dissolved with DMSO and incubated with the cells for 48 h. The drug references used were doxorubicin and etoposide for PC-3 and HT1080, respectively. Furthermore, the IC_{50} were calculated to evaluate the cytotoxicity of each natural product. The concentration of sample required to inhibit 50% of the cell proliferation (IC_{50}) was calculated from a calibration curve by a linear regression[7] using Microsoft Excel. Three independent experiments are carried out for each sample.

Results and Discussion

The crude organic extract was subjected to repeated columns chromatography yielding two new ceramides (**1**, **2**) together with twenty one known compounds namely lanosta-7,24-dien-3-one (**3**),[8] lanosta-8,24-dien-3-one (**4**),[8] β-amyrine (**5**),[9] lupeol (**6**),[9] 6-prenylpinocembrin (**7**),[10] bergapten (**8**),[11] chiricanine A (**9**),[12] genistein (**10**),[13] wighteone (**11**),[13] 6-prenylapigenin (**12**),[14] 3,4-dihydroxybenzoic acid (**13**),[15]

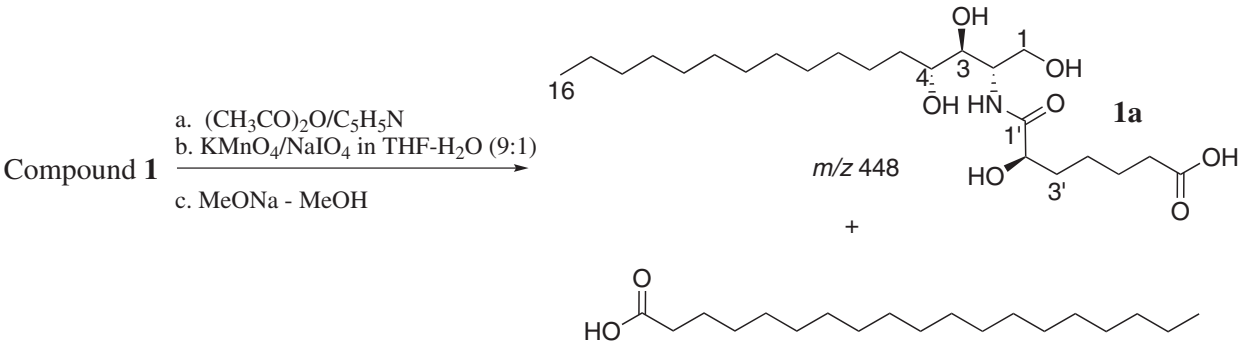
2,4,5-trihydroxybenzoic acid (**14**),[16] alpinumisoflavone (**15**),[17] 4'-*O*-methylalpinumisoflavone (**16**),[13] luteolin (**17**),[18] catechine (**18**),[19] β-sitosterol-3-*O*-(6'-*O*-heptadecanoyl)-β-D-glucopyranosyl (**19**),[20] polystachyol (**20**),[21] lyoniresinol-2a-*O*-β-D-xylopyranoside (**21**),[21] β-sitosterol-3-*O*-β-D-glucopyranoside (**22**),[22] and dongnoside E (**23**).[23]

Compound **1** was obtained as a white powder. Its positive mode HR-ESI-MS spectrum gave a *pseudo*-molecular ion at *m/z* 682.6319 (calc. 682.6344) corresponding to the molecular formula $[C_{42}H_{84}O_5N+H]^+$ accounting for 2 double bond equivalents. The IR spectrum exhibited characteristic absorption bands for free OH group (3330 cm^{-1}), olefinic function (1649 cm^{-1}) and secondary amide (3203, 1620 and 1544 cm^{-1}). The ^1H and ^{13}C NMR spectra (Table 1) of **1** displayed a triplet of 6H at δ_H/δ_C 0.82, (*t*, 6.3 Hz)/14.0 assigned to the two terminal CH_3 groups, a broad singlet at δ_H/δ_C 1.10-1.30/(29.3-29.6) corresponding to a sequence of CH_2 groups, an exchangeable nitrogen-attached proton appearing as doublet at δ_H 7.38 (*J* 8.7 Hz) and a proton geminated to the amide at δ_H/δ_C 4.03 (*m*)/51.8. Furthermore, an oxymethylene group was observed at δ_H/δ_C 3.66 (*dd*, 4.8, 11.4), and 3.75 (*dd*, 4.2, 11.7)/61.3, along with three oxymethine groups at δ_H/δ_C 3.44 (br *s*) 75.8, 3.47 (br *s*)/72.4, and 3.98 (*dd*, 3.6, 8.4)/72.0, and a carbonyl at δ_C 175.5 suggesting a phytoceramide structure.[24] The ceramide core was confirmed by long-range correlations exhibited on the HMBC spectrum between the proton at δ_H 7.38 and the carbonyl at δ_C 175.5. Moreover, the proton of oxymethine group at δ_H 3.47 (H-4) correlated with the carbons at δ_C 61.3 (C-1), 51.8 (C-2), and 75.8 (C-3) which matched with a trihydroxylamine-sphingosine in the phytoceramide skeleton. The sphingosine was attached to a α-hydroxyfatty acyl by an amide function since both protons at δ_H 4.03 (H-2) and 3.98 (H-2**'**) showed correlations with the carbonyl at δ_C 175.5.

The resonances of two overlapped olefin protons in *trans*-geometry[21] were further observed at δ_H/δ_C 5.34 (br *s*)/129.7 and 5.34 (br *s*)/130.8 and located in the fatty acid chain, as deduced from the mass spectrum of the methanolysis product of **1**, which displayed a molecular ion at *m/z* 424 $[C_{27}H_{52}O_3]^{+\cdot}$. The position $\Delta^{7'}$ of this double bond was assigned from its oxidative cleavage (**1a**) which formed a fragment with a *pseudo*-molecular ion at *m/z* 448 $[M+H]^+$ (Scheme 1). NOESY correlations and comparison of ^1H and ^{13}C NMR data with those of the literature allowed to deduce the relative and absolute configuration at C-2, C-3, C-4, and C-2' to be (*S*), (*S*), (*R*), and (*R*) respectively.[24] The foregoing data led to identify **1** as (2*R*,7*E*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxyhexadecan-2-yl]hexacos-7-enamide, trivially named glumoamide.

Compound **2** was isolated as a white solid. Its molecular formula $C_{48}H_{93}NO_{10}$ was determined on the basis of HR-ESI-MS which displayed a *pseudo*-molecular ion peak at *m/z* 844.6897 $[M+H]^+$, and the NMR data (Table 1) accounting for three double bond equivalents. The presence of OH and NH functions with absorption bands on the IR spectrum at 3402, 1620 and 1544 cm^{-1} was characteristic of cerebrosides.[25]

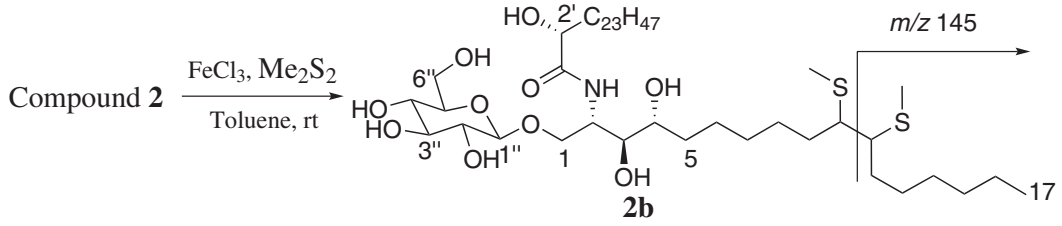
This compound gave a positive test to Molish reagent indicative of glycosides. The NMR spectra of **2** presented similar signals to those of **1**, with additional shifted resonances at δ_H/δ_C 4.22 (*d*, 7.6)/102.9, 3.21 (br *d*, 7.2)/73.2, 3.36 (*t*, 8.0)/76.1, 3.36 (*t*, 8.0)/69.5, 3.22 (br *s*)/76.0, 3.69 (*m*), and 3.78 (br *d*, 11.2)/61.1 characteristic of a β-D-glucopyranosyl moiety.[25] Methanolysis of **2** led to a saturated fatty acid methyl ester (**2a**) identified by the EI-MS molecular ion at *m/z* 426 ($[C_{27}H_{54}O_3]^{+\cdot}$). The position of the double bond observed on the NMR spectra at δ_H/δ_C 5.27 (2H, br *s*)/129.2, 130.1 was determined to be Δ^{10} from the ESI-MS of the sulfenylated derivative (**2b**), which exhibited a pseudo-molecular ion at *m/z* 145 (Scheme 2). The olefin *cis*-geometry was assigned on the basis of the carbon chemical shifts of allylic methylene groups at δ_C 27.0 and 27.1 which are around 33 ppm in the *trans* configuration.[25] Further correlation between the anomeric proton at δ_H 4.22 and the carbon at δ_C 68.6 allowed to connect the sugar moiety at C-1. The relative configuration was deduced by NOESY correlations and the absolute configuration at C-2,



Scheme 1. Oxidative cleavage of **1**.

Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data of **1** and **2** ($CDCl_3$+CD_3OD; δ in ppm, *J* in Hz)

Compound **1**			Compound **2**		
Position	δ_H	δ_C	Position	δ_H	δ_C
NH	7.38 (d, 8.7)	-	NH	7.53 (d, 8.8)	-
1	3.66 (dd, 4.8, 11.4, H_a) 3.75 (br d, 11.2, H_b)	61.3	1	3.73.(m) 4.01 (dd, 5.2, 11.4)	68.6
2	4.03 (m)	51.8	2	4.14 (br d, 3.6)	50.0
3	3.44 (br s)	75.8	3	3.52 (t, 10.4)	74.1
4	3.47 (br s)	72.4	4	3.46 (br d, 10.3)	72.0
5	1.13 (m, H_a), 1.51 (m, H_b)	33.1	5	1.10-1.30 (br s)	32.5
6	1.10-1.30 (br s)	25.7	6	1.10-1.30 (br s)	25.9
7-13, 10′-23′	1.10-1.30 (br s)	29.3-29.6	7-8, 13-14, 5′-22′	1.10-1.30 (br s)	29.2-29.6
14, 24′	1.10-1.30 (br s)	32.5	9, 12	1.98 (m)	27.0, 27.1
15, 25′	1.10-1.30 (br s)	22.6	10	5.27 (br s)	129.2
16, 26′ (CH_3)	0.82 (t, 6.3)	14.0	11	5.27 (br s)	130.1
1′ (C=O)	-	175.5	15, 23′	1.10-1.30 (br s)	31.8, 32.0
2′	3.98 (dd, 3.6, 8.4)	72.0	16, 24′	1.10-1.30 (br s)	22.5
3′	1.50 (m, H_a), 1.73 (m, H_b)	34.4	17, 25′ (CH_3)	0.80 (t, 6.4)	13.9
4′, 5′	1.35 (m)	25.2	1′ (C=O)	-	175.6
6′	1.61 (m, H_a), 1.94 (m, H_b)	32.7	2′	3.96 (dd, 3.6, 8.0)	72.0
7′	5.34 (br s)	129.7	3′	1.10-1.30 (br s)	34.2
8′	5.34 (br s)	130.8	4′	1.10-1.30 (br s)	25.2
9′	1.33 (m, H_a), 1.87 (m, H_b)	32.5	Glucosyl		
			1″	4.22 (d, 7.6)	102.9
			2″	3.21 (br d, 7.2)	73.2
			3″	3.36 (t, 8.0)	76.1
			4″	3.36 (t, 8.0)	69.5
			5″	3.22 (br s)	76.0
			6″	3.69 (m), 3.78 (br d, 11.2)	61.1



Scheme 2. Determination of double bond location by disulfenylation and MS analysis.

C-3, C-4, and C-2**'** was determined as being (*S*), (*S*), (*R*), and (*R*) according to the ^{1}H and ^{13}C NMR data compared with those of the literature.[24, 25] The above mentioned data led to characterize **2** as (2*R*)-*N*-{(2*S*,3*S*,4*R*,9Z)-1-*O*-[(β-D-glucopyranosyl]-3,4-dihydroxyheptadec-9-en-2-yl}-2-hydroxypentacosanamide, trivially named glumoside.

The structures of known compounds were identified by using their NMR data and by comparison of those reported in the literature.

Some of compounds were tested against the prostate cancer PC-3 cells (Table 2). Dongnoside E (**23**) showed a significant antiproliferative activity with an IC_{50} of 0.75 μmol L^{-1}, while the value obtained with the reference drug doxorubicin was 0.91 μmol L^{-1}. In contrast, lanosta-7,24-dien-3-one (**3**), β-amyrine (**5**), lupeol (**6**), 6-prenylapigenin (**12**) and luteolin (**17**) showed moderated activities, whereas other compounds were not active. Compound **23** was further tested against fibrosarcoma cancer HT1080 cells lines and presented an interesting antiproliferative cells growth with 0.7 μmol L^{-1} as IC_{50} after 48 h. This natural product was more active than the reference drug etoposide which showed an IC_{50} 1 μmol L^{-1}.

Table 2. Cytotoxicity of isolated compounds and doxorubicin against prostate cancer PC-3 cell line

Compound	IC_{50} / (μmol L^{-1})
1	> 30
2	> 30
3	28.53 ± 0.16
4	> 30
5	19.86 ± 0.22
6	21.58 ± 0.35
7	> 30
8	> 30
9	> 30
11	> 30
12	25.06 ± 0.01
13	> 30
14	> 30
15	> 30
16	> 30
17	28.10 ± 0.28
18	> 30
19	> 30
20	> 30
23	0.75 ± 0.06
Doxorubicin	0.91 ± 0.12

Conclusions

The higher concentration of flavonoids, benzoic acid derivatives, saponine, steroids and triterpenes highlights the use of this plant in traditional remedy against lung diseases, since several compounds belonging to the above-mentioned classes are present in plants having antitussive and expectorant activities.[26] Besides, some polyphenols are active against viral respiratory infections, bacteria and fungi.[27] The foregoing results clearly indicated that *Ficus glumosa* is a promising source of drug development, and **23** can be a potential antitumour lead.

Acknowledgments

The authors thank T. W. A. S. (The Academy of Sciences for the Developing World) for the fellowship providing to Frederic Nana.

Supplementary Information

Spectra (1D/2D NMR and MS) and NMR data of compounds (**1**-**23**) are available free of charge at http://jbcs.sbq.org.br as PDF file.

References

1. Berhaut, J.; *Ministère du Développement Rural et de l'Hydraulique*, vol. VI, Dakar, Senegal, 1979.
2. Koné, W. M.; Atindehou, K. K.; *S. Afr. J. Bot.* **2008**, *74*, 76.
3. Boulesteix, M.; Guinko, S.; *Quatrième Colloque du Conseil Africain de Malgache pour l'Enseignement Supérieur (C. A. M. E. S.)*, Libreville, Gabon, 1979.
4. Madubunyi, I. I.; Onoja, S. O.; Asuzu, I. U.; *Comp. Clin. Pathol.* **2010**, DOI 10.1007/s00580-010-1103-5.
5. Forguson, L. R.; *Mutat. Res.* **1994**, *307*, 395.
6. Bras, M.; Queenan, B.; Susin, S. A.; *Biochemistry (Moscow)* **2005**, *70*, 231.
7. Joshi, S. C.; Verma, A. R.; Mathela, C. S.; *Food Chem. Toxicol*. **2010**, *48,* 37.
8. Mendes, C. C.; Sandes, L. Q.; Cruz, F. G.; Roque, N. F.; *Chem. Biodivers.* **2009**, *6*, 1463.
9. Mahato, S. B.; Kundu, A. P.; *Phytochemistry* **1994**, *37*, 1517.
10. Caffaratti, M.; Ortega, M. G.; Scarafia, M. E.; Espinar, L. A.; Juliani, H. R.; *Phytochemistry* **1994**, *36*, 1083.
11. Stevenson, P. C.; Simmonds, M. S. J.; Yule, M. A.; Veitch, N. C.; Kite, G. C.; Irwin, D.; Legg, M.; *Phytochemistry* **2003**, *63*, 41.
12. Ioset, J.-R.; Marston, A.; Gupta, M. P.; Hostettmann, K.; *J. Nat. Prod.* **2001**, *64*, 710.
13. Lane, G. A.; Newman, R. H.; *Phytochemistry* **1987**, *26*, 295.
14. Abegaz, B. M.; Ngadjui, B. T.; Dongo, E., Tamboue, H.; *Phytochemistry* **1998**, *49*, 1147.
15. Zhu, C.-C.; Wang, K.-J.; Wang, Z.-Y.; Li, N.; *Bull. Korean Chem. Soc.* **2010**, *31*, 224.
16. Saito, S.; Kawabata, J.; *Helv. Chim. Acta* **2009**, *89*, 1395.
17. El-Masry, S.; Amer, M. E.; Abdel-Kader, M. S.; Zaatout, H. H.; *Phytochemistry* **2002**, *60*, 783.
18. Peng, W.; Han, T.; Wang, Y.; Xin, W.-B.; Zheng, C.-J.; Qin, L.-P.; *Chem. Nat. Compd.* **2011**, *46*, 959.
19. Nechepurenko, I. V.; Polovinka, M. P.; Komarova, N. I.; Korchagina, D. V.; Salakhutdinov, N. F.; Nechepurenko, S. B.; *Chem. Nat. Compd.* **2008**, *44*, 31.
20. Chen, L.; Wang, J.-J.; Zhang, G.-G.; Song, H.-T.; Qin, L.-P.; *Nat. Prod. Res.* **2009**, *23*, 1330.
21. Sadhu, S. K.; Choudhuri, P. P. M. S. K.; Ishibashi, T. O. M.; *J. Nat. Med*. **2006**, *60*, 258.
22. Bayoumi, S. A. L.; Rowan, M. G.; Beeching, J. R.;, Blagbrough, I. S.; *Phytochemistry* **2010**, *71*, 598.
23. Ding, Y.; Chen, Y.-Y.; Wang, D.-Z.; Yang, C.-R.; *Phytochemistry* **1989**, *28*, 2787.
24. Wang, R.-F.; Liu, R.-N.; Zhang, T.; Wu, T.; *Molecules* **2010**, *15*, 7467.
25. Liu, A.; Zou, Z.-M.; Xu, L.-Z.; Yang, S.-L.; *J. Asian Nat. Prod. Res*. **2005**, *7*, 861.
26. Gairola, S.; Gupta, V.; Bansal, P.; Singh, R.; Maithani, M.; *Int. J. Pharm. Sci. Rev. Res.* **2010**, *5*, 5.
27. Mendel, F.; *Mol. Nutr. Food Res*. **2007**, *51*, 116.

Submitted: September 7, 2011
Published online: January 24, 2012