Two new sphingolipids from the stem bark of Synsepalum msolo (Sapotaceae)

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ABSTRACT

Synsepalum msolo commonly known as Bang Bali in Bali-Nguemba, Cameroon is used in traditional medicine against various diseases. The leaves and stem bark extracts were subjected to silica gel and Sephadex LH20 column chromatography to yield pure compounds. The structures of the compounds were determined by detailed analysis of NMR and Mass spectroscopic data and compared to data reported in the literature. Amongst the isolates, were two new sphingolipids: synsepaloside B (1), synsepaloside C (2), and five known compounds: (+)-catechin (3), (−)-epicatechin (4), myricitrin (5), triacontanol (6), and aurantiamide acetate (7). Compounds 1–5 were screened for their antibacterial and anti-yeast activities on several microorganisms. All the tested compounds exhibited weak antibacterial (MIC ≥ 200 μg/mL) and anti-yeast (MIC > 200 μg/mL) activities as compared to standard: ciprofloxacin 0.468 μg/mL and fluconazole MIC = 0.05 μg/mL, respectively.

1. Introduction

Medicinal plants provide major source of molecules with varying medicinal properties due to presence of natural compounds. These plants are useful for curing human diseases and play a vital role in healing due to presence of phytochemical constituents [1]. Cameroon has a very rich flora of medicinal plants serving as phytomedicine, amongst which Synsepalum msolo belonging to the family Sapotaceae and locally known as "Bang Bali" in Bali of the North West region. The plant is found also in Bertoua and Nanga Eboke in the East region and in the East and tropical regions of Africa in Tanzania, Uganda, Kenya, Gabon, D.R. Congo, Ivory Coast and Ghana [2]. In Cameroon, the stem bark and roots are used to treat fever, headache, stomach ache and malaria [3]. The decoction of the dried stem bark of Synsepalum msolo alone or in combination with sugar cane is taken orally as a galactogogue in Tanzania [4,5]. In Taiwan the dried roots decoction is taken orally to treat diabetes mellitus [6]. Nonetheless, many plant species contain active ingredients such as alkaloids, phenols, tannins, cryogenic acids, glycosides, and terpenoids. These ingredients are used and found effective as sweeteners, anti-infections and anti-bacterials [7]. For instance, the stem bark, roots and leaves of Synsepalum msolo contain terpenoids (taraxeryl acetate, taraxerol, herranone, and betulinic acid), steroids (spinasterol and spinasterol-3-O-β-D glucopyranoside) and phenols (catechin, epicatechin and myricitrin) reported in numerous studies to demonstrate anticancer, anti HIV, antibacterial, antimalarial, analgesic, anti-inflammatory, antioxidant, anti-viral, and anti-allergic activities [8–19]. Other classes of compounds isolated from this plant includes, saponins (pachystelanosides A and B) and sphingolipid (pachysteloside A) [13a,b]. Sphingolipids from eukaryotes and higher plant species are shown to exhibit antilucerogenic activity [20], antifungal, antitumor, immunomodulating, antiviral, antitumor, immunostimulatory [21,22]; antiplasmodial, antileishmanial, cytotoxic [23], cell proliferation, apoptosis, fungal pathogenesis and antibacterial activities [24–27]. Therefore, sphingolipids could be considered as active ingredients with...
variant structures that might have potential therapeutic activities. In continuation of our investigation on Synsepalum msolo, we report herein the elucidation of the structures of two new sphenolipids and evaluation of their antibacterial and anti-yeast effects along with three known phenolic compounds on microorganisms.

2. Material and methods

2.1. Plant material

The leaves and stem bark of Synsepalum msolo were collected from Bali at "Maching" in Southern Cameroon, in April 2013. Identification was done by Dr Bathélémy Tchiengue, a botanist of the Cameroon National Herbarium, Yaoundé, where voucher specimen (N° 3849/SRFK) was deposited.

2.2. General experimental procedure

All reagents were purchased from Merck, Darmstadt, Germany and are analytical grade. TLC was performed on silica gel 60 F254, 0.1 mm thick (Merck) of size 20 × 20 cm. TLC spots were detected by fluorescence 254 nm or 366 nm and sprayed with 10% H2SO4 followed by heating at 70 °C. 1H, 13C DEPT, COSY, HMQC, HSQC, HMB spectra were recorded in deuterated solvent on either a Bruker Avance 600 MHz spectrometer or on Varian 500 MHz instrument. Chemical shifts are referenced to internal tetramethylsilane (δ = 0) and coupling constants J are reported in Hz. The Low-resolution electrospray-ionization mass spectrometry (ESI-MS) was carried out on a Micromass Quattro Micro mass spectrometer, HRTOFESI-MS and TOFESI-MS on microOTOF 10237, Bruker compas Data Analysis 4.0. HRESI-MS data were obtained with an LTQ Orbitrap Spectrometer (Thermo Fisher, Waltham, MA, USA) equipped with a HESI-II source. IR spectra was recorded on a Perkin Elmer spectrophotometer. Melting points were recorded using SMP3 melting point apparatus and is uncorrected.

2.3. Extraction and isolation

The powdered dry leaves (0.3 kg) and stem bark (3.3 kg) of S. msolo were extracted twice with CH2Cl2-Ch2OH (1:1 v/v) at ambient temperature for 2 days. The stem bark and leaves extract were concentrated under reduced pressure to yield dark brown viscous syrups (86 g) and black viscous syrup (100 g) respectively. 80 g of the stem bark extract was subjected to silica gel column and eluted with mixtures of n-hexane, ethyl acetate and methanol, in order of increasing polarities to give about 146 fractions. The similar fractions were combined using TLC analysis. Synsepaloside B 1 (6.4 mg), was directly obtained from fractions 121–124 (EtOAc-MeOH 20%) and synsepaloside C 2 (8.7 mg) from fractions 108–120. Aurantiamidine acetate 7 (12 mg) and (-)-epicatechin 4 (30 mg) were directly obtained from fractions 82–87 and 100–106 respectively. 100 g of the leaf extract was washed with n-hexane, ethyl acetate and methanol to yield 16 g. 18 g and 48 g respectively. The ethyl acetate fraction (18 g) was subjected over silica gel column and eluted with increasing polarity of n-hexane, ethyl acetate and methanol to give 124 fractions. The combination of similar fractions yielded triantocanol 6 (5 mg) and (+)-catechin 3 (6 mg). Fractions 121–124 from EtOAc-MeOH (80:20) was purified over Sephadex LH2 (100 MeOH) repeatedly to afford myricitrin 5 (19.2 mg). The compounds were identified using spectroscopic methods (1D and 2D NMR, MS).

2.3.1. Synsepaloside B (1)

2.3.1.1. Methanalysis of synsepaloside B (1). Synsepaloside B (3.5 mg) was refluxed with 0.9 mol L−1 HCl in 82% aqueous MeOH (5 mL) for 20 h at a temperature of 60 °C. The resulting solution was extracted three times with n-hexane. The n-hexane solution was washed with water (5 mL) and dried over anhydrous Na2SO4 then concentrated to yield the fatty acid methyl ester (1.5 mg), identified as methyl hexadecanoate by analysis of GC-MS. Methyl hexadecanoate also known as hexadecanoic acid, 2-hydroxy-methylster was obtained as colorless oil, GC-MS: GC-MS, tR 17.416 min, m/z 286 (Calc. for C18H33O2, 286.25), EI-MS: m/z: 227 [M – C6H12O]+ (27), 71 [C11H13]+ (40), 57 [C18H3]+ (90). See Supplemental Table S1 and supplementary data file.

2.3.2. Synsepaloside C (2)

White amorphous solid, mp 196.9 °C, [α]D20 +13.6 (c 0.1, MeOH); 1H NMR (DMSO-d6, 600 MHz) and 13C NMR (DMSO-d6, 150 MHz). HTOFESI-MS (positive-ion mode), m/z 866.6593 [M+Na]+, TOFESI-MS (positive-ion mode), m/z 866.7 [M+Na]+, TOFESI-MS (negative-ion mode) m/z 842.7 [M – H]1− (Calcd. for C42H65O38, 843.6799), 1H NMR (6, 600 MHz): 7.42 (d, 9.0, 2-Н), 5.38 (dd, 3.0, 13.2, H2), 5.30 (m, 4-H), 4.90 (d, 3.9, 2′-OH), 4.71 (s, 3-ОH), 4.47 (t, 5.7, 6′-OH), 4.28 (d, 5.7, 2′-OH), 4.14 (d, 7.8, 1-H), 4.09 (m, 2-H), 3.87 (m, 2-H), 3.85 (m, 5-H), 3.82 (m, 1-Ha), 3.66 (m, 1-Hb), 3.66 (dd, 6.0, 11.4, 6′-Hb), 3.45 (dd, 5.2, 11.4, 6′-Ha), 3.38 (dd, 8.1, 8.7, 2′-Hb), 3.19 (m, 5-H), 3.16 (m, 3-H), 4.05 (m, 4-H), 2.94 (dt, 3.6, 8.4, 3-H), 1.94 (m, 5-H), 1.76 (m, 6-H), 1.24 (brs, 6-H to H-15 and H-6′ to H-23′), 1.23 (s, H-16, H-24′) and 0.85 (t, 6, 9, H-17, H-25′). 13C NMR (6, 150 MHz): 173.7 (C-1′), 130.2 (C-3), 129.8 (C-5′), 103.4 (C-1′), 74.0 (C-2′), 73.4 (C-3), 73.4 (C-3′), 70.8 (C-4 and C-2′), 68.8 (C-1), 61.0 (C-6′), 49.8 (C-12), 31.8 (C-5′), 29.2 (C-6 to C-15; C-6′ to C-23′), 22.0 (C-16, C-24′) and 13.8 (C-17, C-25′), see Supplemental Table S2.

2.4. Preparation of stock solution

The stock solution of each compound, ciprofloxacin, and florfenicol were prepared in pure DMSO for a final concentration of 1 mg/mL. The stock solutions were filtered with a 0.2 μm sterilized syringe and stored at –20 °C until use.

2.5. Bacterial and yeast strains

Six bacterial strains: Staphylococcus aureus ATCC 43300, Pseudomonas aeruginosa NR48892, Klebsiella pneumoniae ATCC 700603, Escherichia coli ATCC 25922, Shigella flexneri NR518, Streptococcus pneumoniae HM145 and 3 yeast strains: Candida albicans NR 29445, Candida albicans NR 29451 and Candida albicans ATCC 29444 were assayed. Isolates were obtained from Yaounde Centre Hospital, Cameroon and the reference strains from BEI resources and the American Type Culture Collection Bacteria and yeast strains were cultivated.
in petri dishes containing Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) respectively, followed by an incubation period of 24 h at 37°C. Each microorganism was sub-cultured in a new Agar plate and incubated prior to each experiment.

2.6. Antimicrobial assays

The antimicrobial activity of each compound was assessed as recommended by the Clinical and Laboratory Standards Institute with minor modifications by the use of resazurin dye (CLSI, 2012) [28]. Briefly, forty μL of the compounds with concentration of 1 mg/mL was introduced in the microtiter plate containing 60 μL of culture medium for a final volume of 100 μL. 2-fold serial dilutions of samples were performed in 96-well microplates. The bacterial and yeast inoculum were prepared with sterile saline solution (NaCl 0.9%) using freshly cultured microorganisms of 24 h. Each suspension was adjusted to 0.5 McFarland standard then diluted in culture medium to yield a final concentration of 10^8 CFU/mL for bacteria and 1.5 × 10^4 CFU/mL for yeast. Fifty μL of each microbial suspension were added in each well and plates were incubated for 24 h at 37°C. Thereafter, 10 μL of resazurin (0.15 mg/mL in PBS) was added followed by additional 4 h incubation and MIC was recorded visually as the lowest concentration of the compound required to inhibit 50% growth of pathogens. A negative control experiment was conducted using 0.1% DMSO.

3. Statistical analysis

All data were performed in triplicate and resulting MIC values expressed as mean ± standard deviation. The data were processed using the software SPSS 17.0 for Windows.

4. Results and discussion

Synsepaloside B (1) was isolated as white amorphous powder. Its molecular formula C_{35}H_{39}NO_{10} was assigned on the basis of the HRESI-MS (positive-ion mode) at m/z 880.68431 [M+H]^+, and 1D and 2D NMR experiments. The IR spectrum showed absorption bands of hydroxyl (3341 cm\(^{-1}\)) and amide (1643 cm\(^{-1}\)) groups. The \(^1\)H and \(^13\)C NMR spectrum of 1 presented the characteristic signals of a \(\beta\)-D-glucopyranoside moiety (δ_H 4.11, 1H, d, J = 7.8 Hz, anomeric proton, δ_C 103.3 (CH), 70.2 (CH), 76.5 (CH), 76.6 (CH), 73.6 (CH), and 61.3 (CH)), an amide linkage 175.6 (C-1'), four olefinic methines δ_H 5.35 (1H, m, H-9), 5.36 (1H, m, H-8), 5.37 (1H, m, H-12), 5.39 (2H, m, H-13, H-23), 5.43 (2H, m, H-17, H-22), 5.44 (1H, m, H-16) with carbons at δ_C 129.4 (C-13), 129.5 (C-9, C-23), 130.2 (C-8, C-16, C-22), and 130.0 (C-12, C-17), an amido methine (δ_H 4.28 (d, 4.8, 7.8, H-2), δ_C 50.2 (C-2)), an oxygenated methylene (δ_H 4.07 and δ_C 3.82; δ_C 68.5), three oxygenated methines (δ_H 3.62, 3.54, 4.05; and δ_C 74.1, 71.5, and 71.6), two terminal methyls (δ_H 0.93, 6H, t, J = 6.6 Hz) and two long aliphatic chains appearing as broad singlets (δ_H 1.28). Comparing these spectra data with literature studies indicates that compound 1 is a glycosphingolipid [29-31], (Fig. 1).

The methanolation of 1, afforded fatty acid methylster, identified as hexadecanoic acid, 2-hydroxy- methylster and its molecular formula was established to be C_{17}H_{35}O_{3} at m/z 286, in the GC-MS spectrum. See supplementary material.

In the HRESI-MS spectrum, the long-chain fatty acid (LCFA) [30] was determined to be 2-hydroxy-hexadecanoic acid due to the fragment ion at m/z 255 [M – C_{16}H_{33}O_{2}] + (Fig. 2). The position of the 2-hydroxy group in the LCF was confirmed by the \(\alpha\)-cleavage of alcohol at m/z 227 [C_{15}H_{25}O] +, in the HRESI-MS and GC-MS spectra (Fig. 2).

The long-chain base (LCB) [32] of compound 1 was derived as 2-amino-8,12,16,22-tetraen-1,3,4-triol by analysis of the \(^1\)H-\(^1\)H COSY, HMOC and HRESI-MS, which showed a peak at m/z 463 [M-163-255+2H] +. The ion peaks appearing at m/z 536 [M – C_{29}H_{31}] + and 476 [M-C_{27}H_{27}O_{2}] + indicated the presence of oxymethylene carbons in LCB, assigned to positions C-3 and C-4 respectively, which were supported by the COSY correlations of H-1 through H-4 (Fig. 3). This was further supported by the ion peaks at m/z 532 [M-18-C_{25}H_{31}] +, 476 [M-C_{27}H_{27}O_{2}] + and 337 [M-2H-C_{25}-2H_{2}] + resulting from Mc Lafferty rearrangement and the \(^1\)H-\(^1\)H COSY correlations between the oxymethylene δ_H 3.62 (dd, 6.0, 12.0 Hz, H-3) and δ_H 3.54 (dt, 6.6, 12.6 Hz, H-4), respectively (Figs. 2 and 3).

The positions of the olefinic double bonds at C-8/C-9 and C-12/C13 were affirmed by the ion peaks at m/z 301 [C_{28}H_{35}] +, 275 [C_{30}H_{39}] +, 221 [C_{18}H_{29}] +, and 207 [C_{18}H_{27}] + in the positive ion mode HRESI-MS spectrum due to \(\alpha/\beta\)-cleavages of the double bonds. In addition, the Mc Lafferty rearrangements resulting to the ion peaks at m/z 532 [M-18-C_{24}H_{31}] +, 547 [M-18-C_{26}H_{29}] + and 451 [M-18-C_{18}H_{31}] + strongly confirmed the position of the double bonds at C8/C-9 and C12/C-13, respectively (Fig. 2). The ion peak appearing at m/z 207 [C_{19}H_{35}] + and 153 [C_{11}H_{21}] + corroborates a third double bond at C16/C-17 on the LCB. The peaks of the cleavages of an allylic bond at m/z 768 [M – C_{18}H_{31}] + and 794 [M – C_{19}H_{33}] + affirmed the fourth double bond position at C22/C-23, on the LCB.

The E geometry at positions 12, 16 and 22 was supported by the chemical shift of the carbons next to the double bond at (δ_C 34.0 (C-11), δ_C 32.3 (C-14), δ_C 32.3 (C-15), δ_C 32.3 (C-18), δ_C 32.3 (C-21), δ_C 34.0 (C-24)), while the Z geometry at C-8/C-9 was affirmed by the chemical
shifts of the carbons next to the olefinic double bonds at $\delta C_{26.6}$ (C-7) and $\delta C_{26.6}$ (C-10). The chemical shifts for the adjacent carbons to a cis (Z) double bond appear in the range of $\delta C_{26.6}$–28 [33], while those of a trans (E) double bond appear in the range of $\delta C_{32.6}$–34 [30]. Thus, the $\Delta^8$, was determined to be cis (Z) due to the down field chemical shift values in the range of 26–28 [23], and $\Delta^{12}$, $\Delta^{16}$ and $\Delta^{22}$ double bonds were
determined to be trans (E), due to their upfield chemical shift values in the range of 32–34 [29]. Therefore, the structure of compound 1 was determined as 1-O-β-D-glucopyranosyl-(8Z,12E,16E,22E)-2-{(2′E)-2′-hydroxyhexadecanoylaminol}-nonacos-8,12,16,22-tetraen-1,3,4-triol, an unreported sphingolipid.

Synsepaloside C (2) was isolated as white amorphous solid. Its molecular formula C_{36}H_{53}NO_{10} was deduced from its HRTOFESI-MS showing a pseudomolecular ion peak [M+Na]^+ at m/z 866.6593. The 1H and 13C NMR spectrum of 2 showed similar features to that of synsepaloside B. Compound 2 displayed resonances of a β-D-glucopyranoside moiety at δ_{H} 4.15, 1H, d, J = 7.8 Hz, anomic proton, δ_{C} 103.4 (CH), 70.0 (CH), 76.5 (CH), 76.9 (CH), 73.4 (CH), and 61.0 (CH_{2}), an amide linkage at δ_{H} 7.42, 1H, d, J = 9.0 Hz, δ_{C} 173.7 (C-1′), an olefinic methine at δ_{H} 5.38 (dd, J = 3.0, 13.2 Hz, H-3′) and δ_{C} 5.30 (m, H-4′), assigned to carbon at δ_{C} 130.2 (C-3′) and δ_{C} 129.8 (C-4′), an oxygenated methylene (δ_{H} 3.66 and δ_{C} 3.82; δ_{C} 68.8), an amido methine (δ_{H} 4.09, m, H-2′; δ_{C} 49.8), three oxygenated methines (δ_{H} 2.94, 3.85, 3.87; and δ_{C} 73.4, 70.8, and 70.8), two terminal methyls (δ_{H} 0.87, 6H, t, J = 6.9 Hz) and two long aliphatic chains appearing as broad singlets (δ_{C} 1.24). The comparison of these spectra data with literature values suggest that compound 2 is a glycosphingolipid [29–31] (Fig. 1). Compound 2 differs from 1 in that, 2 have a very long-chain fatty acid (VLCPA) [32]. The VLCPA was determined to be 2′-hydroxyoctacos-3-en-1′-ol due to the characteristic fragment ion at m/z 331.3 (Fig. 4).

The molecular ion peak at m/z 295 was attributed to the α-cleavage that supported the location of the olefinic double bond at H-3′/H′4 in the VLCPA (Fig. 4). Furthermore, the fragment ion at m/z 335.1 resulted from the Mc Lafferty fragmentation processes strongly supports the position of the double bond and hydroxy function at H-2′ (Fig. 4). The signal of the olefinic double bond was observed at proton δ_{H} 5.38 (dd, J = 3.0, 13.2 Hz, H-3′) and δ_{C} 5.30 (m, H-4′), attributed to carbon at δ_{C} 130.2 (C-3′) and δ_{C} 129.8 (C-4′) respectively, in the VLCPA. The Δ^{2} double bond of 2 was determined to be trans (E), by comparing the large vicinal coupling constant of the proton at δ_{H} 5.38 (1H, dd, J = 13.2 Hz, H-3′) and upfield chemical shift value of C-5′ in the range of 31.8, with literature data of flavuside B [27]. The LCB of 2 was determined to be 2-aminoheptadecan-1,3,4-triol due to fragment ion at m/z 301.2 (Fig. 4). The ion peaks at m/z 183 and 169 were attributed to the β-cleavages that confirmed the location of hydroxyl functions on the LCB. This was further supported by the COSY correlations for H-1 through H-5 (Fig. 3).

The connections through C1–O–C1″ and C2–NH–C1′ in compound 2 were strengthened using HMBC connectivity from H-2 to C-1 and C-3; from 2-NH to C-1′; from H-3 to C-4; from H-2′ to C-1′; from H-3′ to C-1′ and C-2′; from H-4′ to C-1′, C-5′ and C-6′; from 2′-OH to C-2′; from 3-OH to C-3 and C-4; from H-1 to C-1′, respectively. Therefore, the structure of 2 was established as 1-O-β-D-glucopyranosyl-2-{(2′E,3′E)-2′-hydroxyoctacos-3-enoylamino-heptadecan-1,3,4-triol, reported for the first time (Fig. 1).

Compounds 1–5, were screened for their antibacterial and anti-yeast potency against 6 bacterial strains including Staphylococcus aureus ATCC 43300, Pseudomonas aeruginosa NR48582, Klebsiella pneumoniae ATCC 700603, Escherichia coli ATCC 25922, Shigella flexneri NR518, Streptococcus pneumoniae HM145 and 3 yeast strains Candida albicans NR 29445 Candida albicans NR 29451 and Candida albicans ATCC 29444. See Supplementary Table S3.

All the tested phytoconstituents exhibited weak activity on the bacteria and yeast strains with minimum inhibitory concentration (MIC) ≥ 200 μg/mL. However, as compared to the reference drugs ciprofloxacin and fluconazole, the tested products were much less active. These data suggest that the tested sphingolipids and phenolics of Synsepalum msolo were almost inactive in this experiment against the evaluated

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**Fig. 4.** TOFESI-MS mass fragmentation pattern of synsepaloside C (2).
microorganisms.

5. Conclusion

Two new sphingolipids [synepsaloside B (1) and synepsaloside C (2)], three phenolics [catechin (3), epicatechin (4) and myricitrin (5)], a fatty alcohol [triacontanol] and a peptide derivative [aurantiamide ac] were isolated from the leaves and stem bark of a folk medicine Synepalos moslo. Compounds 3–7 have known biological activities, and have not been reported previously as constituents of Synepalos moslo. Analysis of the new sphingolipids and known compounds were done using NMR and mass spectra data and by comparison to those publish in the literature. The screening of the sphingolipids, catechin, epicatechin and myricitrin with several microorganisms demonstrated weak antibacterial and antifungal activity. However, based on the weak activity observed, we recommend that other classes of compounds isolated from this plant in the future, should be subjected for investigations to identify the components with potent antibacterial and antifungal activity.

CRediT authorship contribution statement

Ache Roland Ndifor: Conceptualization, methodology, data curation, investigation, writing original draft. Njanga Ngaitad Stanislaus: Data curation, writing original draft. Chi Godloves Fr: Data curation, writing original draft. Ferdinand Talontsi: Data curation, writing original draft. Turibio Kulae Tabopda: Co-supervision, project administration, data curation, writing original draft. Elisabeth Zeuko Menkem: Data curation, writing of original draft. Ngadjui Bonaventure Tchaleu: Supervision, project administration, funding acquisition. Yeboah Samuel Owusu: Project administration, funding acquisition, Investigation.

Declaration of competing interest

None.

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