New Sphingolipids and Other Constituents of *Pancovia laurentii*

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Phytochemical investigation of the bark and leaves of *Pancovia laurentii* (Sapindaceae) resulted in the isolation of a new ceramide and a new cerebroside, named pancoviamide (**1**), and pancovioside (**2**) respectively, together with six known compounds: uracil, (*R*)-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)heptadec-5-en-1-yl]-2-hydroxytetracosanamide, stigmasta-7,22-dien-3-ol, *β*-stitosterol, *β*-sitosterol 3-*O*-*β*-D-glucopyranoside, and 2,3-dihydroxypropyl pentadecanoate. The structures of **1** and **2** were determined by means of spectroscopic methods. Compounds **1** and **2** were tested *in vitro* for their antiprotozoal properties against several protozoa and for their cytotoxicity.

Introduction. – *Pancovia laurentii* (Sapindaceae) is a small tree that grows in the tropical regions of Cameroon [1]. Plants of Sapindaceae family are largely used in Cameroonian folk medicine to treat several diseases such as malaria, typhoid, stomach ache, ulcer, skin diseases, dysentery, and rheumatism [2][3]. Previous phytochemical investigations of plants of the Sapindaceae family revealed the presence of triterpenoid saponins, polyprenols, flavonoids, sphingolipids, alkaloids, coumarins, and ellagic acid derivatives as major constituents [4–8]. Some of these secondary metabolites exhibited interesting biological activities such as antiplasmodial, anti-inflammatory, antiulcer, cytotoxic, antioxidant, antibacterial, scorpion venom activity, and migraine target [9–14]. No phytochemical or pharmacological studies have been reported to date on *P. laurentii*. In a continuing search for bioactive or new compounds from Cameroonian medicinal plants, we have investigated an AcOEt extract of the bark and MeOH extract of the leaves of *P. laurentii*. In this contribution, we report the isolation and structure elucidation of a new ceramide, pancoviamide (**1**), and a new cerebroside, pancovioside (**2**). The structures of compounds **1** and **2** were elucidated on the basis of spectroscopic analysis. In addition, the known constituents uracil, (*R*)-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)heptadec-5-en-1-yl]-2-hydroxytetracosanamide, stigmasta-7,22-dien-3-ol, *β*-stitosterol, *β*-sitosterol 3-*O*-*β*-D-glucopyranoside, and 2,3-dihydroxypropyl pentadecanoate were isolated.

Results and Discussion. – The air-dried and ground bark and leaves of *P. laurentii* were extracted separately with MeOH. The residue obtained after evaporation of the solvent from the bark extract was partitioned with H_2O/AcOEt 2:3. These extracts were subjected to successive column chromatographies, and led to the isolation of eight constituents, including one new ceramide, **1**, and one new cerebroside, **2**.

Compound **1** was isolated as an amorphous powder from the fraction with hexane/AcOEt 1:1. The molecular formula, $C_{42}H_{83}NO_5$, with two degrees of unsaturation, was deduced from the combination of ESI-MS, and ^{1}H- and ^{13}C-NMR spectral analyses (*Table 1*). The IR spectrum showed vibration for OH groups at 3329 cm^{-1}. The typical IR absorptions at 1618 and 1539 cm^{-1} suggested an amide linkage, which was confirmed by a signal of N-attached C-atom at δ(C) 53.1 and a signal of a CO group at δ(C) 175.4 in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum in (D_5)pyridine (*Table 1*) exhibited signals of an amide H-atom (*doublet* at δ(H) 8.60 ($J = 9.2$)), of long-chain of CH_2 H-atoms (δ(H) 1.28–1.33. (br. *s*)), and of two terminal Me groups (δ(H) 0.88 (*t*, $J = 6.6$)), indicating a sphingolipid skeleton [7]. The ^{1}H-NMR spectrum also displayed five signals, characteristic of H-atoms geminal to OH groups, at δ(H) 4.42–4.47 (*m*, H_a–C(1)), 4.51–4.56 (*m*, H_b–C(1)), 4.36–4.39 (*m*, H–C(3)), 4.30–4.35 (*m*, H–C(4)), and 4.64–4.65 (*m*, H–C(2′)). The ^{13}C-NMR spectrum displayed signals for four C-atoms linked to O-atoms at δ(C) 62.2 (C(1)), 76.9 (C(3)), 73.2 (C(4)), and 72.6 (C(2′)), respectively, which were assigned on the basis of HSQC data to the

Table 1. *NMR Data for Pancoviamide* (**1**). In (D_5)pyridine; δ in ppm, J in Hz.

	δ(C)	δ(H)	^{1}H,^{1}H-COSY	^{1}H,^{13}C-HMBC
H_a–C(1)	62.2 (*t*)	4.42–4.47 (*m*)	H_b–C(1), H–C(2), OH	C(2), C(3)
H_b–C(1)		4.51–4.56 (*m*)	H_a–C(1), H–C(2), OH	C(2), C(3)
H–C(2)	53.1 (*d*)	5.11–5.17 (*m*)	H_a–C(1), H_b–C(1), H–C(3), NH	C(1), C(3), C(1′)
H–C(3)	76.9 (*d*)	4.36–4.39 (*m*)	H–C(2), H–C(4), OH	C(2), C(4), C(1), C(5)
H–C(4)	73.2 (*d*)	4.30–4.35 (*m*)	H–C(3), H–C(5), OH	
H_a–C(5)	34.3 (*t*)	1.93–2.01 (*m*)		
H_b–C(5)		2.27–2.32 (*m*)		
CH_2(6–21)	30.0–30.2 (*t*)	1.28–1.33 (br. *s*)		
CH_2(22)	32.3 (*t*)	1.22–1.25 (*m*)		
CH_2(23)	23.1 (*t*)	1.25–1.29 (*m*)		
Me(24)	14.4 (*q*)	0.88 (*t*, $J = 6.6$)		
NH		8.60 (*d*, $J = 9.2$)	H–C(2)	
H–C(1′)	175.4 (*s*)			
H–C(2′)	72.6 (*d*)	4.64–4.65 (*m*)	H–C(3′), OH	C(1′)
H_a–C(3′)	35.8 (*t*)	2.20–2.26 (*m*)	H–C(2′)	C(4′), C(1′)
H_b–C(3′)		2.01–2.12 (*m*)	H–C(2′)	
H–C(4′)	130.8 (*d*)	5.51–5.57 (*m*)	H–C(3′)	C(6′)
H–C(5′)	130.9 (*d*)	5.62–5.66 (*m*)	H–C(6′)	C(6′)
CH_2(6′)	33.1 (*t*)	2.16–2.19 (*m*)	H–C(5′)	C(5′)
CH_2(7′)	25.9 (*t*)	2.01–2.12 (*m*)		
CH_2(8′–15′)	30.0–30.2 (*t*)	1.28–1.33 (br. *s*)		
CH_2(16′)	32.3 (*t*)	1.22–1.25 (*m*)		
CH_2(17′)	23.1 (*t*)	1.25–1.29 (*m*)	H–C(18′)	
Me(18′)	14.4 (*q*)	0.88 (*t*, $J = 6.6$)	H–C(17′)	

previous H-atoms. The locations of four OH groups at C(1), C(3), C(4), and C(2′) were determined from the HMBC spectrum, which showed cross-peaks, between the H-atom signal at δ(H) 4.42–4.47 (*m*, H_a–C(1)) and the C-atom signals at δ(C) 53.1 (C(2)) and 76.9 (C(3)), the H-atom signal at δ(H) 5.11–5.17 (*m*, H–C(2)) and the C-atom signals at δ(C) 62.2 (C(1)), 76.9 (C(3)), and 175.4 (C(1′)), the H-atom signal at δ(H) 4.36–4.39 (*m*, H–C(3)) and the C-atom signals at δ(C) 53.1 (C(2)), 73.2 (C(4)), 62.2 (C(1)), and 34.5 (C(5)), and between the H-atom signal at δ(H) 4.64–4.65 (*m*, H–C(2′)) and the C-atom signal at δ(C) 175.4 (C(1′)). The ^{1}H-NMR spectrum of **1** also showed signals of two olefinic H-atoms of a disubstituted C=C moiety at δ(H) 5.51–5.57 (*m*, H–C(4′)) and 5.62–5.66 (*m*, H–C(5′)), which was substantiated by the signals observed in the ^{13}C-NMR and DEPT-135 spectra at δ(C) 130.8 and 130.9. In the HMBC spectrum, cross-peaks between the H-atom signal at δ(H) 2.20–2.26 (*m*, H_b–C(3′)) and the C-atom signals at δ(C) 130.8 (C(4′)) and 175.4 (C(1′)), and between the H-atom signal at δ(H) 2.16–2.19 (*m*, H–C(6′)) and the C-atom signal at δ(C) 130.8 (C(4′)), evidenced the C(4′)=C(5′) bond. The ^{1}H,^{1}H-COSY spectrum showed cross-peaks between the olefinic H-atom signal at δ(H) 5.51–5.57 (*m*, H–C(4′)) and the H-atom signal at δ(H) 2.01–2.12 (*m*, H_a–C(3′)), the H-atom signal at δ(H) 4.64–4.65 (*m*, H–C(2′)) and the H-atom signal at δ(H) 2.20–2.26 (*m*, H_b–C(3′)), confirming the location of the C=C bond. In addition, the two CH_2 H-atom signals at δ(H) 2.20–2.26 (*m*, H_b–C(3′)) and 2.01–2.12 (*m*, H_a–C(3′)) inter-correlate and also correlate to the signal of a H-atom at the O-bearing C-atom at δ(H) 4.64–4.65 (*m*, H–C(2′)), suggesting the presence of a –CO–CH(OH)–CH_2–CH=CH moiety [15][16]. The other olefinic H-atom signal at δ(H) 5.62–5.66 (*m*, H–C(5′)) showed cross-peaks with CH_2 H-atom signals at δ(H) 2.16–2.19 (*m*, H–C(6′)), which further showed cross-peaks with long-chain CH_2 H-atom signals at δ(H) 1.28–1.33 (br. *s*). The configuration of the C=C bond was found to be (*E*) as evidenced by the chemical shifts of C(3′) (δ(C) 35.8) and C(6′) (δ(C) 33.1). In fact, it is known that the geometry of the C=C bond in the long-chain alkene can be determined on the basis of the ^{13}C-NMR chemical shift of the CH_2 C-atom adjacent to the olefinic C-atom, which is observed around δ(C) 27 in (*Z*)-isomer and δ(C) 33 in (*E*)-isomer [17]. The fatty acid and sphingosine chain lengths were determined by characteristic fragment-ion peaks observed in the different mass spectra. Indeed, the length of the fatty acid was determined from EI-MS (*Fig. 1*), which showed the ion peak at *m*/*z* 289 ([$Me(CH_2)_{12}CH$=$CHCH_2CHOHCOH + Li]^+$)

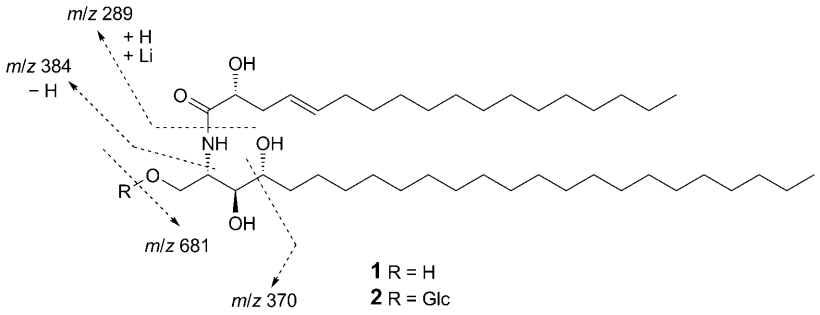
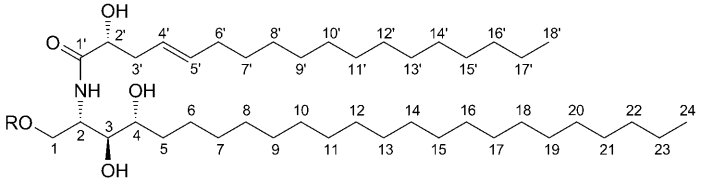


Fig. 1. *Mass fragmentation pattern of compounds* **1** *and* **2**

[18][19]. The length of the long-chain base was also obtained from EI-MS (*Fig. 1*), which showed significant fragment-ion peaks at *m/z* 384 ($[M - C_{16}H_{31}CHOHCONH_2]^+$) and 370 ($[M - CH_3(CH_2)_{19}CHOH]^+$). Consideration of biogenesis and steric hindrance of sphingolipids, and the chemical shift of the H–C(2) signal and the C-atom signals of C(1) to C(4), C(1′), and C(2′) of sphingolipids generally allow us to determine the absolute configuration of the phytosphingosine moiety. The H-atom signal at δ(H) 5.11–5.17 (*m*, H–C(2)) and the C-atom signals at δ(C) 62.2 (C(1)), 76.9 (C(3)), 175.4 (C(1′)), and 72.6 (C(2′)) in **1** were nearly identical to those of previously reported ceramides in the literature [20][21], indicating the same configuration. Thus, the structure of **1** was established as (2*R*,4*E*)-2-hydroxy-*N*-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxytetracosan-2-yl]octadec-4-enamide, named pancoviamide (*Fig. 2*).



1 R = H
2 R = Glc

Fig. 2. *Structures of compounds* **1** *and* **2**

Compound **2** was isolated as an amorphous white powder from the fraction with AcOEt/MeOH 9:1. The molecular formula, $C_{48}H_{93}NO_{10}$, with three degrees of unsaturation, was deduced from HR-ESI-MS, which exhibited a *pseudo*-molecular-ion peak $[M + K]^+$ at *m/z* 882.64245 (calc. 882.64254), and the combination of ^{1}H- and ^{13}C-NMR spectral analyses (*Table 2*). Compound **2** responded positively to the *Molish* test, suggesting that it was a glycoside. The IR spectrum showed an absorption band at 3306 cm^{-1} which indicated the presence of OH groups. The typical IR absorptions at 1633 and 1537 cm^{-1} suggested an amide linkage, which was confirmed by a N-attached C-atom signal at δ(C) 52.1 and a CO signal at δ(C) 176.1 in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum in (D_5)pyridine (*Table 2*) showed signals of an amide H-atom (*doublet* at δ(H) 8.60 ($J = 9.2$)), long-chain CH_2 H-atoms (δ(H) 1.28–1.33 (br. *s*)), two terminal Me groups (δ(H) 0.88 (*t*, $J = 6.9$)), also indicating, as in **1**, a sphingolipid skeleton [18]. Furthermore, the ^{1}H-NMR spectrum also exhibited signals of an anomeric H-atom of sugar at δ(H) 4.96 (*d*, $J = 8.0$) and eleven other carbinol H-atoms appearing as *multiplet*s between δ(H) 3.85 and 4.77, and suggesting the glycosphingolipid nature of **2** [18]. The appearance of C-atom signals at δ(C) 106.0 (C(1″)), 75.6 (C(2″)), 78.8 (C(3″)), 72.8 (C(4″)), 78.9 (C(5″)), and 63.0 (C(6″)) in the ^{13}C-NMR spectrum, and the correlation observed in HMBC spectrum between the signals of H-atom at δ(H) 4.96 and the C-atom at δ(C) 106.0 are characteristic of a β-glucopyranosyl group as a sugar moiety in **2** [22][23]. The other parts of the different spectra of **2** were similar to those of **1**. The EI-MS spectrum (*Fig. 1*), besides the typical fragment-ion peaks of **1**, also showed an additional fragment-ion peak at *m/z* 682 ($[M + H - 162)]^+$), revealing the elimination of one terminal hexosyl moiety [24], and confirming the existence of the glucopyranosyl group in **2**. All these findings indicated that **2** is the

Table 2. *NMR Data for Pancovioside* (**2**). In (D_5)pyridine; δ in ppm, J in Hz.

	δ(C)	δ(H)	^{1}H,^{1}H-COSY	^{1}H,^{13}C-HMBC
H_a–C(1)	70.9 (*t*)	4.50–4.56 (*m*)	H_b–C(1), H_b–C(2), OH	C(2), C(3), C(1″)
H_b–C(1)		4.73–4.77 (*m*)	H_a–C(1), H_b–C(2), OH	C(2), C(3), C(1″)
H–C(2)	52.1 (*d*)	5.31–5.36 (*m*)	H_b–C(1), H_a–C(1), H_b–C(3), NH	C(1), C(3), C(1′)
H–C(3)	76.3 (*d*)	4.33–4.36 (*m*)	H–C(2), H–C(4), OH	
H–C(4)	71.9 (*d*)	4.20–4.26 (*m*)	H–C(3), H–C(5), OH	C(3)
H_a–C(5)	34.3 (*d*)	1.88–1.96 (*m*)		
H_b–C(5)		2.15–2.31 (*m*)		
CH_2(6–21)	29.9–30.7 (*t*)	1.28–1.33 (br. *s*)		
CH_2(22)	32.5 (*t*)	1.21–1.25 (*m*)		
CH_2(23)	23.4 (*t*)	1.26–1.29 (*m*)		
Me(24)	14.7 (*q*)	0.88 (*t*, J = 6.9)	H–C(23)	
NH		8.60 (*d*, J = 9.2)	H–C(2)	C(2), C(1′)
H–C(1′)	176.1 (*s*)			
H–C(2′)	72.9 (*d*)	4.56–4.60 (*m*)	H–C(3′), OH	C(1′)
H_a–C(3′)	35.9 (*t*)	2.15–2.31 (*m*)	H–C(2′), H_b–C(3′)	C(4′), C(1′)
H_b–C(3′)		1.97–2.05 (*m*)	H–C(2′), H–C(4′), H_a–C(3′)	
H–C(4′)	130.6 (*d*)	5.44–5.57 (*m*)	H–C(3′)	C(6′)
H–C(5′)	130.8 (*d*)	5.44–5.57 (*m*)	H–C(6′)	C(6′)
CH_2(6′)	33.4 (*t*)	1.97–2.05 (*m*)	H–C(5′)	C(5′)
CH_2(7′)	27.8 (*t*)	2.06–2.11 (*m*)		
CH_2(8′–15′)	29.9–30.7 (*t*)	1.28–1.33 (br. *s*)		
CH_2(16′)	32.5 (*t*)	1.21–1.25 (*m*)		
CH_2(17′)	23.4 (*t*)	1.26–1.29 (*m*)	H–C(18′)	
Me(18′)	14.7 (*q*)	0.88 (*t*, J = 6.9)		
Glc				
H–C(1″)	106.0 (*d*)	4.96 (*d*, J = 8.0)	H–C(2″)	C(3″)
H–C(2″)	75.6 (*d*)	4.02–4.07 (*m*)	H–C(1″)	C(3″), C(1″)
H–C(3″)	78.8 (*d*)	4.20–4.26 (*m*)		C(2″)
H–C(4″)	72.8 (*d*)	4.20–4.26 (*m*)	H–C(5″)	C(5″)
H–C(5″)	78.9 (*d*)	3.85–3.90 (*m*)	H–C(4″), H_a–C(6″)	
H_a–C(6″)	63.0 (*t*)	4.35–4.39 (*m*)	H_b–C(6″), H–C(5″)	C(5″)
H_b–C(6″)		4.50–4.56 (*m*)	H_a–C(6″)	

corresponding β-D-glucopyranoside of **1**. On the basis of this evidence, the structure of **2** was assigned as (2*R*,4*E*)-*N*-[(2*S*,3*S*,4*R*)-1-(β-D-glucopyranosyloxy)-3,4-dihydroxytetracosan-2-yl]-2-hydroxyoctadec-4-enamide, named pancovioside (*Fig. 2*). Previous phytochemical studies in some Sapindaceae species led to the isolation of cerebrosides and ceramides in this family [7][8], with various biological activities [25–27].

The structures of the known compounds were established by comparing their spectral and physical data with reported data as uracil [28], (*R*)-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)heptadec-5-en-1-yl]-2-hydroxytetracosanamide [29], stigmasta-7,22-dien-3-ol [30], β-stitosterol [30], β-sitosterol 3-*O*-β-D-glucopyranoside [31], and 2,3-dihydroxypropyl pentadecanoate [32].

Compounds **1** and **2** were tested against *Plasmodium falciparum* K1 chloroquine-resistant strain, *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, and *Trypanosoma cruzi*, protozoa responsible for malaria, visceral leishmaniasis, African trypanosomiasis, and chagaes disease, respectively, according to the method described in [33].

Compounds **1** and **2**, together with crude extracts, were also tested for their cytotoxicities. However, no significant effect was detected in this bioassay with the two compounds ($IC_{50} > 5$; *Table 3*).

Table 3. In vitro *Antiprotozoal and Cytotoxicity Activities of Compounds* **1**, **2**, *and Extracts*

Sample	IC_{50} [μg/ml]				
	T. b. rhodesiense	*T. cruzi*	*L. donovani*	*P. falciparum K1*	Cytotoxicity L6
Bark (AcOEt)	nt[a])	nt	nt	1.14	13.7
Leaves	nt	nt	nt	3.64	46.6
1	53.450	> 90	> 90	> 5	> 90
2	> 90	> 90	> 90	> 5	> 90
Melarsoprol[b])	0.004				
Benznidazole[c])		0.536			
Miltefosine[d])			0.194		
Chloroquine[e])				0.037	
Podophyllotoxin[f])					0.004

[a]) nt = Not tested. [b]) Reference drug for *T. b. rhodesiense*. [c]) Reference drug for *T. cruzi*. [d]) Reference drug for *L. donovani*. [e]) Reference drug for *P. falciparum*. [f]) Reference drug for the cytotoxicity test.

We wish to acknowledge the *Service de Coopération et d'Action Culturelle de l'Ambassade de France au Cameroun* for awarding a grant to *F. Tantangmo* at Strasbourg University, and *The Academy of Science for the Developing World* (TWAS) for awarding a research grant No. 07-141 LDC/CHE/AF/AC-UNESCO FR: 3240171776 to our *TWAS Research Unit*. We also thank Mr. *P. Chabert* and Mr. *C. Antheaume* for their contribution to the structure elucidation and NMR analysis, respectively.

Experimental Part

General. M.p.: *Büchi-540* melting-point apparatus. Optical rotations: in $CHCl_3$/MeOH soln. on a *Jasco* digital polarimeter (model *DIP-360*). IR Spectra: *Jasco Fourier*-transform IR spectrometer. ^{1}H- and ^{13}C-NMR spectra: *Bruker* spectrometer equipped with 5-mm ^{1}H and ^{13}C probes operating at 500 and 125 MHz resp., with TMS as internal standard. Flash column chromatography (CC): silica gel (SiO_2; 230–400 and 70–230 mesh; *Merck*). TLC: percolated aluminium silica gel *60 F_{254}* sheets; with different mixtures of petroleum ether (PE), hexane, AcOEt, and acetone as eluents; spots were visualized under UV light (254 and 365 nm) or with MeOH/H_2SO_4 reagent.

Plant Material. The stem bark and leaves of *Pancovia laurentii* were collected in August 2008 at Mont Kala (Yaoundé) in the Centre province of Cameroon and identified by Mr. *V. Nana*, botanist at the National Herbarium of Cameroon, where a voucher specimen has been deposited (N° 3816/SFRK).

Extraction and Isolation. The dried bark (2 kg) and leaves (800 g) of *P. laurentii* were extracted with MeOH (2 × 10 l) for 48 h. The extracts were concentrated under vacuum at r.t. to afford 90 g (brown) and 20 g (green) extracts, resp. The bark extract was suspended in H_2O and extracted successively with AcOEt and BuOH to afford 30 and 45 g extracts, resp. The latter AcOEt bark extract (25 g) was fractionated by CC (SiO_2, 230–400 mesh; gradient mixtures hexane/AcOEt). Ninety fractions of 400 ml each were collected and combined on the basis of TLC to yield four main fractions: *Fr. 1* (2.0 g), *Fr. 2* (5.1 g), *Fr. 3* (6.4 g), and *Fr. 4* (10.5 g).

Fr. 1 and *Fr. 4* were complex mixtures that were not further studied. *Fr. 2* (5.1 g) was subjected to CC (SiO_2, 70–230 mesh; hexane/AcOEt mixtures of increasing polarity) and resulted in the collection of 176 fractions of 150 ml each, which were combined on the basis of TLC analysis. Further purification of *Subfrs. 20–35* afforded *β*-sitosterol (200 mg) and 2,3-dihydroxypropylpentadecanoate (28 mg). *Subfrs. 123* and *134* yield (*R*)-*N*-[(1*S*,2*S*,3*R*)-2,3-dihydroxy-1-(hydroxymethyl)heptadec-5-en-1-yl]-2-hydroxy-

tetracosanamide (11 mg). *Fr. 3* (6.4 g) was subjected to CC (SiO_2, 70–230 mesh; hexane/AcOEt gradient) and yielded β-sitosterol 3-*O*-β-D-glucopyranoside (400 mg) and pancovioside (**2**; 4 mg).

The MeOH leaves extract (17 g) was fractionated by CC (SiO_2, 230–400 mesh; hexane/AcOEt mixtures of increasing polarity). Seventy fractions of 200 ml each were collected and combined on the basis of TLC analysis to yield three main fractions: *Fr. A* (1.5 g), *Fr. B* (4.2 g), and *Fr. C* (7.0 g). *Fr. C* was a complex mixture that was not further studied. *Fr. B* (4.2 g) was subjected CC (SiO_2, 70–230 mesh; hexane/AcOEt gradient of increasing polarity) and resulted in the collection of 300 fractions of 150 ml each. Further purification of *Subfrs. 200–237* afforded *pancoviamide* (**1**; 5 mg). *Subfrs. 270–290* yielded uracil (100 mg). Chromatography of *Fr. A* (1.5 g) yielded essentially stigmasta-7,22-dien-3-ol (1300 mg).

Pancoviamide (=(*2*R,*4*E)*-2-Hydroxy*-N-*[(2*S,*3*S,*4*R)*-1,3,4-trihydroxytetracosan-2-yl]octadec-4-enamide*; **1**). Colorless powder. M.p. 145.4°. $[\alpha]_D^{20} = +16.66$ ($c = 0.006$, $CHCl_3$/MeOH 1:1). IR (KBr): 3329, 1618, 1539. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 688 ($[M + Li]^+$), 289 ($[Me(CH_2)_{12}CH{=}CHCH_2CHOHCOH + Li]^+$). EI-MS: 384 (25, $[M - C_{16}H_{31}CHOHCONH_2]^+$), 370 (30, $[M - Me(CH_2)_{19}CHOH]^+$).

Pancovioside (=(*2*R,*4*E)-N-*[(2*S,*3*S,*4*R)*-1-(*β*-D-Glucopyranosyloxy)-3,4-dihydroxytetracosan-2-yl)-2-hydroxyoctadec-4-enamide*; **2**). Colorless powder. M.p. 178.0°. $[\alpha]_D^{20} = +10.56$ ($c = 0.00375$, $CHCl_3$/MeOH 1:1). IR (KBr): 3306, 1633, 1537, 1072. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 289 ($[Me(CH_2)_{12}CH{=}CHCH_2CHOHCOH + Li]^+$), 682 ($[M + H - 162)]^+$). HR-ESI-MS: 882.64245 ($[M + K]^+$, $C_{48}H_{93}KNO_{10}^+$; calc. 882.64254). EI-MS: 384 (50, $[M - C_{16}H_{31}CHOHCONH_2]^+$), 682 (100, $[M + H - 162)]^+$), 370 (30, $[M - Me(CH_2)_{19}CHOH]^+$).

REFERENCES

[1] A. Aubreville, J. F. Leroy, 'Flore du Cameroun: Les Sapindaceae', Muséum National d'Histoire Naturelle, Paris, 1973, Vol. 1, p. 3.
[2] W. Löscher, D. Hönack, C. P. Fassbender, B. Nolting, *Epilepsy Res.* **1991**, *8*, 171.
[3] A. Raponda-Walker, R. Sillans, 'Les plantes utiles du Gabon', Paris, 1961, p. 388.
[4] C. S. Weckerle, M. A. Stutz, T. W. Baumann, *Phytochemistry* **2003**, *64*, 735.
[5] L. Voutquenne, C. Lavaud, G. Massiot, C. Delaude, *Phytochemistry* **1998**, *49*, 2081.
[6] K. Sachdev, D. K. Kulshreshtha, *Phytochemistry* **1983**, *22*, 1253.
[7] R. S. Miemanang, K. Krohn, H. Hussain, S. F. Kouam, E. Dongo, *Z. Naturforsch., B* **2006**, *61*, 1123.
[8] R. F. Soh, J. K. Bankeu, B. N. Lenta, B. M. Mba'ning, S. Ngouela, E. Tsamo, N. Sewald, *Z. Naturforsch., B* **2009**, *64*, 1070.
[9] M. L. de Mesquita, P. Grellier, A. Blond, J.-P. Brouard, J. E. de Paula, L. S. Espindola, L. Mambu, *Bioorg. Med. Chem.* **2005**, *13*, 4499.
[10] O. K. Yemitan, O. O. Adeyemi, *Fitoterapia* **2005**, *76*, 412.
[11] N. Uawonggul, A. Chaveerach, S. Thammasirirak, T. Arkaravichien, C. Chuachan, S. Daduang, *J. Ethnopharmacol.* **2006**, *103*, 201.
[12] A. Basile, L. Ferrara, M. Del Pezzo, G. Mele, S. Sorbo, P. Bassi, D. Montesano, *J. Ethnopharmacol.* **2005**, *102*, 32.
[13] A. L. Meyer Albiero, J. A. A. Sertié, E. M. Bacchi, *J. Ethnopharmacol.* **2002**, *82*, 41.
[14] S. E. Besra, R. M. Sharma, A. Gomes, *J. Ethnopharmacol.* **1996**, *54*, 1.
[15] K. Chebaane, M. Guyot, *Tetrahedron Lett.* **1986**, *27*, 1495.
[16] R. C. Bheemasankara, C. Satyanarayana, *Ind. J. Chem., Sect. B* **1994**, *33*, 97.
[17] N. Fusetani, K. Yasumoto, S. Matsunaga, H. Hirota, *Tetrahedron Lett.* **1989**, *30*, 6891.
[18] D. Tazoo, K. Krohn, H. Hussain, S. F. Kouam, E. Dongo, *Z. Naturforsch., B* **2007**, *62*, 1208.
[19] N. Mukhtar, K. Iqbal, I. Anis, A. Malik, *Phytochemistry* **2002**, *61*, 1005.
[20] F. Cateni, J. Zilic, G. Falsone, G. Scialino, E. Banfi, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4345.
[21] N.-Y. Yang, D.-C. Ren, J.-A. Duan, X.-H. Xu, N. Xie, L.-J. Tian, *Helv. Chim. Acta* **2009**, *92*, 291.
[22] L. P. Sandjo, P. Hannewald, M. Yemloul, G. Kirsch, B. T. Ngadjui, *Helv. Chim. Acta* **2008**, *91*, 1326.
[23] K. Bock, C. Pedersen, *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27.

[24] A. L. Tapondjou, A. C. Mitaine-Offer, M. Sautour, T. Miyamoto, M.-A. Lacaille-Dubois, *Biochem. Syst. Ecol*. **2005**, *33*, 1293.
[25] T. Natori, M. Morita, K. Akimoto, Y. Koezuka, *Tetrahedron* **1994**, *50*, 2771.
[26] F. Ramos, Y. Takaishi, K. Kawazoe, C. Osorio, C. Duque, R. Acuña, Y. Fujimoto, M. Sato, M. Okamoto, T. Oshikawa, S. U. Ahmed, *Phytochemistry* **2006**, *67*, 1143.
[27] F. Cateni, J. Zilic, G. Falsone, F. Hollan, F. Frausin, V. Scarcia, *Il Farmaco* **2003**, *58*, 809.
[28] R.-F. Wang, X.-W. Yang, C.-M. Ma, M.-Y. Shang, J.-Y. Liang, X. Wang, S.-Q. Cai, Y. Shoyama, *Phytochemistry* **2003**, *63*, 475.
[29] H. S. Garg, S. Agrawal, *J. Nat. Prod.* **1995**, *58*, 442.
[30] N. V. Kovganko, Z. N. Kashkan, E. V. Borisov, E. V. Batura, *Chem. Nat. Compd*. **1999**, *35*, 646.
[31] F. M. Moghaddam, M. M. Farimani, S. Salahvarzi, G. Amin, *Evid. Based Complem. Altern. Med.* **2007**, *4*, 95.
[32] T. Sabudak, E. Işik, S. Öksüz, *Nat. Prod. Res.* **2007**, *21*, 828.
[33] B. N. Lenta, C. Vonthron-Sénécheau, R. F. Soh, F. Tantangmo, S. Ngouela, M. Kaiser, E. Tsamo, R. Anton, B. Weniger, *J. Ethnopharmacol.* **2007**, *111*, 8.

Received February 25, 2010