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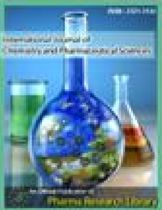

Research Article Open Access

Characterization of constituents from the Roots of *Pachystela msolo* Engler (Sapotaceae)

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A B S T R A C T
The investigation of the cyclohexane fraction of the roots of *Pachystela msolo* led to the isolation of pachysteloside A [1], a new cerebroside, along with five known compounds identified as taraxeryl acetate [2], -taraxerol [3], spinasterol [4], betulinic acid [5], and spinasterol-3-O- -D-glucopyranoside [6]. The structure of the cerebroside was determined by comprehensive analysis of 1D and 2D NMR, EIMS, ESIMS, and HRESI-TOF spectra data.
Keywords: Sapotaceae, *Pachystela msolo,* cerebroside

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1. Introduction

Pachystela msolo (Engler) also known as *Synsepalum msolo* (Engler) T.D. Penn (Sapotaceae) is a medium or tall evergreen tree with many branches which grows up to 20-50 m high. The plant is found widely distributed in the Bali-Nguemba forest in the Northwest region of Cameroon, and in the Northeast region in Bertoua and Nanga-Eboko. It is found as well in the flora of East and tropical regions of Africa [1]. *Pachystela msolo is* known as "Msamvia'' in Swahili language in Tanzania. The ripe fruits can be soaked in water, squeezed then filtered; sugar is added to the juice and served as beverage [2]. The decoction of the dried stem bark of *P. msolo* alone or in combination with sugarcane is taken orally as a galactogogue in Tanzania [3, 4]. In Taiwan a decoction of the dried roots is taken orally to treat diabetes mellitus [4]. However, the medicinal constituents of the roots were not reported until now.

In our research searching for the biologically active constituents from the roots of *P. msolo* Engler, we have isolated one new cerebroside for the first time from this plant along with five known compounds. Cerebrosides are important class of compounds. It has been reported that cerebrosides exhibit biological activities such as antifungal, antitumor, immunomodulating, and nitric oxide release inhibiting activities [5]. Qi reported that cerebrosides from edible Chinese mushroom shown to induce neuronal differentiation in rat PC12 cells [6]. Some exhibit antibacterial and antifungal activities against different strain of microorganisms [7]. Here in we report the structure determination of pachysteloside A (**1**), and some identified components from the roots of *P. msolo*.

2. Materials and Methods

General experimental procedures

All reagents were analytical grade purchased from Merck, Darmstadt, Germany. TLC was performed on silica gel 60 F_{254}, 0.1 mm thick (Merck) of size 20 x 20 cm. The TLC plates were developed in various solvent systems at room temperature. All spots were detected by fluorescence 254 nm or 366 nm and sprayed with 10% H_2SO_4 followed by heating at 70 ^oC. ^1H, ^{13}C, DEPT, 2D ^1H-^1H COSY, HMQC, HMBC, and NOESY spectra were recorded with Bruker Avance 300 and 600 MHz spectrometer and on Varian Inova 500 spectrophotometer.

Chemical shifts are referenced to internal TMS (δ = 0) and coupling constants *J* are reported in Hz. Column chromatography was performed using silica gel 60 (Merck, 0.040–0.063 mm). Low-resolution electrospray-ionization mass spectrometry (ESI-MS) was carried out on a Micromass Quattro Micro mass spectrometer. HRESITOF-MS were recorded on microTOF-Q 98 instrument (Bruker-Daltonik, France) and Q-TOF ULTIMA-III (Waters) quadrupole TOF mass spectrometer. Nitrogen was used as sheath gas (50 arbitrary units) and helium served as the collision gas. The calibration graphs of all compounds were linear (R^2> 0.99) from 5 ng/ml up to a concentration of 4000 ng/ml. The positive mode EI was performed using VG Autospec X (Miromass co), at Electron impact 70 eV. Optical rotations were measured on a Perkin-Elmer polarimeter, model 241. IR spectra were recorded in KBr disks on a Perkin-Elmer spectrophotometer. Melting point was taken using SMP3 melting point apparatus and is uncorrected.

Plant material

The whole plant of *Pachystela msolo* (sapotaceae) was collected from the Bali-Nguemba forest North West region of Cameroon in March 2012 and identified by Dr. Bathélémy Tchiengue, botanist at the National Herberium Cameroon. A voucher specimen (N° 3849/SRFK) has been deposited at the National Herbarium, Yaounde, Cameroon.

Extraction and Isolation

Dried and powdered roots (3.80 kg) of *P. msolo* were extracted with a 1:1 mixture of CH_2Cl_2-MeOH at r.t. for 48 h and filtered. The filtrate was concentrated under vacuum to give 102 g of crude extract. The crude extract was repartitioned in cyclohexane, ethylacetate, and methanol respectively to yield cyclohexane fraction (20 g), ethyl acetate fraction (24 g), and methanol fraction (58 g). Part of cyclo hexane fraction (5 g) was subjected to column chromatography (silica gel) using increasing polarity of n-hexane, n-hexane-EtOAc, EtOAc, and EtOAc-MeOH to yield 18 sub-fractions (A to R).

Column chromatography of sub-fraction B [n-hexane-EtOAc (90:10)] yielded taraxeryl acetate (25.5 mg), while sub-fraction D eluted with n-hexane-EtOAc (85:15) afforded -taraxerol (5.2 mg). The sub-fraction G [n-hexane-EtOAc (80:20)] afforded -spinasterol (18.7 mg) and sub-fraction I [n-hexane-EtOAc (70:30)] afforded betulinic acid (30 mg). Sub-fraction Q eluted with a mixture of MeOH-EtOAc (5:95) yielded spinasterol-3-O- -D-glucopyranoside (40 mg). The sub-fraction R eluted with a mixture of MeOH-EtOAc (20:80) yielded pachysteloside A as a white powder (9.2 mg).

3. Results and Discussion

Pachysteloside A (**1**) (Fig. 1), was obtained as white solid (9.2 mg) from MeOH-EtOAc (20:80) and have been assigned the molecular formula $C_{48}H_{93}NO_{10}$ on the basis of HRESI-TOF at *m/z* = 844.6878[M + H]$^+$, and 1D and 2D NMR experiments. The IR spectrum showed absorptions for hydroxyl groups at 3380 cm^{-1}. The typical IR absorptions at 1730–1643 cm^{-1} suggested an amide linkage, which was confirmed by azomethine carbon signal at $_C$ 50.2 and a carbonyl signal at $_C$ 175.7 in the ^{13}C NMR spectrum. The ^1H NMR spectrum in CD_3OD exhibited signals of a broad singlet at $_H$ 1.33 (methylene protons), triplet of 6H at $_H$ 0.94 (two terminal methyls), assigned to the two terminal methyl groups, an anomeric H-atom signal at $_H$ 4.30 (d, *J* = 7.8 Hz), and four oxymethine signals at $_H$ 3.19 (t, J = 8.4 Hz), 3.29 (*br.m*), 3.37 (*br.m*), and the oxymethylene protons appearing as doublets at $_H$ 3.89 (d, *J* = 12 Hz) and 3.68 (d, *J* = 12 Hz), suggesting a cerebroside structure [8].

Figure 1: Structure of Pachysteloside A (1)

The ^{1}H NMR spectrum showed two olefinic proton signals at $_{H}$ 5.43 (*m*, 12-H) and 5.37 (*m*, 13-H), assignable to the disubstituted double bond. The *trans* (*E*) configuration of the double bond was evidenced by the chemical shifts of the carbons next to the double bond at $_{C}$ 32.3 (C-11) and 32.3 (C-14) in compound **1** [9, 10]. The chemical shift for the carbon signals of *cis* (*Z*) double bonds are usually in the range of 27 - 28 ppm [11, 12]. In the ^{13}C NMR spectrum, the carbon resonances appearing at $_{C}$ 61.2 (CH_2), 70.4 (CH), 73.4 (CH), 76.5 (CH), 76.6 (CH), and 103.3 (CH), revealed the presence of a -D-glucopyranoside [9].

The anomeric proton at $_{H}$ 4.30 (d, *J* = 7.8 Hz) correlated to the carbon signal at $_{C}$ 103.3 in the HMQC spectrum, further confirming the -configuration of the glucoside unit and the -orientation of the proton in glucose moiety. The conclusion was also confirmed by HRESI-TOF spectrum which showed prominent peak at *m/z* 316 [M-CH_3 $(CH_2)_7$-CH_3 $(CH_2)_{17}$-162]$^+$, due to the loss of the glucosyl moiety from the fragment ion at *m/z* 478 [$CH_2CH{=}CH(CH_2)_7$ $(CHOH)_2CH(NHCOCHOH)CH_2OC_6H_{11}O$]$^+$ (Figure2). In addition to the methine signals of the glucose unit, the ^{1}HNMR spectrum of compound 1, also showed three other methine signals at $_{H}$ 3.55 (m), 3.68 (t, *J* = 7.2 Hz), and 4.04 (m), with corresponding carbon resonances at $_{C}$ 71.5 (C-2′), 74.1(C-3), and 71.6(C-4), respectively, suggesting the presence of hydroxyl groups in the fatty acid chain and the long chain base of the sphingolipid. The length of the fatty acid chain was determined by ESI (negative mode), which showed significant fragment ion peaks at *m/z* 311 [$CH_3(CH_2)_{17}CH(OH)CO$] and 325 [$CH_3(CH_2)_{17}CHOHCO$ NH_2H]$^+$. This was confirmed by the EIMS spectrum with molecular ion peak at m/z 326 [$CH_3(CH_2)_{17}CH(OH)CO$ NH_2]$^+$.

The fragment ion observed at *m/z* 369 [CH_3 $(CH_2)_8$ $CH{=}CH$ $(CH_2)_7(CHOH)_2$ $CHNHCH_2O$]$^+$, indicated the length of the long chain base in the ESIMS (negative mode). The ESI (negative mode) gave a fragment ion at *m/z* 183 [CH_3 $(CH_2)_8CH{=}CH$ $(CH_2)_2$+2H]$^+$, indicating the presence of a double bond in the long chain base. Further indication was observed in the HRESI-TOF spectrum with a prominent molecular ion peak appearing at *m/z* 731 [M-CH_3 $(CH_2)_7$+H]$^+$, resulting from the allylic cleavage of the double bond. The double bond position was further confirmed by the EIMS spectrum with the molecular ion peak at *m/z* 128 [$CH_3(CH_2)_8$+H]$^+$, and the typical fragment ions formed at *m/z* 153 [$CH_3(CH_2)_8CH{=}CH$]$^+$, *m/z* 167 [$CH_3(CH_2)_8CH{=}CHCH_2$]$^+$, *m/z* 181 [$CH_3(CH_2)_8$ $CH{=}CH$ $(CH_2)_2$]$^+$, respectively. The position of the hydroxyl group at C-3 and C-4 was ascertained by the mass fragmentation pattern from ESIMS (negative mode) with the molecular ion peak at *m/z* 311 [$(CHOH)_2$ $(CH_2)_7$ $CH{=}CH$ $(CH_2)_8CH_3$], (Figure 2).

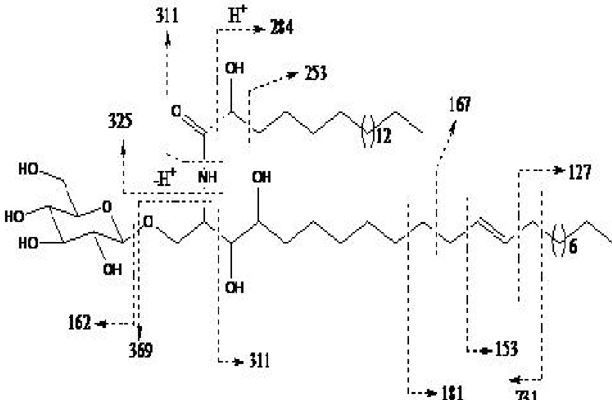

Figure 2: Mass fragmentations pattern of Pachysteloside A (1)

The strong correlation observed in the HMBC spectrum between the proton signal at $_{H}$ 4.04 to C-1′ ($_{C}$ 175.7) suggest that the fourth hydroxyl group is present at C-2′ of the fatty acid chain (Figure 3), thus the long chain base and fatty acid of 1 must be 1-O- -D-glucopyranosyl-2-amino-12-docosene-1,3,4-triol and 2-hydroxyicosanoic acid, respectively. In the NOESY spectrum, correlations were observed between the proton signals at $_{H}$ 5.43 and 2.01, 5.37 and 2.01, 4.08 and 3.82, 4.04 and 1.67, 4.28 and 3.62, 3.62 and 3.89, 4.28 and 3.19 (Fig. 4). On the basis of these evidence, the structure of **1** was determined to be 1-O- -D-glucopyranosyl-3, 4-dihydroxy-2-icosanoyl-2-(2′-hydroxy icosanoyl amino)-12*E*-docosene.

The configuration at chiral centers C-2, C-2′, C-3, and C-4 could not be established without chemical transformation which will require much more material [9,13]. We have named the compound pachysteloside A after the producing organism *Pachystela msolo.* Taraxeryl acetate [14], -taraxerol[15], Spinasterol [16], betulinic acid [17], and spinasterol-3-O- -D-glucopyranoside [18] were identified by comparison with published data. Some biological properties of the known constituents from literature sources are cited in table1.

Table 1: Structures of known constituents isolated from the cyclohexane fraction of *P. msolo* roots and their biological activity from literature sources

Name of Compounds	Structures	Activities reported
Taraxeryl acetate	2	Anti-inflammatory [19]
β-taraxerol	3	Antibacterial, Cytotoxic activity against human cancer cell line H157, Antitumor [20]
Spinasterol	4	Anti-inflammatory [21]
Betulinic acid	5	Inhibition of human immunodeficiency virus (HIV), Antibacterial, Antimalarial, Anti-inflammatory, Anthelmintic and Antioxidant properties [22]
spinasterol-3-O- -D-glucopyranoside	6	Promotes production of collagen in UV-irradiated fibroblast cells *via* stimulation of procollagen synthesis and *via* inhibition of matrix Metalloprotease-1 expression [23]

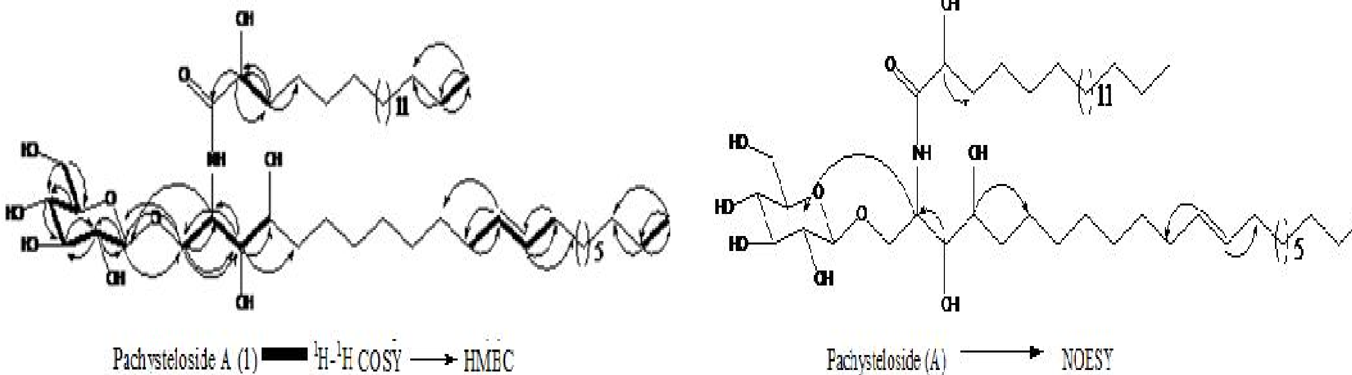

Figure 3: 1H-1H COSY and HMBC correlations for Pachysteloside A (1)

Figure 4: NOESY correlations for Pachysteloside A (1).

Pachytsteloside A (1):

White solid, m.p. 158.4 ^oC; $[\alpha]^{20}_D$ +12.7 (*c* 0.1, MeOH);IR (KBr): ν_{max} = 3380, 3341, 2931, 2869, 1737, 1658, 1456, 1029 cm^{-1};^1H NMR (600 MHz, CD_3OD): δ_H0.94 (t, *J* =7.6 Hz, 6H, 20′-H 22-H), 1.33 (br.s, 5-H, 7-10-H, 15-21-H, 4′-19′-H), 1.35 (m, 2H, 6-H), 1.63 (m, 1H, 3a′-H), 1.67 (m, 1H, 3b′-H), 2.01 (m, 2H, 11-H, 14-H), 3.19 (dd, *J* = 16.8, 8.4 Hz, 1H, 2″-H),3.29 (br.m, 1H, 4″-H), 3.37 (br.m, 1H, 3″-H),3.55 (m, 1H, 4-H), 3.62 (dd, *J* = 12.0, 7.2 Hz, 1H, 3-H), 3.68 (d, *J* = 12.0 Hz, 1H, 6b″-H), 3.89 (d, *J* = 12.0 Hz, 1H, 6a″-H), 3.82 (m, 1H, 1a-H), 4.08 (m, 1H, 1b-H), 4.04 (m, 1H, 2′-H), 4.28 (m, 1H, 2-H), 4.30 (d, 1H, *J* = 7.8 Hz, 1H, 1″-H), 5.37 (m, 1H, 13-H), 5.43 (m, 1H, 12-H);^{13}C NMR (150 MHz, CD_3OD): δ_C 13.1 (C-20′, C-22), 22.6 (C-19′, C-21),24.5 (C-4′), 25.8 (C-6),28.9 (C-17′), 29.5 (C-5′-16′, C-8-10, C-15-19),29.6 (C-7), 31.4 (C-18′, C-20), 31.7 (C-5), 32.3 (C-11, C-14), 34.1 (C-3a′), 50.2 (C-2), 61.2 (C-6″),68.5 (C-1), 70.4 (C-7″),71.5 (C-2′), 71.6 (C-4), 73.4 (C-2″), 74.1 (C-3), 76.5 (C-3″), 76.6 (C-4″), 103.3 (C-1″),175.7 (C-1′); HRESI-TOF: *m/z* 844.6878 $[M+H]^+$, (calcd. for $C_{48}H_{93}NO_{10}$+H 844.6878), 478 $[CH_2CH=CH(CH_2)_7(CHOH)_2CH(NHCOCHOH)CH_2OC_6H_{11}O]^+$; ESIMS (negative mode): *m/z* 369$[CH_3(CH_2)_8 CH=CH (CH_2)_7 (CH OH)_2 CHNHCH_2O_5]^+$,325$[CH_3(CH_2)_{17} CH(OH) CONH-H]^+$, 311$[CH_3(CH_2)_{17} CH (OH) CO]^+$ and $[(CHOH)_2 (CH_2)_7 CH=CH (CH_2)_8CH_3]^+$, 183$[CH_3 (CH_2)_8CH = CH(CH_2)_2+2H]^+$. 326 $[CH_3(CH_2)_{17} CHOHCO NH_2]^+$, *m/z* 181 $[CH_3 (CH_2)_8CH=CH(CH_2)_2]^+$, *m/z* 167 $[CH_3(CH_2)_8 CH=CH CH_2]^+$, *m/z* 153 $[CH_3(CH_2)_8CH=CH]^+$, 128 $[CH_3 (CH_2)_8+H]^+$; EIMS data and important^1H-^1H COSY, HMBC and NOESY correlations are illustrated in Figure 3 and 4; biological activity not yet determined.

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