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Bioactive Seco-Lanostane-Type Triterpenoids from the Root of *Leplaeamayombensis*

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ABSTRACT: Fractionation of the ethyl acetate-soluble extract of the root of *Leplaeamayombensis*afforded two new 3,4-seco-lanostane-type triterpenoids,leplaeric acids A and B (**1, 2**), a new lanostane-type triterpenoidleplaeric acid C (**3**), and six known natural products **5-10**. Derivatization of the main constituent **1**, afforded the dimethyl ester **4**, the monoamide**11**, and diamide**12** for SAR studies. The structures of these compounds were established through spectroscopic methods and a single crystal X-ray diffraction analysis was used to confirm the relative configuration of compound **1**. These lanostane derivatives are unique since they are the first C-21 oxygenated lanostanes isolated from plant sources. Preliminary biological assays against the MDA MB 231 breast cancer cell line showed that compounds **1, 2, 4,**and **11** have modest cytotoxic activity. Compound **2** was the most active with an IC_{50} of 55μM. From these results, the amides (**11**, **12**) of compound **1** were less active compared to the esters (**2**, **4**).

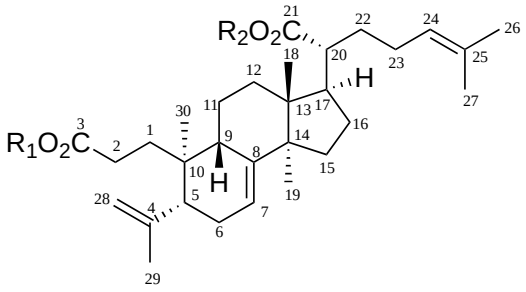
*Leplaea*is a genus which was originally placed under the genus *Guarea* as a species. However, recent morphological studies show that the group is distinctfrom the South-American members of *Guarea* and deserves generic status. Therefore, the genus *Leplaea*has been reinstated to accommodate five of the species formerly placed in *Guarea.*These species are:*Leplaealaurentii*(De Wild.) E. J. M. Koenen& J.J. de Wilde, *Leplaeathompsonii*(Sprague & Hutch.) E. J. M. Koenen& J.J. de Wilde, *Leplaeaadenopunctata*E. J. M. Koenen& J.J. de Wilde, *Leplaeacedrata*(A. Chev.) E. J. M. Koenen& J.J. de Wilde,and*Leplaeamayombensis*(Pellegr.) Staner.[1]

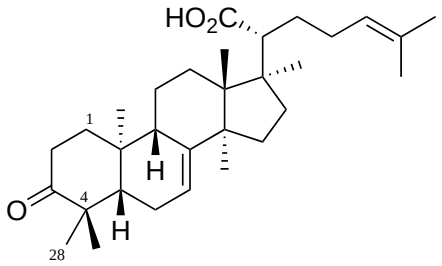
Leplaeamayombensis is distributed in Gabon, West Cameroon, Congo, D.R. Congo (Zaire) and was first identified in 2010.[2]This plant is used in African folk medicine forthe treatment of cancer and microbial infections. Phytochemical studies on this speciesreveal that triterpenoids are its main constituents.[3]

Triterpenoids are the C-30 class of natural products derived from squalene. The biosynthetic origin of most triterpenoids lies in cascade cyclizations and rearrangements of the acyclic precursors squalene (S) and 2,3-oxidosqualene (OS), leading to thetetra- and pentacyclictriterpenoidskeletons.[4,5,6,7]Lanostanes aretetracyclic triterpenoids and are the principal constituents in some genera such as the genus KadsuraJuss., e.g*K. coccinea*(Lem.) A.C. Sm. andgenus AbiesL. such as*A. balsamea*(L.) Mill.Several of these lanostanetriterpenoids have potential anti-HIV, anticancer, and cholesterol biosynthesis inhibitory activities.[8] For example, schisanlactone E and changnanic acid isolated from *K. longipedunculata*showedantiproliferative effects against murine leukemia P388 cells,[9] and ananosic acids B and C isolated from *K. ananosma*exhibited cytotoxicity against human CCRF-CEM cells and HeLa cells.[10]

In the present study,the isolation and structure elucidation of three new lanostane-type

triterpenoids (**1-3**, Figure 1) from the air dried roots of *L. mayombensis*are reported.Derivatives, **4**, **11,**and **12** were prepared by esterification and amidation of compound **1**for SAR studies. Compounds **1**, **2, 4,** and **11** possess modest cytotoxicity against MDA MB 231 breast cancer cell lines.





Leplaeric acid A (**1**)**:** R_1= R_2 = H
Leplaeric acid B (**2**)**:** R_1= Me, R_2 = H
Dimethyl ester (**4**)**:** R_1 = R_2 = Me

Leplaeric acid C (**3**)

Figure 1. Lanostane-typetriterpenoids from*L. mayombensis*.

RESULTS AND DISCUSSION

The CH_2Cl_2-MeOH (1:1) extract of the air-dried roots of *L. mayombensis* was partitioned with hexanes, EtOAc, and *n*-BuOH successively. Three new lanostane-type triterpenoids**1-3** and six known compounds, **5-10**(Figure 4) were obtained from the EtOAc fraction through a series of column chromatographic separations.Esterification and amidationof compound **1** afforded its dimethyl ester**4**, monoamide**11,** and diamide**12** for structure-activity relationship (SAR) studies (Scheme 1).

Compound **1** was obtained as a white powder from silica gel column chromatography eluting with 30% hexanes-EtOAc. It is soluble in CH_2Cl_2, stains on TLC with iodine or H_2SO_4, melts at 160^{o}Cand has an $[\alpha]_D^{20}$ - 3.4 (*c* 2.0, $CHCl_3$). Its molecular formula was determined as $C_{30}H_{46}O_4$

on the basis of HRESIMS(*m/z* 471.3468 $[M+H]^+$) indicating eight idicesof hydrogen deficiency. The IR spectrum indicated the presence of a carboxylic acid group (1702 cm^{-1}). The ^1H NMR(600 MHz, $CDCl_3$) spectrumshows six methyl groupsatδ_H 1.80 (3H, m), 1.68 (3H, d, J = 1.3 Hz), 1.61 (3H, d, J = 1.3 Hz), 1.01 (3H, s), 0.92 (3H, s), and 0.88(3H, s). The ^{13}C NMR(150MHz, $CDCl_3$) spectrum exhibited 30 carbon signals that were sorted by a DEPT experiment as six methyl(δ_C27.0, 25.7, 21.7, 21.4, 17.7, 16.2), nine methylene (δ_C33.5, 32.3, 30.5, 30.2, 30.0, 27.8, 27.6, 26.0, 18.1), one terminal double bond (=CH_2, δ_C 114.1), sixmethinecarbons (four -CH (δ_C50.4, 50.0, 48.8, 40.9) and two =CH(δ_C123.4, 118.0)),two carbonyls(δ_C183.4, 181.3), three fully substituted olefinic carbons (δ_C147.5, 145.7, 123.4), and three quaternary carbons (δ_C51.4, 43.3, 36.4). On the basis of the above datacompound **1**was either a 3,4-seco-lanostane or a lupeol-type triterpenoidsince they are the only triterpenoids in these classes with terminal olefinic functionalities.[11]

The lupeol-type structure was however discarded based on the indices of hydrogen deficiency. The ^1H and ^{13}C NMR data were assigned using^1H-^1H COSY, HSQC, HSQC-TOCSY, HMBC,and NOESYspectra (Figure S1-S17, Supporting Information). Typical in the HMBC spectrum are correlations between H-6 (δ 2.23, 2.02), H-7 (5.26),H_2-28(4.83), H_3-29 (1.80) withC-5(δ 50.0), and the deshielded methyl protonsH_3-29 (δ 1.80) with C-5(δ50.0), C-4(147.5), and C-28(114.1) which implied a $\Delta^{4(28)}$ terminal double bond (Figure 2). Also observed are HMBC correlations between H-24 (δ 5.12) with Me-26 (δ25.7) andMe-27(17.7) for a 2-methylprop-1-enyl unit. HMBC correlations of the olefinicH-7 (δ 5.26) withC-9 (δ 40.9) and C-14 (51.4) indicate a$\Delta^{7(8)}$ double bond(Figure 1).COSY correlations were observed between H-7(δ 5.26) and H_2-6 (2.25,2.20), H-24 (δ 5.12) and H_2-23(2.09, 1.99),and NOESY correlations

between H-24 (δ 5.12)and Me-26(δ 1.68) andMe-27 (1.61) as well as H_2-28 (δ 4.83) with Me-29 (δ 1.80). Collectively, the NMR data supported the assigned structure of compound **1** and the connectivity and relative configuration were unambiguously confirmed using single crystal X-ray diffraction[12](Figure 2).Compound **1** was thus defined as (5α, 9β, 10α, 13β, 14α, 17α, 20α)-3,4-secolanostan-4(28),7,24-triene-3,21-dioic acid, a new seco-lanostane-typetriterpenoidand named, leplaeric acid A.In an attempt to unambiguously assign the absolute configuration of leplaeric acid**(1)**by X-ray analysis,*p*-bromobenzamides**11** and **12**possessing a heavy atomwere prepared (Scheme 1). However, none of the crystals obtained of these derivativeswere of X-ray quality.

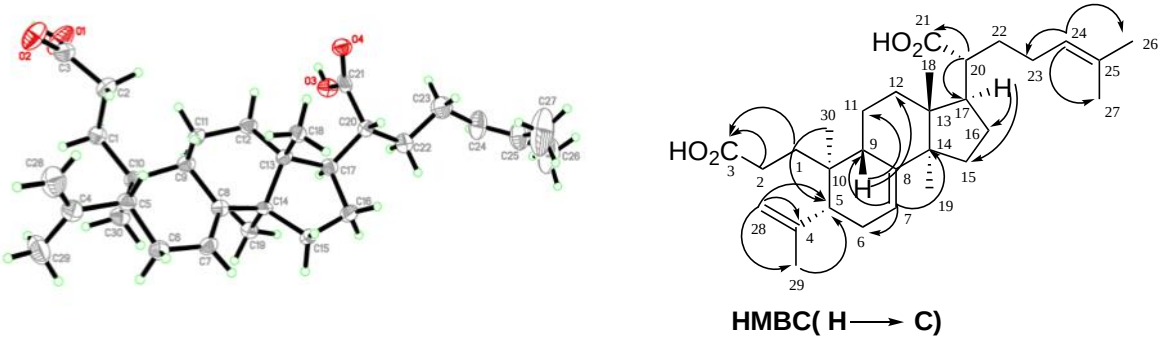


Figure 2. ORTEP drawing (left) and main HMBC correlations of **1** (right).

Compound **2** was obtained as a white powder after silica gel column chromatography eluting with 88% hexanes-EtOAc.It is soluble in CH_2Cl_2,stains on TLC with iodine or $H_2SO_4$and melts at 174°C.Its molecular formula was determined as $C_{31}H_{48}O_4$ on the basis of HRESIMS (*m/z* 485.3627 $[M+H]^+$) indicating eight indicesof hydrogen deficiency. The IR spectrum of compound **2**showed the presence of a carboxylic acid (1700cm^{-1}) and an ester (1739cm^{-1})group. The ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150MHz, $CDCl_3$) spectra were similar to compound **1** except for the presence of a three-proton singlet at δ_H 3.60 attributed to an

estergroup. The HMBC spectrum shows correlations between H_2-2 protons (δ 2.39, td, *J*=6.0,12.0Hz and 2.19) andMeO-31 atδ 51.5 indicating that the methoxy group is at C-3. Theshielding of H_2-2 as compared to compound **1** (δ2.62 and 2.42) indicatesa difference in its chemical environment.The ^{13}C NMR spectrum of compound **2** indicates a 3-methoxycarbonyl (δ174.4)as compared to a 3-hydroxycarbonyl function(δ 181.3) in compound **1**. On the basis of these 1D and 2D spectroscopicvalues (Table 1), the structure of compound **2**, leplaeric acid B, was assigned as the 3-methyl ester ofleplaeric acid A (Figure 1).

Compound **3**was obtained as a white powder from a silica gel column chromatography eluting with 82% hexanes-EtOAc. Its IR spectrum indicated the presence of two carbonyl groups(1704 and 1654 cm^{-1}) and it has a melting point range of 274-276^{o}C.The HRESIMSshowed (*m/z* 455.3427 $[M+H]^+$) and a molecular formula of $C_{30}H_{46}O_3$. It is soluble in $CH_2Cl_2$and stains on TLC with iodine or H_2SO_4.The ^{1}H NMR (600 MHz, $CDCl_3$) spectrumof compound **3**was similar to compound **1** except for the absence of the terminal double bond protons H_2-28 (δ_H 4.83), thevinylic Me-29(1.80), and the presence of two methyl groupsMe-28 (δ_H 1.11, s) and Me-29 (1.04, s) respectively.Compound **3** also exhibited differences from compound **1** in the ^{13}CNMR spectrum with the presence of a carbonyl group (δ_C 216.9)indicative of a ketone and the absence of the terminal olefinic moiety$\Delta^{4(28)}$ (δ_C 147.5, 114.1). In the HMBCspectrum,correlations of H_2-2 (δ2.76,2.32), H_2-1 (1.97 and 1.45) with the C-3 (δ 216.9) carbonyl groupwereobserved. AditionalHMBC correlations were also observed between the methyl protons H_3-28 (δ 1.11) and H_3-29 (1.04)with C-3(δ 216.9), confirming the position of the carbonyl group of leplaeric acid C(Figures1 and 3).It is likely that leplaeric acidsA (**1**) and C (**3**) are derived biosynthetically from the same lanosterol precursor. Oxidation of the C-3 hydroxy group of the lanosterol would give rise to the C-3 carbonyl in ketone**3**, while an

oxidosqualenecyclase-catalyzed Grob fragmentation of the A-ring[13] of the sterol precursor yields an intermediate that would provideleplaeric acid A (**1**) after additional oxidation and olefin isomerization reactions.

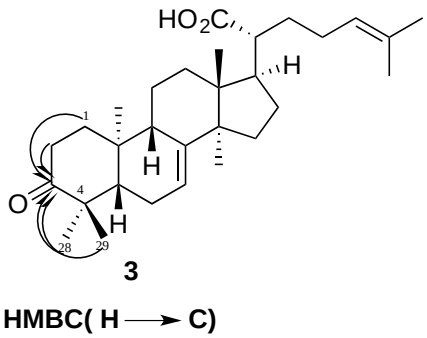


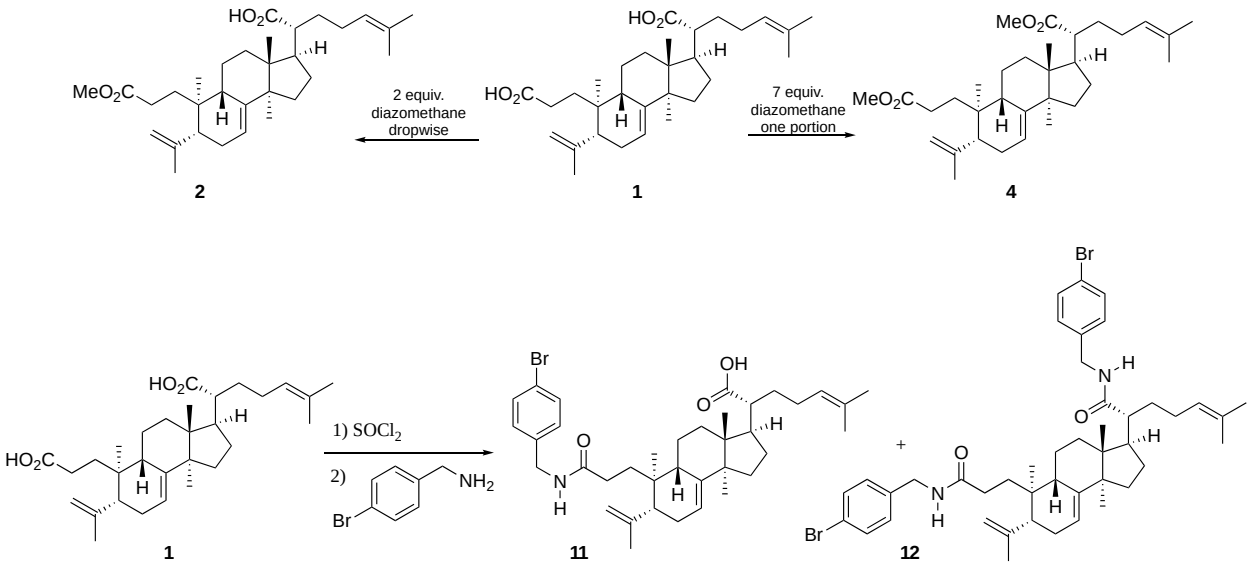
Figure 3. Main HMBC correlations of ring A in **3**.

Table 1. ^{1}H and ^{13}C NMR Data (600 and 150 MHz) of Compounds 1-4, 11-12 (δ_H, $CDCl_3$, *J* in Hz)

	1		2		3		4		11	12
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{1}H
1	2.11(1H, m) 1.33(1H,m)	27.8	2.00(1H, m) 1.33 (1H,m)	27.3	1.97(IH, m) 1.45(1H, m)	38.7	2.00(1H, m) 1.33(1H,m)	26.1	2.11(1H, m) 1.33(1H,m)	2.11(1H, m) 1.33(1H,m)
2	2.62 (1H, m) 2.42(1,H, m)	27.6	2.39(1H,td, *J*=12.0, 6.0Hz) 2.25(1H, m)	28.1	2.76(1H, m) 2.32(1H, m)	35.0	2.37 (1H, m) 2.11(1,H, m)	27.1	2.48 (1H, m) 2.42(1,H, m)	2.48 (1H, m) 2.42(1,H, m)
3		181.3		174.4		216.9		174.0		
4		147.5		147.3		35.2		147.3		
5	2.00(1H, m)	50.0	2.09 (1H, m)	49.9	1.72 (1H, t, *J*=6Hz)	52.5	2.00 (1H, m)	49.9	2.00(1H, m)	2.00(1H, m)
6	2.25(1H, m) 2.02 (1H, m)	30.2	2.23(1H, m) 2.05(1H, m)	30.2	2.10(2H, m)	24.6	2.12(1H, m) 1.99(1H, m)	30.2	2.25(1H, m) 2.02 (1H, m)	2.25(1H, m) 2.02 (1H, m)
7	5.26(1H, m)	117.9	5.28 (1H, m)	118.3	5.31 (1H, m)	118.4	5.18(1H,t, *J*=6Hz)	118.2	5.26(1H, m)	5.26(1H, m)
8		145.7		145.8		145.6		145.9		
9	2.60(1H, m)	40.9	2.48(1H, m)	40.6	2.04 (1H, t, *J*=6Hz)	50.0	2.45(1H,m)	40.6	2.48(1H, m)	2.48(1H, m)
10		43.3		43.3		43.5		43.1		
11	1.55(1H, m) 1.35(1H, m)	18.1	1.55(1H, m) 1.45(1H, m)	18.1	1.53 (2H, m)	18.0	1.53 (2H,m)	17.9	1.55(1H, m) 1.35(1H, m)	1.55(1H, m) 1.35(1H, m)
12	1.52(2H, m)	33.5	1.55(2H, m)	33.8	1.55(2H, m)	33.7	1.57(2H,m)	33.8	1.52(2H, m)	1.52(2H, m)
13		36.4		36.9		35.0		36.8		
14		51.4		51.3		51.1		51.6		
15	1.50(2H, m)	32.3	1.56(2H, m)	32.2	1.56(2H, m)	32.5	1.57 (2H,m)	32.4	1.50(2H, m)	1.50(2H, m)
16	1.45 (2H, m)	30.5	1.75(1H, m) 1.55(1H, m)	30.0	1.75(1H, m) 1.45(1H, m)	30.2	1.46 (2H,m)	30.1	1.45 (2H, m)	1.45 (2H, m)
17	2.27(1H, m)	48.8	2.33 (1H,td,*J*=12.0, 6.0Hz)	47.3	2.29 (IH, m)	48.3	2.25 (1H,td, *J*=6..0;12.0Hz)	47.3	2.27(1H, m)	2.27(1H, m)
18	0.88(3H, s)	16.2	0.84(3H, s)	15.8	0.95(3H, s)	12.9	0.75(3H,s)	15.9	0.88(3H, s)	0.88(3H, s)
19	1.01 (3H, s)	27.0	1.01(3H, s)	27.4	1.01(3H, s)	27.5	0.91(3H,s)	27.4	1.01 (3H, s)	1.01 (3H, s)
20	2.48(1H, m)	50.4	2.44(1H, m)	49.3	2.32 (1H, m)	47.3	2.35(1H, m)	49.4	2.41(1H, m)	2.41(1H, m)
21		183.4		182.4		181.4		176.5		
22	1.60(2H, m)	32.0	1.62(2H, m)	31.9	1.94 (1H, m) 1.34 (1H, m)	27.3	1.57(1H, m) 1.23(1H,m)	31.8	1.60(2H, m)	1.60(2H, m)
23	2.09(1H,m) 1.99(1H, m)	26.0	2.00(2H, m)	26.0	1.99(2H, m)	26.2	1.82(2H,m)	26.1	2.09(1H,m) 1.99(1H, m)	2.09(1H,m) 1.99(1H, m)
24	5.12 (1H, m)	123.4	5.12 (1H, m)	123.5	5.10 (1H, m)	123.7	5.01(1H, t)	123.9	5.07 (1H, m)	5.07 (1H, m)
25		132.4		132.3		132.5		132.1		
26	1.68(3H, d, *J*=1.3Hz)	25.7	1.70(3H, d, *J*=1.3Hz)	25.7	1.68(3H, s)	25.8	1.50(3H,s)	25.7	1.68(3H, s)	1.68(3H, s)
27	1.61(3H, d, *J*=1.3Hz)	17.7	1.60(3H, d, *J*=1.3Hz)	17.6	1.58 (3H, s)	17.8	1.61(3H,s)	17.6	1.61(3H, s)	1.61(3H, s)
28	4.83(2H, m)	114.1	4.86 (1H, m) 4.81 (1H, d, *J*=2.1Hz)	113.9	1.11 (3H, s)	21.7	4.76 (1H,d, *J*=6Hz) 4.72 (1H,d, *J*=6Hz)	113.7	4.83(2H, d, *J*=12.0 Hz)	4.83(2H, d, *J*=12.0 Hz)
29	1.80 (3H, m)	21.7	1.80 (3H, m)	22.5	1.04 (3H, s)	24.7	1.71(3H,s)	22.3	1.80 (3H, m)	1.80 (3H, m)
30	0.92(3H, s)	21.4	0.95(3H, s)	21.8	0.89(3H, s)	21.8	0.82(3H, s)	21.9	0.92(3H, s)	0.92(3H, s)
31			3.61(3H, s)	51.5			3.60(s, OMe)	51.1	7.48(2H, t, *J*=6Hz), 7.25(2H, d, *J*=12Hz), 5.71(NH, t, *J*=6Hz), 4.58 (1H, dd, *J*= 12.0 6.0Hz) 4.29 (1H, dd, *J*= 12.0 6.0Hz) for 4-bromobenzylamide	7.49(2H, t, *J*=6Hz), 7.25(2H, d,*J*=12Hz), 5.71(NH,t, *J*=6Hz), 4.58 (1H, dd, *J*= 12.0 6.0Hz) 4.29 (1H, dd, *J*=12.0 6.0Hz) for 4-bromobenzylamide
32							3.59(s, OMe)	51.1		7.49(2H, t, *J*=6Hz), 7.19(2H, d,*J*=12Hz),

										5.77(NH, t, *J*=6Hz) 4.42 (2H, d,*J*=6.0Hz) for 4-bromobenzylamide

To obtain derivatives for structure-activity relationship (SAR) analysis, ester derivatives**2** and **4** wereprepared fromleplaeric acid A(**1**) using diazomethane generated in situ from 1-methyl-3-nitro-1-nitrosoguanidine (MNG) and amidation of**1**with 4-bromobenzylamine provided amide derivatives**11** and **12**(Scheme 1).



Scheme 1. Diazomethane esterification and amidationof compound **1**.

Treatment of compound **1**with 2 equivalents of diazomethane[14]afforded compound **2** in a biomimetic-type transformation. When excess diazomethane(7 equiv.)was used, the dimethyl diester**4**was produced. Synthesized compound **2** was identical withthe natural productby comparing their TLC R_f values and NMR spectroscopic data.

Compound **4** was purified as a white amorphous compound from a silica gel column eluting with 95% hexanes-EtOAc from the diazomethane reaction of compound **1**. Its IR spectrum shows an intense peak for an ester group at 1733 cm^{-1}. Its ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150MHz, $CDCl_3$) spectra were similar to compound **1** except for the presence of two

methoxyproton resonances at δ3.60(s, OMe) and 3.59(s, OMe)in the ^{1}H NMR spectrum and at δ51.1(OMe),174.0 and176.5(two -COOMe groups) in the ^{13}C NMR spectrum (Table 1). These results were supported by the HRESIMS (*m/z* 499.3782 $[M+H]^{+}$) for $C_{32}H_{50}O_4$.Based on spectroscopic analysis, compound **4** was determined to be3, 21-dimethyl-leplaeric ester.Compound **1** was treated with thionyl chloride followed by 2 equiv. of 4-bromobenzylamine in a two pot reaction to afford compounds**11** and **12** in a 1:3 ratio that was purified on a silica gel column eluting with 30% (**12**) and 45% (**11**) hexanes-EtOAc respectively.

In addition to the lanostane derivatives, five known compounds were also isolated and included: andirobin[15](**5**), 1,2-dihydroandirobin[15](**6**), β-sitosterol[16](**7**), stigmasterol[16](**8**), α-spinosterol[17](**9**),and sigmoidin B[18](**10**) (Figure 4).

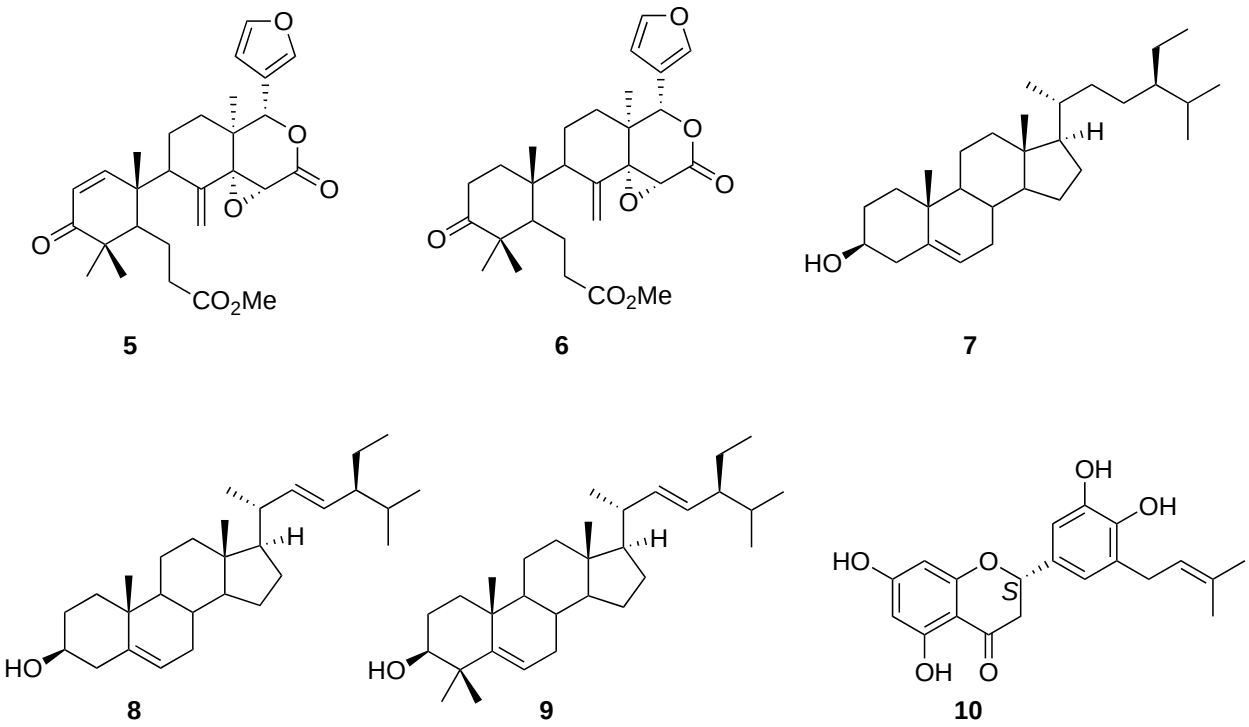


Figure 4: Known compounds from the EtOAc extract of *Leplaeamayombensis*.

To ascertain if any of the isolated compounds exhibited cytotoxic activity, the relative viability of the MDAMB231 breast cancer cell line exposed to triterpenoids at multiple concentrations was assayed. For this, MDAMB231 cells were plated in a 96 well format. The indicated compounds, dissolved in DMSO were serially diluted in culture media prior to addition to the culture. After a 3-day incubation time, relative viability was determined indirectly through the measurement of total ATP in each well using CellTiter-Glo (Promega, Figure 5). Following this assay, the IC_{50} value was determined with 95% confidence interval (Table 2).The methyl esters **2** and **4** were more active than the diacidagainst the MDA MB 231 breast cancer cell line which may be due to greater cell permeability. The methylesters **2** and **4** were equally more active than the amides **11** and **12.** Additionally, a significant loss in activity of the diamide**12** compared to diacid**1** was observed.

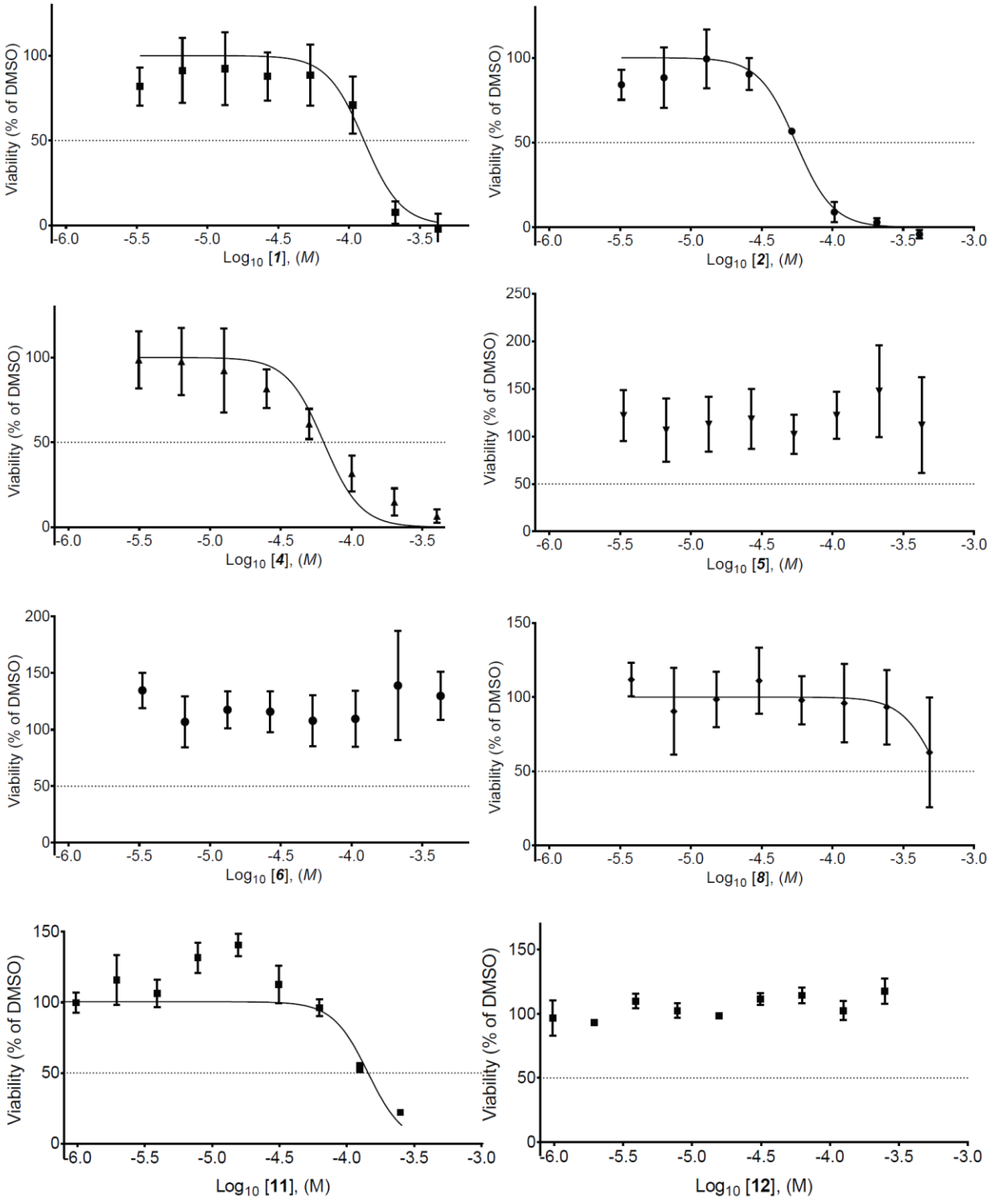


Figure 5. Triterpenoids**1, 2** and **4** have modest cytotoxic activity against MDAMB231 breast cancer cells. MDAMB231 cells were plated at a density of 2000 cells per well and treated with the indicated compounds for 3 days followed by cell viability assay. Error bars indicate the

standard deviation of three independent measurements. The curves indicate the best fit of a non-linear curve fitting the equation, $Y=100/\{1+10^{[(X-LogIC50)]}\}$.

Table 2. IC_{50}Values Calculated from Scheme 1 and Figure 4for the Indicated Compounds Toward MDAMB231 Breast Cancer Cells after 3 Day Exposure.

	1	***2***	***4***	**8**	***11***
IC_{50} (μM)	127	55.0	63.5	564	140
95% Confidence Intervals (μM)	102 to 158	47.5-63.8	51.5-78.3	409-779	107-183

EXPERIMENTAL SECTION

General Experimental Procedures.Melting points were measured on a MEL-TEMP^R apparatus. Optical rotations were determined using a Rudolph Autopol IV polarimeter. IR spectra were recorded on a BrukerTensor 27 Spectrometer. 1D (^{1}H,^{13}C and DEPT) and 2D (^{1}H-^{1}H COSY, HSQC, HSQC-TOCSY, HMBC, TOCSY, NOESY, and ROESY) NMR spectra were recorded on a Bruker Ascend 600 (^{1}H 600 MHz and ^{13}C 150 MHz) NMR spectrometer equipped with a 5 mm cryoprobe. ^{1}H NMR chemical shifts are reported as δ values in ppm relative to $CDCl_3$ (7.26 ppm,), coupling constants (*J*) are reported in Hertz (Hz), and multipilicity follows convention. Unless indicated otherwise, $CDCl_3$ served as an internal standard (77.2 ppm) for all ^{13}C spectra. The chemical shifts are quoted relative to TMS. X-ray data were recorded on a Bruker400 Machine.HRESIMS data were aquired on a Thermo LTQ Orbitrap mass spectrometer

with ESI direct infusion and a Bruker Amazon SL for LRMS. Flash column chromatography was performed using 60Å Silica Gel (200-300 mesh).

Sample Collection.*L. mayombensis* fruits and roots were harvested in Yaoundé-Cameroon, (October, 2014) and identified by Mr. Victor Nana (Plant taxonomist) of the Cameroon National Herbarium (HNC), where voucher specimens were deposited (46220/HNC). The root was collected, cut into small pieces, dried at room temperature and powdered.

Extraction and Isolation. Powdered roots (1.5 kilograms) were extracted by maceration in MeOH:CH_2Cl_2(1:1 v/v) at room temperature. The filtrates were concentrated ona rotary evaporator under reduce pressure to yield 250 g of crude extract. The crude extract (240 g) was subjected to fractionation using hexanes, EtOAc and *n*-BuOH. The EtOAc rich fraction (70 g) was subjected to silica gel 60 (0.063 – 0.200 mm) column chromatography using hexanes, hexanes-EtOAc gradient and EtOAc. Fractions (150) of 150 mL each were collected and concentrated under vacuum. Eight compounds namely, leplaericacid A (**1**, 200mg), leplaeric acid B(**2**, 30 mg), leplaeric acid C (**3**, 15 mg**)**,andirobin (**5**, 20mg), 1,2-dihydroandirobin (**6**, 15mg), *β*-sitosterol (**7**, 13 mg), stigmasterol (**8**, 20mg), and α-spinosterol (**9**, 15 mg), were obtained.

Preparation of Compound 4. To a stirred solution of 50% KOH (10 mL) under diethyl ether (5.0 mL) at 0 °C, MNG (11.25 mg, 0.08mmol) was added gently. The resulting yellow coloured organic layer was separated, dried over KOH pellets, and added to compound **1** (18 mg, 0.04mmol) in Et_2O (2 mL), the reaction mixture was stirred for 2 h and the solvent was removed under reduced pressure. Column chromatographic purification of the crude material over silica gel using 5% EtOAc in hexanes as solvent afforded pure compound **4** (70% yield).

Preparation of Compound 2.To a stirred solution of of 50% KOH (1 mL), MNG (3.75 mg, 0.021 mmol) in Et_2O (1 mL) at 0 °C was added gently. The organic layer was separated and

added to compound **1** (6mg, 0.0128mmol). Silica gel column chromatograpy eluting with 12% EtOAc in hexanes afforded compound **2** (60% yield).

Biological Screening.MDAMB231 cells were cultured in Dulbecco's modified Eagle medium (Corning), supplemented with 10% fetal bovine serum (Gibco) and antibiotics (Lonza). For the determination of cytotoxicity, cells were plated in 96-well plates (Nest Scientific) at a density of 2000 cells per well in 100 μL of media per well. On the following day, the media was aspirated, and 100 μL of media in which a serial dilution of DMSO-dissolved compound (or DMSO as a control) was added to wells in triplicate. Three days later, the relative number of metabolically active cells was determined by addition of the CellTiter-Glo reagent (Promega) and measurement of chemiluminesence according to the manufacturer's protocolusing a Fluoroskan Ascent FL (Thermo Scientific). To calculate the percent viability, the signal from background wells (media only) was subtracted from each well and then the remaining chemiluminesence value was normalized to the signal from wells with the equivalent amounts of DMSO. Graphing was done using GraphPad Prism 6 (GraphPad Software). IC_{50} values were calculated by fitting a non-linear curve using the "log-inhibitor vs normalized response" function given by the equation $Y=100/\{1+10^{[(X-LogIC50)]}\}$.

*Leplaeric acidA***(1):** White micro-crystallinepowder obtained from 70% hexanes-EtOAc. Soluble in CH_2Cl_2, mp 160°C, IR ν_{max} 2953, 1702, 1449 cm^{-1}, and has an $[\alpha]_D^{20}$–3.4 (*c* 2.0, $CHCl_3$).^{1}H and ^{13}C NMR data, see Table 1; HRESI (*m/z* 471.3468 $[M+H]^+$) for $C_{30}H_{46}O_4$.

*Leplaeric acid B***(2)**: white micro-crystalline powder from 88% hexanes-AcOEt. Soluble in CH_2Cl_2, mp 174°C, IR ν_{max}1700, 1739 cm^{-1}, and has an $[\alpha]_D^{20}$ +21.7 (*c* 0.35, $CHCl_3$).^{1}H and ^{13}C NMR data, see Table 1; HRESIMS (*m/z* 485.3627 $[M+H]^+$) for $C_{31}H_{48}O_4$.

*Leplaeric acid C***(3)**. White micro-crystalline powder from 82% hexanes-EtOAc. Soluble in CH_2Cl_2, mp 274-276°C. IR ν_{max} 3382, 3300-2600, 1704, 1654 cm^{-1}, and has an $[\alpha]_D^{20}$ –40.0 (*c* 0.20, $CHCl_3$).^{1}H and ^{13}C NMR data, see Table 1; HRESIMS (*m/z* 455.3427 $[M+H]^+$) for $C_{30}H_{46}O_3$.

Leplaericacid-3,21-dimethyl ester **(4)**. IR ν_{max} 1733 cm^{-1}. ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS (m/z 499.3782 $[M+H]^+$) for $C_{32}H_{50}O_4$.

X-ray Crystallographic Analysis. White crystals of compound **1** were obtained from $CHCl_3$-hexanes. Data for complex**1**were collected at 150 K on a Bruker D8 Quest with IμS microfocus source using Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods and refined by full-matrix least-squares refinement on F^2 after multiscan absorption correction of the data using SADABS.[19,20] Data was processed using the Bruker AXS SHELXTL software, version 6.14.[21]Crystallographic data of 1: $C_{30}H_{46}O_4$ (M = 470.67); Monoclinic crystal (0.336 x 0.032 x 0.018 mm); space group $P2_1$; unit cell dimensions a = 16.1809(12) Å, b = 6.7481(4) Å, c = 16.4245(13) Å, β = 116.917(3)°, V = 1599.1(2) $Å^3$; Z = 2; ρcalcd = 0.977Mg/m^3; μ = 0.063 mm^{-1}; 16 926 reflections measured (2.520° ≤ θ ≤ 25.636°), 5983 unique (Rint = 0.0810), which were used for all calculations; the final refinement produced R1 = 0.0607 (>2σ(I)) and wR2 = 0.1249 (all data); and Flack parameter = -0.3(10), which does not allow for absolute stereostructure determination by X-ray. All non-hydrogen atoms were refined anisotropically, all hydrogen atoms attached to carbon were placed in calculated positions and the carboxylate hydrogens were located in the difference map and their positions refined. A disordered water molecule was removed from the structure (8 e-) using the SQUEEZE procedure implemented in PLATON.[22]Thecrystallographic data of **1** were deposited at the CambridgeCrystallographic Data Centre under deposition number CCDC 1491114. Copies

of the crystallographic data can be obtained for freefrom the Cambridge Crystallographic Data Centre, 12 Union Road,Cambridge CB2 1EZ, UK e-mail: deposit@ccdc.cam.ac.uk or fax:(+44) 1223-336-033] or by www.ccdc.cam.ac.uk/conts/retrieving.html

ASSOCIATED CONTENT

Supporting Information

NMR data for lanostane-type terpenoids (**1-4**) and (**11-12**). This material is available free of charge via the internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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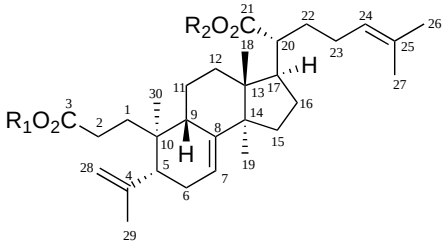
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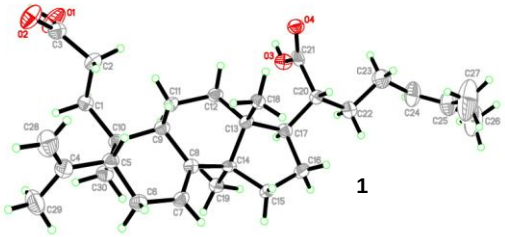
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Table of Contents/Abstract Graphic:





Leplaeric acid A (**1**)**:** R_1= R_2 = H
Leplaeric acid B (**2**)**:** R_1= Me, R_2 = H



1