Triterpenoid Derivatives from *Cylicodiscus gabunensis*

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Three new olean-12-ene derivatives (1—3), together with known urs-12-ene-3β, 28-diol (4) were isolated from the stem root of *Cylicodiscus gabunensis*. The structures of the new compounds were established by chemical and spectroscopic means as β-amyrin-*n*-nonyl ether (1), 22α-hydroxyolean-12-en-3β-yl-β-D-galactopyranoside (2), and 24-hydroxyolean-12-en-3β-yl-β-D-glucopyranoside (3).

Key words *Cylicodiscus gabunensis*; Mimosaceae; olean-12-ene; triterpene; β-amyrin derivative; triterpene glycoside

The *Cylicodiscus* genus belongs to the Mimosaceae family comprising 64 genera and 2950 species, mostly tropical.[1] *Cylicodiscus gabunensis* Harms (*C. gabunensis*), known as Denya (Ghana), Edum (Gabon), Adoum, Bokoka (Cameroon), or Bouemon (Ivory Coast), is a large tree, common in the rain forests of Sierra Leone to the Cameroon and Gabon.[2,3] In the traditional medicine its stem bark is used for various therapeutic purposes.[4,5] Preliminary bioassays on the crude EtOAc extract from the stem bark of *C. gabunensis* have shown antidiarrheal and antimicrobial activities.[6] These results may be explained by the presence of triterpenoids, a class of phytochemical compounds known for their broad spectrum of biological properties.[7—10] Recently, we reported that the stem bark of *C. gabunensis* contained coumestan and triterpenoid glycosides.[6,11] As part of our continuing chemical investigation of *C. gabunensis* aimed at the potentially active constituents, phytochemical screening was performed on the stem root, resulting in the isolation of three new triterpenoid derivatives (**1**—**3**), and known urs-12-ene-3β, 28-diol (**4**). This paper describes the isolation and structural elucidation of these new compounds.

Results and Discussion

The crude methanolic extract of the stem root of *C. gabunensis* was successively extracted with hexane, and EtOAc to give 15.8 g and 122 g of extract, respectively. A portion of the EtOAc extract was subjected to column chromatography (CC) as described in the Experimental to afford compounds **1**, **2**, **3**, and **4**.

Compound **1** was isolated as white powder and gave a positive Lieberman–Burchard test for triterpenoids. Combined with broad-band-decoupled ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) analysis, its molecular formula was determined as $C_{39}H_{68}O$ by high resolution-electron ionization-MS (HR-EI-MS) spectrum, showing a molecular ion peak (M^+) at m/z=552.5271.

The IR spectrum exhibited absorptions at 1650 cm^{-1} (double bond), and 1357 cm^{-1} (ether function). Interpretation of ^{1}H–^{1}H shift correlation spectroscopy (COSY), ^{1}H-detected-heteronuclear multiple-quantum coherence (HMQC), and ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) spectra led assignments of ^{1}H- and ^{13}C-NMR data as shown in Table 1. The ^{13}C-NMR spectrum of **1** was almost superimposable with that of β-amyrin[12] except that the former disclosed additional *O*-*n*-nonyl signals [δ_C 63.1, 26.3, 25.7, 29.7, 29.6, 29.3, 31.9, 22.7 (each CH_2), δ_C 14.4 (CH_3)] suggesting that **1** is a *n*-nonyl ether derivative of the latter compound. Location of the *O*-*n*-nonyl group was confirmed by the HMBC spectrum showing correlation between H-1′ (δ_H 3.60) of the *O*-*n*-nonyl group and C-3 (δ_C 78.4) of the β-amyrin moiety. Consequently, the structure of **1** was determined as β-amyrin-*n*-nonyl ether (**1**) (see Fig. 1).

Compound **2** was isolated as white powder. Its high resolution-FAB-MS (HR-FAB-MS) spectrum exhibited a M^+ peak at m/z=604.4338 consistent with the molecular formula $C_{36}H_{60}O_7$. The positive response to the Lieberman–Burchard test, as well as inspection of the ^{13}C- and ^{1}H-NMR spectral data revealed **2** to be a saponin. The ^{1}H-NMR spectrum of aglycone revealed eight tertiary methyl groups [δ_H 0.76, 0.86, 0.88, 0.92, 0.95, 0.98, 1.03, 1.08 (each 3H, s)], an olefinic proton [δ_H 5.23 (1H, t, J=3.6 Hz)], and two oxygenated CH groups [δ_H 3.35 (1H, dd, J=5.2, 11.1 Hz), 3.40 (1H, dd, J=13.7, 4.3 Hz)]. Moreover, the ^{13}C-NMR and ^{13}C-DEPT spectra of aglycone indicated characteristic signals of two oxygenated C-atoms [δ_C 81.4, 76.6 (each CH)], and two

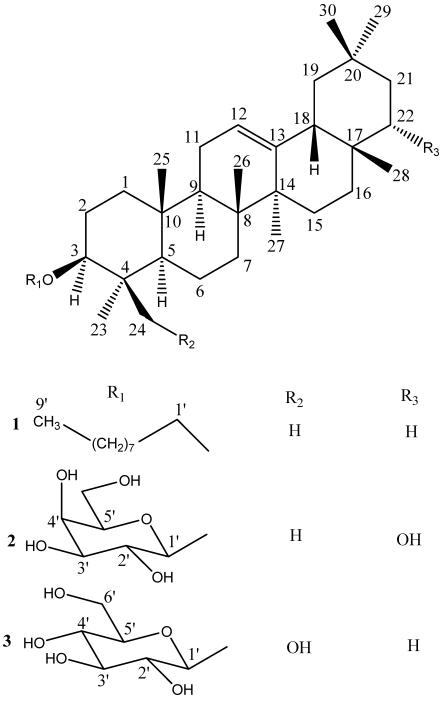

Fig. 1. Structures of Isolated Triterpenoid Derivatives **1**—**3**

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Table 1. The ^{1}H-NMR (δ_H in ppm, 500 MHz) and ^{13}C-NMR (δ_C in ppm, 125 MHz) Data of Compounds **1**—**3**

No.	**1**		**2**		**3**	
	δ_H (mult, J)[a)]	δ_C[a)] (DEPT)	δ_H (mult, J)[b)]	δ_C[b)] (DEPT)	δ_H (mult, J)[b)]	δ_C[b)] (DEPT)
1	1.10 (1H, t-like, 10.9)	38.6 (CH_2)	1.12 (1H, m)	36.6 (CH_2)	1.11 (1H, dd, 13.2, 12.2)	38.4 (CH_2)
	1.60 (1H , m)		1.59 (1H, m)		1.65 (1H, m)	
2	2.01 (1H, m)	27.4 (CH_2)	2.22 (1H, m)	27.3 (CH_2)	2.23 (1H, m)	27.7 (CH_2)
	1.59 (1H, m)		1.88 (1H, m)		1.92 (1H, m)	
3	3.21 (1H, dd, 4.4, 10.6)	78.4 (CH)	3.35 (1H, dd, 5.2, 11.1)	81.4 (CH)	3.63 (1H, dd, 5.1, 10.2)	81.9 (CH)
4		38.7 (C)		37.55 (C)		42.8 (C)
5	0.69 (1H, d, 11.6)	55.2 (CH)	0.75 (1H, d, 11.2)	55.3 (CH)	0.75 (1H, d, 11.3)	55.8 (CH)
6	1. 40 (1H, m)	18.5 (CH_2)	1.50 (1H, m)	18.5 (CH_2)	1.49 (1H, m)	19.7 (CH_2)
	1.29 (1H, m)		1.33 (1H, m)		1.34 (1H, m)	
7	1.26 (1H, m)	32.7 (CH_2)	1.27 (1H, m)	32.9 (CH_2)	1.27 (1H, m)	32.9 (CH_2)
	1.01 (1H, d, 12.3)		1.04 (1H, d, 12.6)		1.05 (1H, d, 12.2)	
8		38.2 (C)		39.9 (C)		40.6 (C)
9	1.59 (1H, dd, 12.2, 4.2)	47.7 (CH)	1.63 (1H, dd, 12.4, 5.3)	47.6 (CH)	1.63 (1H, dd, 12.3, 5.1)	47.0 (CH)
10		36.2 (C)		36.9 (C)		36.7 (C)
11	1.76 (1H, m)	23.5 (CH_2)	1.79 (1H, m)	23.5 (CH_2)	1.77 (1H, m)	23.3 (CH_2)
	2.01 (1H, m)		2.03 (1H, m)		2.05 (1H, m)	
12	5.16 (1H, t, 3.5)	121.7 (CH)	5.23 (1H, t, 3.6)	122.5 (CH)	5.19 (1H, t, 3.9)	121.7 (CH)
13		145.2 (C)		143.9 (C)		145.2 (C)
14		41.7 (C)		42.1 (C)		41.7 (C)
15	1.03 (1H, dd, 11.3, 5.4)	26.2 (CH_2)	1.03 (1H, dd, 10.8, 4.6)	25.9 (CH_2)	1.02 (1H, dd, 10.5, 5.5)	26.9 (CH_2)
	1.48 (1H, m)		1.52 (1H, m)		1.50 (1H, m)	
16	1.44 (1H, m)	27.2 (CH_2)	1.48 (1H, m)	29.8 (CH_2)	1.47 (1H, m)	26.1 (CH_2)
	1.05 (1H, dd, 11.3, 5.7)		1.07 (1H, 10.8, 4.9)		1.06 (1H, dd, 10.5, 4.8)	
17		32.6 (C)		37.4 (C)		32.4 (C)
18	2.15 (1H, dd, 9.7, 3.6)	47.2 (CH)	2.18 (1H, dd, 10.2, 4.1)	44.7 (CH)	2.15 (1H, dd, 9.9, 3.8)	47.3 (CH)
19	1.89 (1H, d, 3.6)	46.7 (CH_2)	1.92 (1H, d, 4.1)	46.2 (CH_2)	1.88 (1H , d, 3.8)	46.8 (CH_2)
	1.64 (1H, d, 9.7)		1.66 (1H, d, 10.2)		1.63 (1H, d, 9.9)	
20		31.1 (C)		30.5 (C)		31.1 (C)
21	1.69 (1H, dd, 11.6, 5.2)	34.8 (CH_2)	1.96 (1H, d, 4.3)	41.5 (CH_2)	1.70 (1H, dd, 11.8, 5.1)	34.7 (CH_2)
	1.14 (1H, m)		1.58 (1H, d, 13.7)		1.16 (1H, m)	
22	1.68 (1H, 11.6, 5.8)	37.1 (CH_2)	3.40 (1H, dd, 13.7, 4.3)	76.6 (CH)	1.71 (1H, dd, 11.8, 5.3)	37.1 (CH_2)
	1.12 (1H, m)				1.18 (1H, m)	
23	1.14 (3H, s)	28.2 (CH_3)	0.88 (3H, s)	28.1 (CH_3)	0.86 (3H, s)	22.3 (CH_3)
24	0.94 (3H, s)	15.5 (CH_3)	0.76 (3H, s)	15.6 (CH_3)	3.35 (1H, d, 11.2, 24a-H)	64.4 (CH_2)
					4.24 (1H, d, 11.2, 24b-H)	
25	0.99 (3H, s)	16.3 (CH_3)	0.98 (3H, s)	15.6 (CH_3)	1.10 (3H, s)	16.1 (CH_3)
26	0.87 (3H, s)	16.7 (CH_3)	1.03 (3H, s)	16.9 (CH_3)	0.88 (3H, s)	16.8 (CH_3)
27	1.25 (3H, s)	26.1 (CH_3)	1.08 (3H, s)	20.1 (CH_3)	1.32 (3H, s)	26.0 (CH_3)
28	0.83 (3H, s)	28.4 (CH_3)	0.86 (3H, s)	28.2 (CH_3)	1.13 (3H, s)	28.5 (CH_3)
29	0.83 (3H, s)	33.2 (CH_3)	0.95 (3H, s)	32.2 (CH_3)	0.88 (3H, s)	33.3 (CH_3)
30	0.97 (3H, s)	23.7 (CH_3)	0.92 (3H, s)	25.4 (CH_3)	0.94 (3H, s)	23.7 (CH_3)
1′	*n*-nonyl. 3.60 (2H, t, 4.4)	*n*-nonyl. 63.1 (CH_2)	Gal. 4.80 (1H, d, 7.8)	Gal. 100.7 (CH)	Glc. 5.32 (1H, d, 7.9)	Glc. 101.5 (CH)
2′	1.45 (2H, m)	26.3 (CH_2)	4.33 (1H, dd, 8.9, 8.1)	73.5 (CH)	3.50 (1H, dd, 8.8, 7.8)	74.5 (CH)
3′	1.31 (2H, m)	25.7 (CH_2)	4.04 (1H, dd, 9.2, 9.1)	76.4 (CH)	3.44 (1H, dd, 9.1, 8.8)	77.4 (CH)
4′	1.29 (2H, m)	29.7 (CH_2)	4.54 (1H, dd, 9.2, 9.3)	70.5 (CH)	3.42 (1H, dd, 9.7, 9.1)	71.2 (CH)
5′	1.30 (2H, m)	29.6 (CH_2)	3.83 (1H, m)	76.8 (CH)	3.43 (1H, m)	77.8 (CH)
6′	1.26 (2H, m)	29.3 (CH_2)	4.62 (1H, m)	61.5 (CH_2)	3.79 (1H, m)	62.1 (CH_2)
			4.15 (1H, m)		4.10 (1H, d, 10.5)	
7′	1.27 (2H, m)	31.9 (CH_2)				
8′	1.35 (2H, m)	22.7 (CH_2)				
9′	0.79 (3H, t, 3.5)	14.4 (CH_3)				

a) Measured in $CDCl_3$; *b*) Measured in DMSO-d_6.

olefinic C-atoms [δ_C 122.5 (CH), 143.9 (C)]. Comparison of the ^{1}H- and ^{13}C-NMR assignments of **2**, which were established by analysis of the ^{1}H–^{1}H COSY, HMQC, and HMBC spectra, with reported data suggested that the aglycone moiety was olean-12-ene-3β, 22-diol (α-sophoradiol).[13)]

Compound **2** was subjected to acid hydrolysis with 15% HCl/MeOH, to yield an aglycone, identified as α-sophoradiol by comparison of its physical and spectral data with those published,[13)] along with a sugar component, identified by direct HPLC analysis. The sugar was confirmed as D-galactose by comparison of its retention time and optical rotation with those of an authentic sample.

Meanwhile, the configuration of the D-galactopyranosyl unit was deduced to be β from the coupling constant value observed for the H-1′ of Gal (J=7.8 Hz). HMBC correlations from H-1′ (δ_H 4.80) of galactose unit to C-3 (δ_C 81.4) of the aglycone confirmed the attachment of the D-galactopyranosyl group to C-3. On the basis of the above data, the structure of compound **2** was elucidated to be 22α-hydroxy-olean-12-en-3β-yl-β-D-galactopyranoside (**2**) (see Fig. 1).

Compound **3** was obtained as colourless needles. Its molecular formula was deduced as $C_{36}H_{60}O_7$ by HR-FAB-MS

[m/z=604.4338 (M^+)] and confirmed by broad band decoupled ^{13}C-NMR and ^{13}C-DEPT analysis. The olean-12-ene glycoside nature of **3** was evident from the positive response to the Lieberman–Burchard test combined with the ^{13}C- and ^{1}H-NMR spectral data analysis. Comparison of the ^{1}H- and ^{13}C-NMR assignments of **3**, which were established under the same conditions as in the case of **1** and **2**, with reported data suggested that aglycone moiety and carbohydrate unit were olean-12-ene-3β, 24-diol[14] and D-glucose,[15,16] respectively. Acid hydrolysis followed by spectroscopic analysis of the aglycone and direct HPLC analysis of the sugar component, confirmed this suggestion. Furthermore, the configuration of the D-glucopyranosyl moiety was regarded to be β by the J value of its anomeric proton signal at δ 5.32 (d, J=7.9 Hz). The site of glycosylation was suggested by a downfield shift observed for C-3 (δ_C 81.9 in **3**, this signal appeared at 80.7 ppm in olean-12-ene-3β, 24-diol), and confirmed by HMBC experiments showing correlations between H-1 (δ_H 5.32) of glucose unit and C-3 (δ_C 81.9) of the aglycone. Consequently, the structure of **3** was determined as 24-hydroxyolean-12-en-3β-yl-β-D-glucopyranoside (**3**) (see Fig. 1). The known compound urs-12-ene-3β, 28-diol (**4**) was isolated as white powder and identified by comparison with the reported data.[12]

Experimental

General Procedures Melting points were determined on X-4 digital micro-melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 341 digital polarimeter. IR spectra were recorded with KBr pellets on a Perkin-Elmer 577 spectrometer. HPLC was performed by using a system comprised of a CCPM pump, a CCP PX-8010 controller, an RI-8010 detector and a Shodex OR-2 detector, and a Rheodyne injection port with a 20 μl sample loop. The EI-MS was recorded on a JEOLMSRoute massspectrometer. The FAB-MS was obtained with a Kratos MS 25 instrument with a DS-55 data system, and collision gas Xe (ion gun conditions 6 kV and 10 mA). The NMR spectra were recorded with a Bruker AMX-500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). Samples were run in DMSO-d_6 or $CDCl_3$. Chemical shifts were given in (ppm) with tetramethylsilane as an internal standard, and coupling constants (J) were reported in Hertz (Hz). CC was performed using a silica-gel (Kieselgel 60, 70—230 mesh, 230—400 mesh, Merck, Germany), Sephadex LH-20 (Pharmacia), and TLC using a pre-coated silica-gel 60 F254 (0.25 mm, Merck, Germany).

Plant Material The stem root of *Cylicodiscus gabunensis* HARMS was collected in May 2002 on Mount Eloudem, Yaounde-Cameroon. The plant was identified at the National Herbarium, Yaounde, where a voucher specimen is deposited (No. 21574/SRF/CAM).

Extraction and Isolation The air-dried, powdered stem root of *C. gabunensis* (4.8 kg) was immersed in MeOH (25 l) and kept for 72 h. The MeOH extract was filtered and concentrated to dryness under reduced pressure. The crude extract (225 g) was successively extracted with *n*-hexane, (5×500 ml) and EtOAc (5×500 ml) to give 15.8 g and 122 g of extract, respectively and 67.5 g of residue. A 90 g portion of the EtOAc extract was subjected to CC on silica gel (400 g) using a gradient solvent system of hexane, hexane–EtOAc, EtOAc, EtOAc–MeOH and MeOH in increasing polarity. A total of 200 fractions of 250 ml each were collected and combined on the basis of TLC analysis leading to six main series I—VI. Series III (9.5 g) [fractions 20—89] was rechromatographed on silica gel, using hexane–EtOAc (70 : 30) to give fifty fractions (F1—F50). Fractions F3 and F7 afforded compound **1** (200 mg) and **4** (150 mg) respectively. Series V (3 g) [fractions 150—190] was separated on Sephadex LH-20 eluted with MeOH to give twenty fractions [F1—F20]. Fraction F18 was further purified by preparative TLC, using MeOH/CH_2Cl_2/cyclohexane (1.5 : 5 : 3.5) to give compounds **2** (32 mg) and **3** (28 mg).

Compound **1**: White powder, mp 99—101 °C; $[\alpha]_D^{22}$ +57° (c=0.25, $CHCl_3$); IR (KBr) cm^{-1}: 1650, 1380, 1357, 1034; ^{1}H-NMR (see Table 1); ^{13}C-NMR (see Table 1); HR-EI-MS m/z=552.5271 (M^+) (Calcd for $C_{39}H_{68}O$: m/z=552.5270).

Compound **2**: White powder; mp 285—286 °C; $[\alpha]_D^{22}$ +62° (c=0.02, MeOH); IR (KBr) cm^{-1} 3545—3250, 1450, 1385, 1010; ^{1}H-NMR (see Table 1); ^{13}C-NMR (see Table 1); HR-FAB-MS m/z=604.4338 (M^+) Calcd for $C_{36}H_{60}O_7$: m/z=604.4339).

Compound **3**: Colourless needles; mp 258—260 °C; $[\alpha]_D^{22}$ +78° (c=0.02, MeOH); IR (KBr) cm^{-1}: 3550—3250, 1450, 1390, 1010; ^{1}H-NMR (see Table 1); ^{13}C-NMR (see Table 1); HR-FAB-MS m/z=604.4338 (M^+) Calcd for $C_{36}H_{60}O_7$: m/z=604.4339).

Acid Hydrolysis and Identification of Sugars Compounds **2** and **3** (6 mg each) were separately refluxed with 15% HCl/MeOH (6 ml) at 80 °C for 4 h. After cooling, each reaction mixture was concentrated and the residue partitioned with CH_2Cl_2/H_2O. The organic layer was concentrated to dryness to yield 2.9 mg of white material from **2** (M2) and 2.4 mg of white material from **3** (M3). After purification by preparative-TLC, M2 and M3 yielded respectively white powder identified as α-sophoradiol and colourless needles identified as olean-12-ene-3β, 24-diol, by comparison of its physical and spectroscopic data with those published.[13,14] The aqueous layer was evaporated and the residue was analysed by HPLC under the following conditions: column, Aminex HPX-87H (7.8 mm i.d.×300 mm); solvent, 5 mM H_2SO_4; flow rate, 0.6 ml/min; detection, refractive index and optical rotation. The sugar was confirmed as D-galactose (D-glucose) by comparison of its retention time and optical rotation with those of an authentic sample: retention times (min), 9.62 (D-galactose, positive optical rotation), 8.98 (D-glucose, positive optical rotation).

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References

1) Coates P. K. (revised and updated by Meg C. P.) "Trees of Southern Africa," 3rd ed., Struik, South Africa, 2002.
2) Le Thomas A., "Mimosaceae in Flore du Gabon," ed. by Aubreville, Orstom, Paris, 1969.
3) Chudnoff M., "Ag, Handbook," USDA Foresr Service, Washington D.C., 1984, p. 607.
4) Tane P., Bergquist K.-E., Téné M., Ngadjui B. T., Ayafor J. F., Olov S., *Tetrahedron*, **51**, 11595—11600 (1995).
5) Adjanohoun J. E., Aboubakar N., Dramane K., Ebot M. E., Ekpere J. A., Enow-Orock E. G., "Contribution to Ethnobotanical and Floristic Studies in Cameroon," Traditional Medicine and Pharmacopoeia, Technical and Research Commission of Organisation of African Unity (OAU/STRC), Lagos, Nigeria 1996, p. 241.
6) Kouam J., Tane P., Meli L. A., Noundou S. X., Muhammad I. C., Fomum Z. T., *Nat. Prod. Commun.*, **2**, 835—840 (2007).
7) Morinaga O., Fukuda N., Tanaka H., Shoyama Y., *Glycobiology*, **15**, 1061—1066 (2005).
8) Katerere D. R., Gray A. I., Nash R. J., Waigh R. D., *Phytochemistry*, **63**, 81—83 (2003).
9) Gaidi G., Correia M., Chauffert B. , Beltramo J.-L., Wagner H., Lacaille D. M.-A., *Planta Med.*, **68**, 70—72 (2002).
10) Pawar R. S., Bhutani K. K., *Phytomedecine*, **12**, 391—393 (2005).
11) Kazie N., Kouam J., Tane P., Kuete V., Watchueng J., Fomum Z. T., *Nat. Prod. Commun.*, **4**, 931—934 (2009).
12) Shashi B. M., Asish P. K., *Phytochemistry*, **37**, 1517—1575 (1994).
13) Ndom J.-C., Kouam, Vardamides J. C., Wansi J. D., Kamdem A. W., Mbafor J. T., Fomum Z. T., *Bull. Chem. Soc. Ethiop.*, **15**, 151—156 (2001).
14) Tanaka R., Tabuse M., Matsunaga S., *Phytochemistry*, **27**, 3563—3567 (1988).
15) Nguyen X. N., Nguyen H. T., Phan V. K., Chau V. M., Yan D., Jae-Hee H., Hee-Kyoung K., Young H. K., *Chem. Pharm. Bull.*, **57**, 986—989 (2009).
16) Hao-Bin H., Xu-Dong Z., Hong C., Xiao-Qiang G., Huai-Sheng H., *Chem. Pharm. Bull.*, **57**, 1000—1003 (2009).