Laportoside A and Laportomide A: A New Cerebroside and a New Ceramide from Leaves of *Laportea ovalifolia*

Dagobert Tazoo[a], Karsten Krohn[b], Hidayat Hussain[b], Simeon F. Kouam[c], and Etienne Dongo[a]

[a] Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P. O. Box 812, Yaounde, Cameroon
[b] Department of Chemistry, University of Paderborn, Warburger Straße 100, 33098 Paderborn, Germany
[c] Department of Chemistry, Higher Teachers' Training College, University of Yaounde I, BP 47, Yaounde, Cameroon

Reprint requests to Prof. Karsten Krohn. Fax: (+49)-5251-603245.
E-mail: k.krohn@uni-paderborn.de

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Dedicated to Prof. Peter Welzel on the occasion of his 70th birthday

Laportoside A (**1**), a new cerebroside, and a new ceramide named laportomide A (**2**), have been isolated from *Laportea ovalifolia*. Their structures were determined by comprehensive analyses of their 1D, 2D NMR, and HREIMS spectral data. The remaining two known compounds were identified as β-sitosterol acetate, and β-sitosterol glucopyranoside.

Key words: Cerebroside, Ceramide, *Laportea ovalifolia*

Introduction

The genus *Laportea* (Urticaceae) comprises 25 species coming from tropical areas. Five of them are present in Cameroon, including *Laportea ovalifolia* [1], which is common in tropical Africa, especially Senegal, Angola and East Africa [2]. *Laportea ovalifolia* is commonly found growing in the swampy area of Cameroon, in both the dry and rainy seasons [1]. A decoction of the leaves of this plant is used in traditional medicine for the treatment of bacterial infections, headaches, and dysentery [1]. It is also used to cure urinary infections, pneumonia, female infertility and to solve central nervous system disorders [3]. Recent pharmacological investigations in Cameroon have shown that the methanol/methylene chloride extract of this species possesses antidiabetic and hypolipidaemic activities [4]. As part of our systematic search for new bioactive lead structures from African medicinal plants, one new cerebroside, laportoside A (**1**), and laportomide A (**2**), a new ceramide, together with two known compounds identified as β-sitosterol acetate and β-sitosterol glucopyranoside, have been isolated from *Laportea ovalifolia*. Ceramides and cerebrosides are biologically important classes of compounds. For some more recently recognized biological activities see preceding papers [5 – 8].

Results and Discussion

Dried and powdered leaves of *L. ovalifolia* were extracted with a 1 : 1 mixture of CH_2Cl_2/MeOH at r. t. The residue obtained after evaporation of the solvent was fractionated between *n*-hexane and water, followed by conventional purification procedures of the *n*-hexane extract, and resulting in the isolation of four constituents, including one new cerebroside (**1**) and one new ceramide (**2**) and the known compounds β-sitosterol acetate and β-sitosterol glucopyranoside.

Laportoside A (**1**) was assigned the molecular formula $C_{48}H_{93}NO_{10}$ on the basis of ESI-TOF at *m/z* = 844.6839 $[M+H]^+$ and ^1H and ^{13}C NMR spectral analyses (see Experimental Section). An IR absorption band at 3410 cm^{-1} indicated the presence of hydroxyl groups. The typical IR absorptions at 1630 and 1530 cm^{-1} suggested an amide linkage, which was confirmed by a nitrogen-attached carbon signal at δ = 49.9 and a carbonyl signal at δ = 177.7 in the ^{13}C NMR spectrum. The ^1H NMR spectrum in [D_6]DMSO (see Experimental Section) exhibited a doublet at δ = 7.51

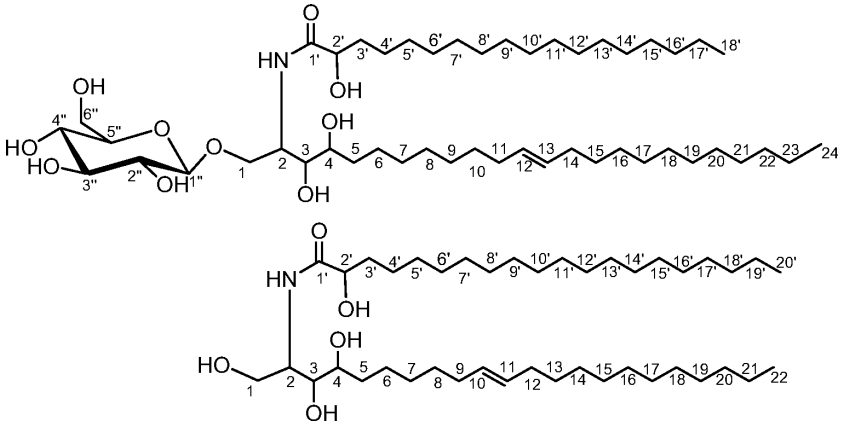

Fig. 1. Structures of laportoside A (**1**) (top) and laportomide A (**2**) (bottom).

(J = 8.8 Hz) due to an NH proton, a broad singlet at δ = 1.27 (methylene protons), a triplet at δ = 0.84 (two terminal methyls), an anomeric proton at δ = 4.12 (J = 8.0 Hz), and carbinol protons appearing as multiplets between δ = 4.12 and 3.03, suggesting a cerebroside structure [9]. The ^{1}H NMR spectrum also showed two olefinic proton signals at δ = 5.32 (m, 13-H) and 5.34 (m, 12-H), attributable to the presence of one 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 δ = 32.8 (C-11) and 32.5 (C-14) in **1** [10]. The chemical shifts for carbon signals of *cis* (*Z*) double bonds are usually in the range of δ = 27 – 28 [5, 10 – 12]. 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 [9]. In the ^{13}C NMR spectrum, the carbon resonances appeared at δ = 61.0 (CH_2), 69.9 (CH), 73.4 (CH), 76.4 (CH), 76.8 (CH), and 103.4 (CH), revealing the presence of a β-glucopyranoside. The anomeric proton at δ = 4.12 (d, J = 8.0 Hz) correlated to the carbon signal at δ = 103.4 in the HMQC spectrum, further confirming the β-configuration of the glucoside unit and the α-orientation of the proton in the glucose moiety. The conclusion was also confirmed by EIMS which showed a prominent peak at *m/z* = 681.2 due to the elimination of the glucosyl moiety. Besides the methine signals for the glucose unit, the ^{1}H NMR spectrum of **1** also showed three other methine signals at δ = 3.32 (m, CHOH), 3.37 (m, CHOH), 3.84 (m, CHOH), 70.5 (CHOH), 70.9 (CHOH), and 74.0 (CHOH) in the ^{13}C NMR spectrum. The above data suggested the presence of three hydroxyl groups in the ceramide skeleton of **1** (Fig. 1).

The length of the fatty acid chain was determined by EIMS, which showed significant fragment ion peaks at *m/z* = 283 $[CH_3(CH_2)_{15}CHOHCO]^+$, 298 $[CH_3(CH_2)_{15}CHOHCONH]^+$, and 517 $[CH_3(CH_2)_{15}$-$CH(OH)C(OH)=NC(=CH_2)CH_2OC_6H_{11}O_5]^+$. The length of the long chain base was determined by the characteristic ions at *m/z* = 504 $[M–CH_3$-$(CH_2)_{17}(CH)_2(CHOH)_2]^+$, 339 $[CH_3(CH_2)_{17}(CH)_2$-$(CHOH)_2]^+$ and 545 $[CH_3(CH_2)_{17}(CH)_2(CHOH)_2$-$CHCH_2C_6H_{11}O_6]^+$ in the EIMS [12 – 19]. This also confirmed the position of the double bond in the long chain base. The typical fragment ion at *m/z* = 541 was formed by elimination of decene from that at *m/z* = 681.2 through McLafferty rearrangement [9, 19], further confirming 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).

Cross peaks in the ^{1}H-^{1}H COSY spectrum were observed between an amide proton (δ = 7.51) and 2-H methine (δ = 4.08), which, in turn, was coupled to three protons at δ = 3.80 (1a-H), δ = 3.32 (3-H), and δ = 3.62 (1b-H). Furthermore, 3-H (δ = 3.32) showed correlations with 2-H (δ = 4.08) and with 4-H (δ = 3.37). No cross peaks were observed of the signal at 3.84 to any downfield proton signals, but in the HMBC spectrum it showed strong correlation to C-1′ (δ = 173.7). This suggested that the fourth hydroxyl group is present at C-2′ of the fatty acid chain. The positions of the three hydroxyl groups in the long chain base were further confirmed by the mass fragmentation pattern (Fig. 2) as well as by the HMBC correlations (Fig. 3). Thus the long chain base and fatty acid of **1** must be 1-*O*-β-D-glucopyranosyl-2-amino-12-tetracosene-1,3,4-triol and

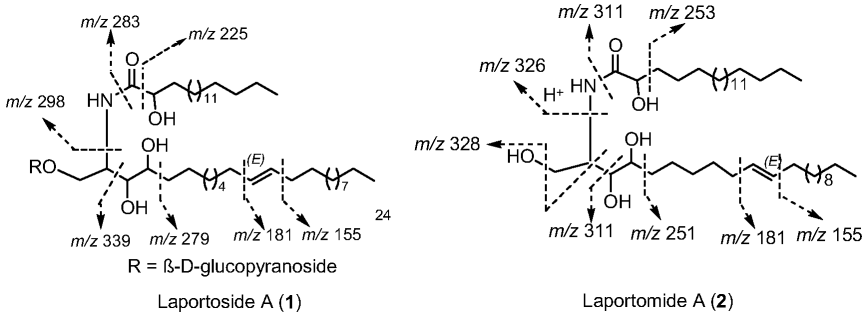

Fig. 2. Mass fragmentation pattern of compounds **1** and **2**.

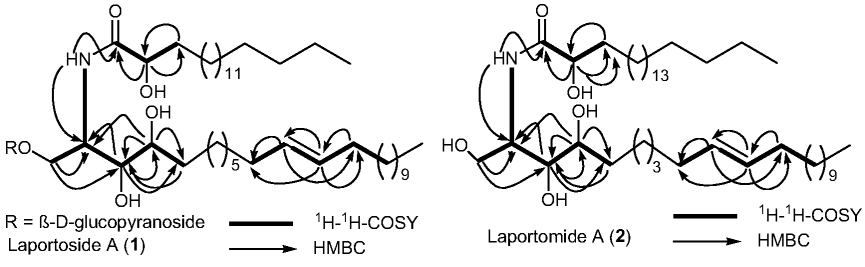

Fig. 3. ^{1}H-^{1}H COSY and HMBC correlations for **1** and **2**.

2-hydroxyoctadecanoic acid, respectively. On the basis of this evidence, the structure of **1** was determined to be 1-*O*-β-D-glucopyranosyl-3,4-dihydroxy-2-octadecanoyl-2-(2′-hydroxyoctadecanoylamino)-12*E*-tetracosene. The configuration at the chiral centers at C-2, C-2′, C-3, and C-4 could not be established without chemical transformations which would require much more material [19]. We have named the compound laportoside A after the producing organism, *Laportea ovalifolia*.

Laportomide A (**2**) was obtained as a colorless powder, m. p. 119 °C, and was assigned the molecular formula $C_{42}H_{83}NO_5$ on the basis of HREIMS. The IR spectrum presented bands at 1620 and 1540 cm^{-1} due to the amide group. The ^{1}H NMR spectrum (in C_5D_5N, see Experimental Section) showed five characteristic signals of protons geminal to hydroxyl groups at δ = 4.66 (m, 2′-H), 4.54 (dd, J = 5.0, 10.0, 1a-H), 4.46 (dd, J = 5.0, 10.0, 1b-H), 4.38 (m, 3-H), and 4.32 (m, 4-H), further supported by the absorption band at 3610 cm^{-1} for hydroxyl group(s) in the IR spectrum. A sixth signal was present at δ = 5.11 (m, 2-H), identified as a methine proton vicinal to the nitrogen atom of the amide group and further confirmed by an amide carbonyl signal at δ = 176.5 in the ^{13}C NMR spectrum. Compound **2** also showed two olefinic protons [δ = 5.55 (dt, J = 16.0, 6.0 Hz, 10-H) and 5.48 (dt, J = 16.0, 6.0 Hz, 11-H)], two terminal methyls at δ = 0.86, and methylenes at δ = 1.29 (br. s, CH_2 chain). The geometry of the C_{10}/C_{11} alkene bond was *trans*, as evidenced by the large vicinal coupling constant (J_{10-11} = 16.0 Hz). The ^{13}C NMR spectrum showed four methines at δ = 78.1 (CHOH), 74.3 (CHOH), 73.7 (CHOH), and 54.2 (CHNH) and a methylene group at δ = 63.3 (CH_2OH). The length of the fatty acid chain was determined by EIMS, which showed significant fragment ion peaks at *m/z* = 283 $[CH_3(CH_2)_{17}CH(OH)CO]^+$, 326 $[CH_3(CH_2)_{17}CH(OH)CONH]^+$, and 383 $[CH_3(CH_2)_{17}CH(OH)C(OH)=NC(=CH_2)CH_2OH]^+$. The length of the long chain base was determined by the characteristic ions at *m/z* = 370 $[M–CH_3(CH_2)_{14}(CH)_2(CHOH)_2]^+$, 311 $[CH_3(CH_2)_{14}(CH)_2(CHOH)_2]^+$ and 328 $[CH_3(CH_2)_{14}(CH)_2(CHOH)_2OH]^+$ in the EIMS [12 – 19]. This also confirmed the position of the double bond in the long chain base. The typical fragment ion at *m/z* = 541 was formed by elimination of decene from $[M]^+$ through McLafferty rearrangement [9, 19]. The positions of the hydroxyl groups at C-3 and C-4 were ascertained by the mass fragmentation pattern (Fig. 2) and especially by the ^{1}H-^{1}H COSY and HMBC spectra (Fig. 3), which are similar to those of compound **1**. Thus the long chain base and fatty acid of **2** must be 2-amino-10-docosene-1,3,4-triol and 2-hydroxyicosanoic acid, respectively. Thus the structure of laportomide A (**2**) was determined to be 1,3,4-trihydroxy-2-(2’-hydroxyicosanoylamino)-10*E*-docosene. The configurations at the chiral centers C-2, C-2′, C-3, and C-4

could not be established without chemical transformations which would require much more material [19].

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 *Laportea ovalifolia* (Urticaceae) were collected at Mvolye, neighbourhood of Yaounde, Cameroon, in September 2003, and identified by Dr. Louis Zapfack (plant taxonomist), Department of Biology and Physiology, University of Yaounde I, Cameroon. A voucher specimen (Nr. 50623/HNC) has been deposited at the National Herbarium, Yaounde, Cameroon.

Extraction and isolation

Dried and powdered leaves (3.0 kg) of *L. ovalifolia* were extracted with a 1 : 1 mixture of CH_2Cl_2/MeOH at r. t. for 48 h and then filtered. The filtrate was concentrated under vacuum to give 160 g of crude residue. The crude extract was suspended in water and extracted with *n*-hexane and EtOAc to yield an *n*-hexane and an ethyl acetate fraction. The mixture of *n*-hexane and ethyl acetate fractions (90 g) was then subjected to column chromatography (silica gel, *n*-hexane, *n*-hexane-EtOAc, EtOAc and EtOAc-MeOH, in order of increasing polarity) yielding 15 fractions. Fraction F_{10} was eluted with a mixture of MeOH-EtOAc (0.5 : 9.5) yielding laportoside A (**1**) (10.1 mg). Column fraction F_8 [*n*-hexane-EtOAc (6 : 4)] was similarly subjected to a CC, yielding laportomide A (**2**) (10.4 mg). Finally fraction F_9 gave β-sitosterol glucopyranoside (10.2 mg) on subjecting it to CC using *n*-hexane-EtOAc (2 : 8).

*Laportoside A (**1**)*

Colorless powder, m. p. 187 °C. – $[\alpha]_D^{20} = +15.21$ (c = 0.91, $CHCl_3$ + MeOH). – IR ($CHCl_3$ + MeOH): ν_{max} = 3410, 2940, 2870, 1630, 1533, 1297 cm^{-1}. – ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta = 0.84$ (t, J = 6.5 Hz, 6 H, 18′-H, 24-H), 1.23 (s, 7-10-H, 16-21-H, 4′-16′-H), 1.46 (m, 1 H, 3b′-H), 1.47 (m, 2 H, 4′-H), 1.57 (m, 1 H, 3a′-H), 1.91 (m, 4 H, 11-H, 14-H), 2.92 (m, 1 H, 2″-H), 3.03 (m, 1 H, 4″-H), 3.06 (m, 1 H, 5″-H), 3.12 (m, 1 H, 3″-H), 3.32 (m, 1 H, 3-H), 3.37 (m, 1 H, 4-H), 3.42 (dd, J = 4.0, 11.5 Hz, 1H, 6b″-H), 3.62 (dd, J = 4.0, 10.0 Hz, 1H, 1a-H), 3.65 (dd, J = 6.0, 11.5 Hz, 1H, 6a″-H), 3.80 (dd, J = 4.0, 10.0 Hz, 1H, 1a-H), 3.84 (m, 1 H, 2′-H), 4.08 (m, 1 H, 2-H), 4.12 (d, J = 8.0 Hz, 1 H, 1″-H), 5.32 (m, 1H, 13-H), 5.34 (m, 1 H, 12-H), 7.51 (d, J = 8.8 Hz, 1 H, NH). – ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 13.9 (C-18′, C-24), 22.0 (C-15), 24.5 (C-17′, C-23), 28.6 (C-16′), 29.5 (C-7-10, 15-21, C-4′-15′), 32.0 (C-6), 32.3 (C-5), 32.8 (C-11), 32.5 (C-14), 34.3 (C-3′), 49.9 (C-2), 61.0 (C-6″), 68.9 (C-1), 69.9 (C-4″), 70.5 (C-3), 70.9 (C-2′), 73.4 (C-2″), 74.0 (C-4), 76.4 (C-3″), 76.8 (C-5″), 103.4 (C-1″), 129.6 (C-12), 130.2 (C-11), 173.7 (C-1′). – HRMS (ESI-TOF): m/z = 844.6839 $[M+H]^+$ (calcd. 844.6852 for $C_{48}H_{93}NO_{10}$ + H). EIMS data and important, ^{1}H-^{1}H COSY and HMBC correlations are illustrated in Fig. 3.

*Laportomide A (**2**)*

Colorless powder, m. p. 119 °C. – $[\alpha]_D^{20} = +8.17$ (c = 0.04, $CHCl_3$ + MeOH). – IR ($CHCl_3$ + MeOH): ν_{max} = 3610, 2940, 2860, 1640, 1297 cm^{-1}. – ^{1}H NMR (500 MHz, C_5D_5N): $\delta = 0.86$ (t, J = 6.5 Hz, 6H, 20′-H, 22-H), 1.29 (s, 7-8-H, 14-19-H, 4′-18′-H), 1.40 (m, 2H, 21-H), 1.71 (m, 2H, 4′-H), 1.74 (m, 2H, 7-H), 1.95 – 2.01 (m, 4H, 9-H, 12-H), 2.05 (m, 2H, 5-H), 2.10 (m, 3′-H), 4.32 (m, 1H, 4-H), 4.38 (m, 1H, 3-H), 4.46 (dd, J = 5.0, 10.0 Hz, 1H, 1b-H), 4.54 (dd, J = 5.0, 10.0 Hz, 1H, 1a-H), 4.66 (m, 1H, 2′-H), 5.11 (m, 1H, 2-H), 5.48 (dt, J = 6.0, 16.0 Hz, 1H, 21-H), 5.55 (dt, J = 6.0, 16.0 Hz, 1H, 20-H), 7.06 (s, 1H, OH), 7.37 (s, 1H, OH), 7.39 (s, 1H, OH), 7.63 (s, 1H, OH), 8.58 (d, J = 8.0 Hz, 1H, NH). – ^{13}C NMR (125 MHz, C_5D_5N): δ = 15.5 (C-20′, C-22), 24.1 (C-21, C-19′, C-24), 27.9 (C-18′), 30.8 (C-8, C-14-19, C-4′-18′), 31.5 (C-7), 32.2 (C-9, C-12), 33.3 (C-6), 34.2 (C-5), 34.5 (C-3′), 54.2 (C-2), 63.3 (C-1), 73.7 (C-2′), 74.3 (C-4), 78.1 (C-3), 129.9 (C-11), 132.0 (C-10), 176.5 (C-1′). – HREIMS: m/z = 681.6252 (calcd. 681.6267 for $C_{42}H_{83}NO_5$). – EIMS data and important, ^{1}H-^{1}H COSY and HMBC correlations are illustrated in Fig. 3.

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