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Mayombensin, a New Azadirachtin I Derivative with Unusual Structure from *Guarea mayombensis*

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ABSTRACT

A new highly oxidized tetranortriterpenoid, named mayombensin (**1**), was isolated from the twigs of Guarea mayombensis together with five known compounds and, further, two ceramides, 3,4-dimethyl-secotirucalla-4(28),7,24-trien-3,21-dioic acid (**3**), the glucosides of stigmasterol and β-sitosterol, and the respective aglyca. The structure of **1** was elucidated by detailed NMR analysis and confirmed as a novel azadirachtin homologue.

Introduction

The genus *Guarea* belongs to the family Meliaceae and has 150 species of trees and shrubs in tropical America (8 of them occur in Panama [1] and 20 in Africa [2]). Some species are used in folk medicine for the treatment of rheumatism and as an emetic and hemostatic remedy [3]. A few pharmacological studies demonstrated anti-inflammatory, antiviral, and antiprotozoal activities of some *Guarea* extracts [3–6]. Previous phytochemical investigations on this genus led to the isolation and identification of a wide variety of constituents including sesqui-, di-, and triterpenes, limonoids, steroids, flavonoids, and coumarins [3, 7–10]. Although a high number of chemical studies on the genus *Guarea* have been reported, none is known on *Guarea mayombensis* Pellegr., which is a big tree up to 10–15 m high, commonly distributed in tropical Africa. This paper deals with the structure elucidation of a new tetranortriterpenoid **1** isolated together with five known compounds and, further, two ceramides from the methanol extract of the finely powdered twigs of *G. mayombensis* on chromatographic separation.

Results and Discussion

ESI HRMS of compound **1** displayed a pseudo-molecular ion peak at m/z 593.2583 ($[M - H]^-$), consistent with the molecular formula $C_{30}H_{42}O_{12}$. Its IR spectrum exhibited typical absorptions for hydroxy (3423 cm^{-1}), ester carbonyl (1697 cm^{-1}), ether (1268, 1142, 1075 cm^{-1}), and double bond (1652 cm^{-1}) functionalities. The UV spectrum showed a peak at 220 nm due to the π–π * transition of an α,β-unsaturated carbonyl group [8, 11]. The ^{13}C NMR spectrum of **1** provided evidence for a conjugated ester carbonyl group by signals at δ_C = 167.6 (C-1') and of two olefinic carbon atoms at δ_C = 137.8 (C-3') and 130.1 (C-2'). The ^{13}C NMR spectrum displayed a further 27 signals of aliphatic carbons, of which 14 between

δ_C = 60-101 were oxygenated. This agreed well with the ^{1}H NMR spectrum (▶ **Table 1**), which exhibited a set of signals attributable to oxymethine and oxymethylene groups between δ_H = 3.50-6.50. Besides, the ^{1}H NMR and HSQC spectra also showed three aliphatic methyl singlets of H_3-18, H_3-29, and H_3-30, and two broadened signals of olefinic methyls, which coupled with an olefinic proton at δ_H = 7.34 (H-3'). In the HMBC experiment, cross-peaks from both H-3' and H_3-5' to C-1' and C-2', and from H_3-4' to C-2' were observed, indicating the presence of a tigloyl unit. The above data suggested **1** to be a highly oxygenated nortriterpene, structurally related to azadirachtin-type limonoids [12–14].

In particular, the NMR data of C-5–C-10 and C-13–C-18 for **1** were very similar to those of azadirachtin I (**2**) [12], suggesting that these two compounds were structurally related. This was supported by detailed analysis of the 2D NMR data (HSQC, HMBC). The COSY and TOCSY spectra clearly defined the spin system of H-1/H_2-2/H-3, H-5/H-6/H-7, and H-15/H_2-16/H-17. Furthermore, the HMBC spectrum showed correlations from H-1 to C-3, C-5, C10, C-19 and C-1', from H-3 to C-5, from H-5 to C-3, C-4, C-6, C-7, C-10, C-19, C-28, and C-29, from H-7 to C-5, C-6, C-8, C-9, C-14, and C-30, from H_2-28 to C-3, C-4, C-6, and C-29, and from H_3-29 to C-3, C-4, C-5, and C-28, confirming the carbon skeleton and the substitution in rings A and B.

Further comparison of the NMR data has shown that compound **1** differed from azadirachtin I (**2**) by the replacement of the acetyl group in the C-3 position by a hydroxy group, and of the tetrahy-

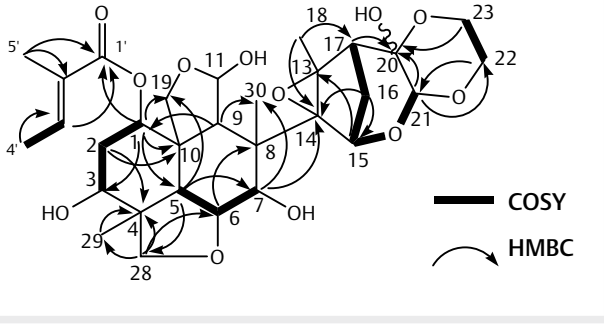
▶ **Table 1** ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR data of mayombensin (**1**) in pyridine-d_5 with HMBC assignments.

Position	δ_H (m, *J* in Hz)	δ_C	Complete list of HMBC (H → C) correlations
1	6.01 (t, 2.8)	75.4	C-3, 5, 10, 19 (w), 1' (w)
2*α*	2.70 (td, 2.3, 15.0)	32.6	C-3, 4, 10
2*β*	2.27 (td, 2.9, 15.8)		
3	4.06 (brs)	70.8	C-5
4	-	44.8	-
5	4.16 (d, 12.6)	35.2	C-3, 4, 6, 7, 10, 19, 28, 29
6	3.97 (m)	75.6	C-7 (vw), 8 (vw)
7	4.96 (brs)	74.5	C-5, 6, 8, 9, 14, 30
8	-	46.0	-
9	3.47 (brs)	50.6	C-1, 8, 10, 13 (w), 14, 19, 30
10	-	49.9	-
11	6.11 (brs)	101.9	C-8, 10, 19
13	-	66.8	-
14	-	70.6	-
15	4.96 (brs)	79.6	C-13, 14, 17, 21
16a	2.22 (brd, 11.1)	28.1	C-13, 14, 15, 17, 20
16b	1.81 (ddd, 11.6, 5.5, 2.9)		C-20 (w)
17	2.66 (d, 5.3)	53.9	C-13, 14, 15, 16 (w), 18 (w), 20, 21
18	2.38 (s)	18.7	C-13, 14, 17
19	4.22 (d, 2.9)	72.3	C-1, 5, 10, 11
20	-	93.1	-
21	6.54 (s)	95.0	C-20, 22
22*α*	4.34 (m)	59.5	C-23 (vw)
22*β*	3.48 (dd, 11.4, 2.0)		C-21
23*α*	3.57 (dd, 11.3, 2.3)	60.0	C-20
23*β*	4.56 (td, 11.7, 2.7)		C-22
28*α*	4.67 (d, 7.2)	77.9	C-3, 4, 6, 29
28*β*	3.75 (d, 7.2)		C-4 (w), 5, 6, 29
29	1.02 (s)	20.5	C-3, 4, 5, 28
30	1.55 (s)	22.7	C-7, 8, 9, 14
1'	-	167.6	-
2'	-	130.1	-
3'	7.33 (dq, 1.4, 7.1)	137.8	C-1', 2', 4', 5'
4'	1.48 (dd, 7.1, 1.3)	14.7	C-2', 3'
5'	1.83 (brs)	12.8	C-1', 2', 3'
OH	6.55, 5.78, 5.69 (3 s br)	-	

w = weak, vw = very weak

drofuran ring at C-20,21 by a 1,4-dioxan moiety. The latter was derived from the ^{1}H and ^{13}C NMR spectra of **1** by the presence of two oxymethylene groups at $\delta_H = 4.38/3.52$ ($\delta_C = 59.5$, C-22) and $\delta_H = 4.60/3.61$ (δ_C 60.0, C-23), which formed an isolated spin system in the ^{1}H-^{1}H TOCSY experiment. Moreover, the HMBC spectrum of **1** (▶ **Fig. 1**) gave important correlations between H_β-22 and an acetal-methine at $\delta_C = 95.0$ (C-21), between H_α-23 and a quaternary acetal carbon at $\delta_C = 93.1$ (C-20), and between H-21 with both C-20 and C-22. Therefore, the planar structure of compound **1** was characterized as 3-deacetyl-20,21-defuranyl-20,21-dioxanyl azadirachtin I and named mayombensin.

The relative configuration of mayombensin (**1**) was established on the basis of NOESY analysis and comparison with data reported in the literature for related compounds [11, 14]. Accordingly, C-11, C-19, and C-30 were assigned as β, and H-5 was α-oriented based on the biosynthesis of azadirachtin-type limonoids [15]. The NOESY spectrum of **1** showed correlations from both H-5 and H-1 to H-9.



▶ **Fig. 1** Selected COSY and HMBC correlations of mayombensin (**1**).

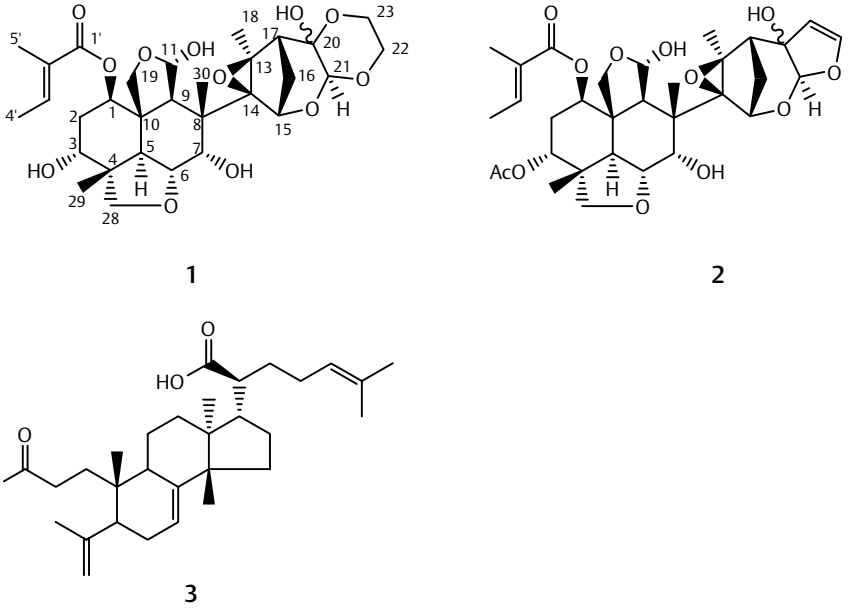
Further NOESY correlations from H-9 to H_3-18, from H_3-18 to H-17 and H-21, and from H-21 to H-15 were observed. The same experiment also exhibited correlations between H_3-30/H-7, H_3-30/H-6, H-6/H_3-29, and H_3-29/H-3. The relative configuration of mayombensin (**1**) was thus defined as shown in ▶ **Fig. 2**, which agrees – with exception for C-11,20 – with all other azadirachtins.

The known compounds were identified as 3,4-dimethyl-secotirucalla-4(28),7,24-trien-3,21-dioic acid (**3**), stigmasterol, β-sitosterol, and the glucosides of the latter two. The structures were determined by comparison of their NMR and mass spectral data with those reported in the literature [8, 16]. Additionally, two ceramides have been isolated (for data, see Supporting Information).

Materials and Methods

General procedures

The optical rotation was measured on a Perkin-Elmer polarimeter 241 at the sodium D line. The IR spectrum was recorded on an FT/IR-4100 Jasco spectrophotometer. NMR spectra were recorded on Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometers. The NMR data of **1** were referenced on pyridine-d_5 with δ_H 7.22 and δ_C 150.35; measurements in $CDCl_3$ were referenced to δ_H 7.24 and δ_C 77.00. ESI HRMS was measured on a microTOF (Bruker mass spectrometer). Melting points were determined on a Mettler FP61 melting point apparatus. Flash chromatography was performed using silica gel (Macherey Nagel & Co; 230-400 mesh). Thin-layer chromatography was performed using Merck pre-coated silica gel 60 F_{254} aluminum foil.



▶ **Fig. 2** Chemical structures of compounds **1**, **2**, and **3**.

Plant material

The twigs of *G. mayombensis* were collected in December 2014 from Kala Mount, Yaounde Cameroon. Authentication was carried out by M. Victor NANA at the National Herbarium Yaoundé, where a voucher specimen has been deposited (accession number: 46220HNC).

Extraction and isolation

The air-dried powdered twigs of *G. mayombensis* (4 kg) were extracted with 10 L of MeOH at room temperature (twice, each 2 days). After evaporation, 75 g of extract were obtained. The crude extract was subjected to column chromatography (100 × 4.5 cm) over silica gel (70–230 mesh), with a gradient system of *n*-hexane–EtOAc. A total of 370 fractions of ca. 200 mL each were collected. The pure compounds were obtained by direct crystallization. Fractions 50-67 (2.29 g), eluted with *n*-hexane–EtOAc (19:1), gave a mixture of stigmasterol and β-sitosterol (36 mg). The combined fractions 117–147 (3.9 g), eluted with *n*-hexane–EtOAc (17:3), gave 3,4-dimethyl-secotirucalla-4(28),7,24-trien-3,21-dioic acid (**3**; 32 mg). Fractions 228-245 (2.21 g), eluted with *n*-hexane-EtOAc (3: 2), afforded ceramide A (**8**) (18 mg). Fractions 271-285 (1.82 g), eluted with *n*-hexane-EtOAc (1:1) precipitated at room temperature, gave glucosides of stigmasterol and β-sitosterol (28 mg). The combined fractions 318-326 (1.04 g), eluted with *n*-hexane–EtOAc (2:3), afforded mayombensin (**1**) (18 mg). Combined fractions 335-358 (2.99 g), eluted with *n*-hexane–EtOAc (1:3), yielded ceramide B (**9**) (8 mg).

Mayombensin (**1**): White powder; m.p. 209-210 °C; $[\alpha]_D^{20}$ + 10.4 (c 0.5, MeOH); IR (film): ν_{max} 3423, 2930, 1697, 1652, 1268, 1142, 1075, 993 cm^{-1}; UV (MeOH): λ_{max} 220 nm (log ε = 3.81); ^1H and ^{13}C NMR data, see ▶ **Table 1**; (–)-ESI HRMS: m/z 593.2583 $[M - H]^-$ (calcd. for $C_{30}H_{42}O_{12}$ 593.2598).

3,4-Dimethyl-secotirucalla-4(28),7,24-trien-3,21-dioic acid (**3**): For ^1H, ^{13}C NMR, and MS data, see Supporting Information.

Ceramide A (**8**): For ^1H, ^{13}C NMR, and MS data, see Supporting Information. (+)-ESI HRMS: m/z = 704.6138 $([M + Na]^+$ (calcd. for $C_{42}H_{83}NNaO_5$ 704.6169). For the formula, see **Fig. S1** in the Supporting Information.

Ceramide B (**9**): For ^1H, ^{13}C NMR and MS data, see Supporting Information. (+)-ESI HRMS: m/z = 720.6092 $([M + Na]^+$ (calcd. for $C_{42}H_{83}NNaO_6$ 720.6118); (–)-ESI HRMS: m/z = 696.6103 $([M - H]^-$ (calcd. for $C_{42}H_{82}NO_6$ 696.6142). For the formula, see **Fig. S1** in the Supporting Information.

Supporting information

Spectral data of compounds **1**, **3**, **8** and **9** are available as Supporting Information.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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