Paullinoside A and Paullinomide A: A New Cerebroside and a New Ceramide from Leaves of *Paullinia pinnata*

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Paullinoside A (**1a**), a new cerebroside, and a new ceramide named paullinomide A (**2a**), have been isolated from *Paullinia pinnata*. Their structures were determined by comprehensive analyses of their 1D and 2D NMR, HREIMS spectral data, and chemical evidence. The remaining four known compounds were identified as β-amyrin, 13β,17β-dihydroxy-28-norolean-12-ene, β-sitosterol, and β-sitosterol glucopyranoside.

Key words: Cerebroside, Ceramide, *Paullina pinnata*

Introduction

The German physician J. L. W. Tudichum was able to isolate an organic base that he called sphingosine in addition to sugar and fatty acids by fractional crystallization of alcoholic brain extracts. The structure was elucidated by Carter in 1947. The isolation and naming of further brain lipids such as ceramide, sphingomyelin, and cerebroside are also attributed to Tudichum [1]. Harouse reported that galactosyl ceramide inhibited the entry of HIV-1 in neural cell lines and Kitagawa reported that cerebrosides from soybean showed ionophoric activity for Ca^{2+} ions [2]. KRN 7000, a cerebroside from *Agelas mauritianus*, is under Phase I clinical trials for cancer treatment [3] and some cerebrosides also exhibit biological activities such as antifungal, antitumor, immunomodulating, and nitric oxide release inhibiting activities [4]. Ceramides are predominant lipids of human epidermal stratum corneum, acting as a water barrier to prevent loss of body water [5]. Some of them also exhibit biological activities such as cytotoxic, antitumour, immunomodulatory, antiviral, antifungal and Ca^{2+}-ATPase activities [3]. As part of our systematic search for new bioactive lead structures from African medicinal plants, one new cerebroside, paullinoside A (**1a**), and paullinomide A (**2a**), a new ceramide, together with four known compounds identified as β-amyrin, 13β,17β-dihydroxy-28-norolean-12-ene, β-sitosterol, and β-sitosterol glucopyranoside have been isolated from *Paullinia pinnata*.

Results and Discussion

Dried and powdered leaves of *P. pinnata* were extracted with methanol. The residue obtained after evaporation of the solvent was fractionated between EtOAc and water, followed by conventional purification procedures, and resulting in the isolation of six constituents, including one new cerebroside (**1a**) and one new ceramide (**2a**).

The molecular formula of paullinoside A (**1a**) was assigned $C_{33}H_{63}NO_9$ on the basis of HREIMS at m/z 617.4475 and ^{1}H and ^{13}C NMR spectral analyses (see Experimental Section). An IR absorption band at 3390 cm^{-1} indicated the presence of hydroxyl groups. The typical IR absorptions at 1630 and 1535 cm^{-1} suggested an amide linkage, which was confirmed by a nitrogen-attached carbon signal at $\delta = 53.3$ and a carbonyl signal at $\delta = 174.2$ in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum in DMSO-d$_6$ (see Experimental Section) exhibited a doublet at $\delta = 7.39$ ($J = 9.5$ Hz) due to a NH proton, a broad singlet at $\delta = 1.29$ (methylene protons), a triplet at $\delta = 0.87$ (two terminal methyls), an anomeric proton at $\delta = 4.13$ ($J = 8.0$ Hz), and carbinol protons appearing as multiplets between $\delta = 4.13$ and 3.03, suggesting a cerebroside structure [6]. The ^{1}H NMR spectrum also showed

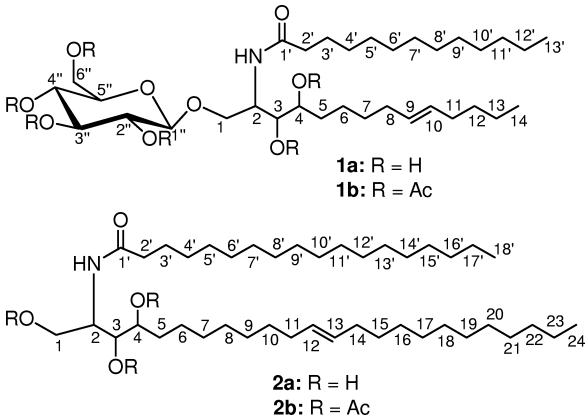

Fig. 1. Structures of paullinoside A (**1a**), paullinomide A (**2a**), and their acetates **1b** and **2b**.

two olefinic proton signals at $\delta = 5.50$ (dt, $J = 15.0$, 6.0 Hz, H-9) and 5.42 (dt, $J = 15.2$, 6.0 Hz, H-10), attributable to the presence of one disubstituted double bond. The large vicinal coupling constants of H-9 and H-10 ($J = 15.0$ Hz) clearly indicated an *E*-geometry for the double bond. The amino alcohol fragment was identified as a sphingosine unit by the characteristic signals that appeared in the ^{1}H and ^{13}C NMR spectra [6]. In the ^{13}C NMR spectrum, the carbon resonances appeared at $\delta = 61.5$ (CH_2), 71.5 (CH), 73.8 (CH), 77.0 (CH), 77.1 (CH), and 103.9 (CH), revealing the presence of a β-glucopyranoside. The anomeric proton at $\delta = 4.13$ (d, $J = 8.2$ Hz) correlated with the carbon signal at $\delta = 103.9$ in the HMQC spectrum, further confirming the β-configuration of the glucoside unit and α-orientation of the proton in the glucose moiety. This was also confirmed by EIMS which showed a prominent peak at m/z 455 due to the elimination of a glucosyl moiety. Besides the methine signals for a glucose unit, the ^{1}H NMR spectrum of **1a** also showed two other methine signals at $\delta = 4.01$ (m, CHOH) and 3.81 (m, CHOH), and 70.5 (CHOH) and 73.8 (CHOH) in the ^{13}C NMR spectrum. The presence of six acetoxy methyl groups resonating between $\delta = 1.99$ and 2.20 in the ^{1}H NMR spectrum of the peracetylated derivative (**1b**) of **1a** further confirmed the presence of two hydroxyls in the ceramide skeleton, in addition to four on the glucopyranose unit of **1a** (Fig. 1).

The length of the fatty acid chain was determined by EIMS, which showed significant fragment ion peaks at m/z 197 $[CH_3(CH_2)_{11}CO]^+$, 214 $[CH_3(CH_2)_{11}CONH_2 + H]^+$, and 269 $[CH_3(CH_2)_{11}$-$C(OH)=NC(=CH_2)CH_2OH]^+$. The length of the long chain base was determined by the characteristic ions at m/z 417 $[M\text{-}CH_3(CH_2)_7(CH)_2(CHOH)_2+H]^+$, 200 $[CH_3(CH_2)_7(CH)_2(CHOH)_2+H]^+$ and 214 $[CH_3(CH_2)_7(CH)_2(CHOH)_2OH\text{–}2H]^+$ in the EIMS [7 – 14]. This also confirmed the position of the double bond in the long chain base. The typical fragment ion at m/z 413, formed by elimination of propene from the ion with m/z 455 through McLafferty rearrangement [6, 14], further confirmed the position of the double bond in the long chain base. Its structure could be established through characteristic fragment ions in the EIMS (Fig. 2).

The positions of the hydroxyl groups were ascertained by the mass fragmentation pattern (Fig. 2), ^{1}H-^{1}H COSY, and HMBC spectra (Fig. 3). Cross peaks in the ^{1}H-^{1}H COSY spectrum were observed between an amide proton ($\delta = 7.39$) and H-2 methine ($\delta = 3.84$), which, in turn, was coupled to three protons at $\delta = 3.93$ (H-1a), $\delta = 3.81$ (H-3), and $\delta = 4.53$ (H-1b). Furthermore, H-3 ($\delta = 3.81$) showed correlations with H-2 ($\delta = 3.84$) and with H-4 ($\delta = 4.00$). The positions of the hydroxyl groups in the long chain base were further confirmed from their HMBC correlations (Fig. 3). Thus, the long chain base and fatty acid of **1a** must be 1-*O*-β-D-glucopyranosyl-2-amino-9-tetradecene-3,4-diol and tridecanoic acid, respectively. On the basis of this evidence, the structure of **1a** was determined to be 1-*O*-β-D-glucopyranosyl-3,4-dihydroxy-2-tridecanoylamino-9*E*-tetradecene. The configuration at the chiral centers at C-2, C-3, and C-4 could not be established without chemical transformations that would require much more material [14]. We have named the compound paullinoside A after the producing organism, *Paullinia pinnata*.

Paullinomide B (**2**) was isolated as colorless oil. The molecular formula was determined to be $C_{42}H_{83}NO_4$ by HREIMS. The IR spectrum presented bands at1620 and 1540 cm^{-1} due to the amide group. The ^{1}H NMR spectrum (in $CDCl_3$+CD_3OD, see Experimental Section) possessed four characteristic signals of protons geminal to hydroxyl groups at $\delta = 3.93$ (m, H-3), 3.66 (dd, $J = 4.5$, 11.5, H-1a), 3.60 (dd, $J = 4.5$, 11.5 Hz, H-1b) and 3.20 (m, H-4), and the formation of a tetraacetate **2b** proved the presence of three hydroxyl groups in compound **2a**, further supported by the absorption band at 3610 cm^{-1} for hydroxyl group(s) in the IR spectrum. A fifth signal was present at $\delta = 3.95$ (m, H-2) and identified as a methine proton vicinal to the nitrogen atom of the amide

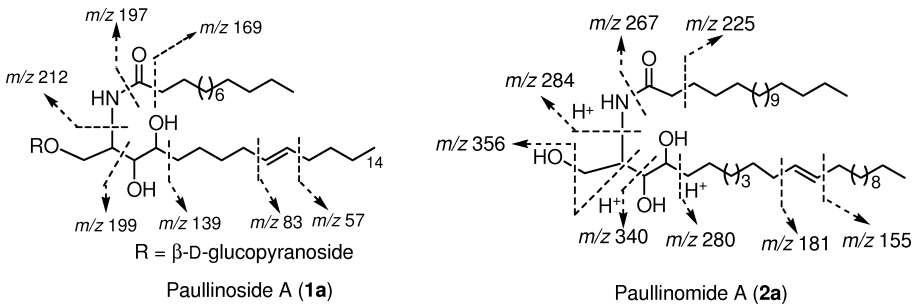

Fig. 2. Mass fragmentation pattern of compounds **1a** and **2a**.

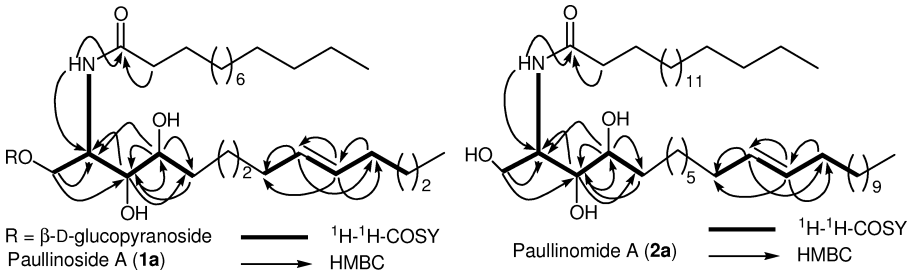

Fig. 3. ^{1}H-^{1}H COSY and HMBC correlations for **1a** and **2a**.

group. Compound **2a** also showed two olefinic protons [$\delta = 5.26$ (dt, $J = 15.2$, 6.0 Hz, H-13), 5.23 (dt, $J = 15.2$, 6.0 Hz, H-12)], one terminal methyl at $\delta = 0.75$, and methylenes at $\delta = 1.29$ (br s, CH_2 chain). The ^{13}C NMR spectrum showed one amide carbonyl at $\delta = 175.6$, three methines at $\delta = 75.3$ (CHOH), 72.1 (CHOH), and 51.4 (CHNH), and a methylene group at $\delta = 60.9$ (CH_2OH). The length of the fatty acid chain was determined by EIMS, which showed significant fragment ion peaks at m/z 267 $[CH_3(CH_2)_{16}CO]^+$, 284 $[CH_3(CH_2)_{16}CONH_2 + H]^+$, and 339 $[CH_3(CH_2)_{16}C(OH)=NC(=CH_2)CH_2OH]^+$. The length of the long chain base was determined by the characteristic ions at m/z 325 $[M-CH_3(CH_2)_{17}(CH)_2(CHOH)_2+H]^+$, 340 $[CH_3(CH_2)_{17}(CH)_2(CHOH)_2+H]^+$ and 364 $[CH_3(CH_2)_{17}(CH)_2(CHOH)_3OH–2H]^+$ in the EIMS [7–14]. This also confirmed the position of the double bond in the long chain base. The typical fragment ion at m/z 525 was formed by elimination of propene from $[M]^+$ through McLafferty rearrangement [6, 14]. The positions of the hydroxyl groups at C-3 and C-4 were ascertained by the mass fragmentation pattern (Fig. 2) and especially from ^{1}H-^{1}H COSY and HMBC spectra (Fig. 3), which are similar to compound **1a**. Thus the long chain base and fatty acid of **2a** must be 2-amino-12-tetracosene-1,3,4-triol and octadecanoic acid, respectively. So the structure of paullinomide A (**2a**) was determined to be 1,3,4-trihydroxy-2-octadecanoylamino-12*E*-tetracosene. The configuration at the chiral centers at C-2, C-3, and C-4 could not be established without chemical transformations that would require much more material [14].

β-Amyrin [15], 13β,17β -dihydroxy-28-norolean-12-ene [16], β-sitosterol [17], and β-sitosterol glucopyranoside [18] were identified by comparison with published data.

Experimental Section

General

^{1}H, 2D ^{1}H-^{1}H COSY, ^{13}C, 2D HMQC and HMBC spectra were recorded with a Bruker Avance 500 MHz spectrometer. Chemical shifts are referenced to internal TMS ($\delta = 0$) and coupling constants *J* are reported in Hz. Optical spectra were recorded with a NICOLET 510P FT-IR spectrometer, a UV-2101PC spectrometer, and Perkin-Elmer 241 polarimeter.

Plant material

The leaves of *Paullinia pinnata* L. (Sapindaceae) were collected at Obala, Central province of the Republic of Cameroon, in April 2004, and identified by Dr. Louis Zapfack (plant taxonomist), Department of Biology and Physiology, University of Yaounde I, Cameroon. A voucher specimen (No. 44641) has been deposited at the National Herbarium, Yaounde, Cameroon.

Extraction and isolation

Dried and powdered leaves (2.5 kg) of *Paullinia pinnata* L. were extracted with MeOH at room temperature for

72 h and filtered. The filtrate was concentrated under vacuum to give 135 g of crude residue. The crude extract was suspended in water and extracted with EtOAc to yield an ethyl acetate fraction (50 g). The EtOAc fraction was then subjected to column chromatography (silica gel, *n*-hexane, *n*-hexane-EtOAc and EtOAc, in order of increasing polarity) yielding 11 fractions. Fraction F_{10} was eluted with a mixture of *n*-hexane-EtOAc (1.5 : 8.5) yielding paullinoside A (**1a**) (15.1 mg), and fraction F_8 [*n*-hexane-EtOAc (5 : 5)] subjected to CC, afforded paullinomide A (**2a**) (38.1 mg). Column fractions F_2 [*n*-hexane-EtOAc (9.5 : 0.5)] and F_3 [*n*-hexane-EtOAc (9 : 1)] were similarly subjected to CC, yielding β-amyrin (8.4 mg) and 13β,17β-dihydroxy-28-nor-olean-12-ene (21.0 mg), respectively. Finally, fraction F_4 eluted with *n*-hexane-EtOAc (8.5 : 1.5) afforded β-sitosterol (15.4 mg) and fraction F_9 gave β-sitosterol glucopyranoside (78.2 mg) when subjected to CC using *n*-hexane-EtOAc (3 : 7).

Paullinoside A (**1a**): Colorless powder, m. p. 137 °C; $[\alpha]_D^{20}$ + 13.23 (c = 0.92, $CHCl_3$ + MeOH); IR ν_{max} ($CHCl_3$ + MeOH): 3390, 2940, 2860, 1630, 1533, 1297 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ = 0.87 (t, J = 6.5 Hz, 6 H, 13'-H, 14-H), 1.29 (s, 7-16-H, 4'-12'-H), 1.58 (m, 2 H, H-13), 1.68 (m, 2 H, 3'-H), 1.71 (m, 2 H, 6-H), 1.83 (m, 2 H, 5-H), 1.92 – 2.01 (m, 4 H, 8-H, 11-H), 2.03 (t, J = 7.3 Hz, 1 H, 2'-H), 3.09 (m, 1 H, 4"-H), 3.12 (m, 1 H, 2"-H), 3.16 (m, 1 H, 5"-H), 3.45 (dd, J = 6.0, 11.5 Hz, 1 H, 6a"-H), 3.53 (dd, J = 4.0, 10.0 Hz, 1 H, 1a-H), 3.68 (dd, J = 6.0, 11.5 Hz, 1 H, 6b"-H), 3.81 (m, 1 H, 3-H), 3.84 (m, 1 H, 2-H), 3.93 (dd, J = 4.0, 10.0 Hz, 1 H, 1b-H), 4.01 (m, 1 H, 4-H), 4.13 (d, J = 8.0 Hz, 1H, 1"-H), 4.51 (s, 1 H, OH), 4.89 (s, 1 H, OH), 4.96 (s, 1 H, OH), 5.42 (dt, J = 6.0, 14.5 Hz, 1 H, 10-H), 5.50 (dt, J = 6.0, 14.5 Hz, 1 H, 9-H), 6.89 (d, J = 6.0 Hz, 1 H, OH), 7.39 (d, J = 8.8 Hz, 1 H, NH), 8.04 (d, J = 6.0 Hz, 1 H, OH). – ^{13}C NMR (125 MHz, DMSO-d_6): δ = 14.3 (C-13', C-14), 22.5 (C-12), 26.8 (C-12', C-13), 27.1 (C-11'), 29.5 (C-6-7, C-4'-10'), 32.2 (C-6), 32.4 (C-5), 32.8 ((C-8), C-11), 34.9 (C-2'), 53.3 (C-2), 61.5 (C-1"), 69.1 (C-1), 70.5 (C-4), 70.9 (C-3), 71.5 (C-4"), 73.8 (C-2"), 77.0 (C-3"), 77.1 (C-5"), 103.9 (C-1"), 130.3 (C-10), 131.3 (C-9), 174.2 (C-1'). – HREIMS: m/z 617.4475 (Calcd. 617.4485 for $C_{43}H_{63}NO_9$). – EIMS data and important ^1H-^1H COSY and HMBC correlations are illustrated in Figs 2 and 3.

Paullinomide A (**2a**): Colorless powder, m. p. 174 °C; $[\alpha]_D^{20}$ + 17.11 (c = 0.92, $CHCl_3$ + MeOH); IR ν_{max} ($CHCl_3$ + MeOH): 3610, 2940, 2860, 1620, 1540, 1297 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ = 0.74 (t, J = 6.5 Hz, 6H, 18'-H, 24-H), 1.29 (s, 7-9-H, 16-20-H, 4'-15'-H), 1.52 (m, 2 H, H-23), 1.65 (m, 2 H, 3'-H), 1.70 (m, 2 H, 6-H), 1.80 (m, 2 H, 5-H), 1.85 – 1.90 (m, 4 H, 11-H, 14-H), 1.95 (t, J = 7.3 Hz, 1 H, 2'-H), 3.40 (m, 1 H, 4-H), 3.60 (dd, J = 4.5, 11.5 Hz, 1 H, 1a-H), 3.66 (dd, J = 4.5, 11.5 Hz, 1 H, 1b-H), 3.93 (m, 1 H, 3-H), 3.95 (m, 1 H, 2-H), 5.21 (dt, J = 6.0, 14.5 Hz, 1 H, 13-H), 5.26 (dt, J = 6.0, 14.5 Hz, 1 H, 12-H). – ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD): δ = 13.7 (C-18', C-24), 22.4 (C-22), 25.0 (C-17', C-23), 25.6 (C-16'), 39.5 (C-6-10, C-15-20, C-4'-15'), 32.3 (C-6), 32.4 (C-5), 33.7 (C-11, C-14), 34.2 (C-2'), 51.4 (C-2), 60.9 (C-1), 71.8 (C-2), 75.3 (C-4), 129.6 (C-13), 130.5 (C-12), 175.6 (C-1'). – HREIMS: m/z 665.6284 (Calcd. 665.6300 for $C_{42}H_{83}NO_5$). – EIMS data and important, ^1H-^1H COSY and HMBC correlations are illustrated in Figs 2 and 3.

Acetylation: Dry pyridine (0.5 ml) and Ac_2O (1.0 ml) were added to compound **1a** (7 mg) and **2a** (8 mg) separately and left overnight. The usual workup yielded **1b** and **2b**, respectively.

Compound **1b**: m. p. 71 °C. $[\alpha]_D^{20}$ + 9.4 (c = 0.92, $CHCl_3$). IR ν_{max} ($CHCl_3$): 2970, 1730, 1620, 1270 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.85 (t, J = 6.5 Hz, 6 H, 13'-H, 14-H), 1.29 (s, 7-16-H, 4'-12'-H), 1.57 (m, 2 H, H-13), 1.63 (m, 2 H, 3'-H), 1.73 (m, 2 H, 6-H), 1.85 (m, 2 H, 5-H), 1.91 – 2.01 (m, 4 H, 8-H, 11-H), 2.05 (t, J = 7.3 Hz, 1 H, 2'-H), 1.99 – 2.24 (18H, s, 6-OAc), 3.29 (m, 1 H, 4"-H), 3.37 (m, 1 H, 2"-H), 3.41 (m, 1 H, 5"-H), 3.59 (dd, J = 6.0, 11.5 Hz, 1 H, 6a"-H), 3.70 (dd, J = 4.0, 10.0 Hz, 1 H, 1a-H), 3.78 (dd, J = 6.0, 11.5 Hz, 1 H, 6b"-H), 3.89 (m, 1 H, 2-H), 3.97 (m, 1 H, 3-H), 3.99 (dd, J = 4.0, 10.0 Hz, 1 H, 1b-H), 4.19 (m, 1 H, 4-H), 4.23 (d, J = 8.0 Hz, 1 H, 1"-H), 5.43 (dt, J = 6.0, 14.5 Hz, 1 H, 10-H), 5.53 (dt, J = 6.0, 14.5 Hz, 1 H, 9-H), 7.39 (d, J = 8.8 Hz, 1 H, NH).

Compound **2b**: m. p. 121 °C. $[\alpha]_D^{20}$ + 15.3 (c = 0.82, $CHCl_3$). IR ν_{max} ($CHCl_3$): 2960, 1735, 1620, 1260 cm^{-1}. δ = 0.75 (t, J = 6.5 Hz, 6 H, 18'-H, 24-H), 1.29 (s, 7-9-H, 16-20-H, 4'-15'-H), 1.52 (m, 2 H, H-23), 1.63 (m, 2H, 3'-H), 1.70 (m, 2H, 6-H), 1.84 (m, 2 H, 5-H), 1.86 – 1.93 (m, 4 H, 11-H, 14-H), 1.99 (t, J = 7.3 Hz, 1 H, 2'-H), 1.99, 2.02, 2.03, (9H, all s, 4 × OAc), 3.60 (m, 1 H, 4-H), 3.75 (dd, J = 4.5, 11.5 Hz, 1 H, 1a-H), 3.86 (dd, J = 4.5, 11.5 Hz, 1 H, 1b-H), 3.99 (m, 1 H, 2-H), 4.07 (m, 1 H, 3-H), 5.22 (dt, J = 6.0, 14.5 Hz, 1 H, 13-H), 5.27 (dt, J = 6.0, 14.5 Hz, 1 H, 12-H).

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