# Steroids and Ceramide from the Stem Bark of *Odyendyea gabonensis*

Emmanuel Ngeufa Happi[a], Simone Véronique Fannang[b], Marie Fomani[a], Suzye Mireille Moladje Donkwe[a], Nkoungou Yomzak Carine Nicaise[a], Jean Duplex Wansi[a,c], and Norbert Sewald[c]

[a] Department of Chemistry, University of Douala, Faculty of Science, 24157 Douala, Cameroon
[b] Department of Pharmaceutical Science, University of Douala, Faculty of Medicine and Pharmaceutical Science, 2701 Douala, Cameroon
[c] Organic and Bioorganic Chemistry, Department of Chemistry, Bielefeld University, 33501 Bielefeld, Germany

Reprint requests to E. N. Happi. Tel: +237-99-86-50-48. E-mail: ngeufa@yahoo.fr

*Z. Naturforsch.* **2013**, *68b*, 924 – 930 / DOI: 10.5560/ZNB.2013-3132
Received May 10, 2013

Two new steroids, 22*E*, 24*R*-stigmast-22-ene-3,6,11-trione (**1**) and 22*E*, 24*R*-3-acetylstigmasta-5,22-diene-7,11-dione (**2**), and one new ceramide, (2*S*,3*S*,4*R*,5*R*) *N*-(1,3,4,5-tetrahydroxyundecan-2-yl)tetradecanamide (**7**), together with eleven known compounds were isolated from the $CH_2Cl_2$ extract of the stem bark of *Odyendyea gabonensis*. The structures of all compounds were determined by comprehensive analyses of their 1D and 2D NMR, mass spectral (EI and ESI) data, chemical reactions, and comparison with previously known analogs. Pure compounds were tested for their activity against the bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, the fungi *Mucor miehei* and *Candida albicans*, and the plant pathogen oomycetes *Aphanomyces cochlioides*, *Pythium ultimum* and *Rhizoctonia solani* using the paper disk agar diffusion assay. For active compounds, MICs were determined by the broth microdilution assay. Cytotoxic activity against the human lung adenocarcinoma cell line A 549 was evaluated by the MTT assay. All compounds delivered low to missing antimicrobial activities in the agar diffusion assay and MICs $> 1$ mg mL$^{-1}$. The alkaloids **10** and **11** displayed cytotoxic activity against the human lung adenocarcinoma cell line A549 with $IC_{50}$ 2.5 and 4.5 $\mu$m respectively.

*Key words:* *Odyendyea gabonensis*, Simaroubaceae, Steroids, Ceramide, Antimicrobial and Cytotoxicity Activities

## Introduction

The plant species *Odyendyea gabonensis* (Pierre) Engl. (Simaroubaceae), synonym *Quassia gabonensis* Pierre [1, 2], is a huge tree growing in primary forests of Central Africa. Bark, leaves and fruits of *O.gabonensis* are used in traditional medicine as purgative as well as for the treatment of malaria, pulmonary disorders and cancer [3]. Previous phytochemical investigations on the bark and fruits yielded scopoletin, 8-hydroxycanthin-6-one, ailanthinone, glaucarubinone, 2′-acetoxyglaucarubinone, 2′-acetoxy-glaucarubin, excelsin, canthin-6-one, 5-methoxycanthin-6-one, 4,5-dimethoxycanthin-6-one, 1-hydroxymethyl-$\beta$-carboline, 1-carboxamide-$\beta$-carboline, (–)-odyendane and (–)-odyendene, (–)-odyendanol, 9-hydroxy-5-methoxycanthin-6-one, 22*E*,24*R*-stigmasta-5,22-diene-3,7-dione, and 22*E*,24*R*-stigmast-22-ene-3,7-dione [4 – 7]. In this report, we describe the isolation and structural elucidation of two new steroids (**1**, **2**) and one ceramide (**7**), together with the antimicrobial and cytotoxic activities of isolated compounds.

## Results and Discussion

The $CH_2Cl_2$ extract of the stem bark of *O. gabonensis* was separated by repeated column chromatography and preparative TLC (PTLC) to afford three new and eleven known compounds (Fig. 1). The known compounds were identified as 22*E*,24*R*-stigmast-22-ene-3,6-dione (**3**), 22*E*,24*R*-3-acetylstigmasta-5,22-diene-7-one (**4**), $\beta$-sitosterone (**5**), stigmasterone (**6**), (–)-odyendene (**8**), (–)-odyendane (**9**), 9-hydroxy-5-

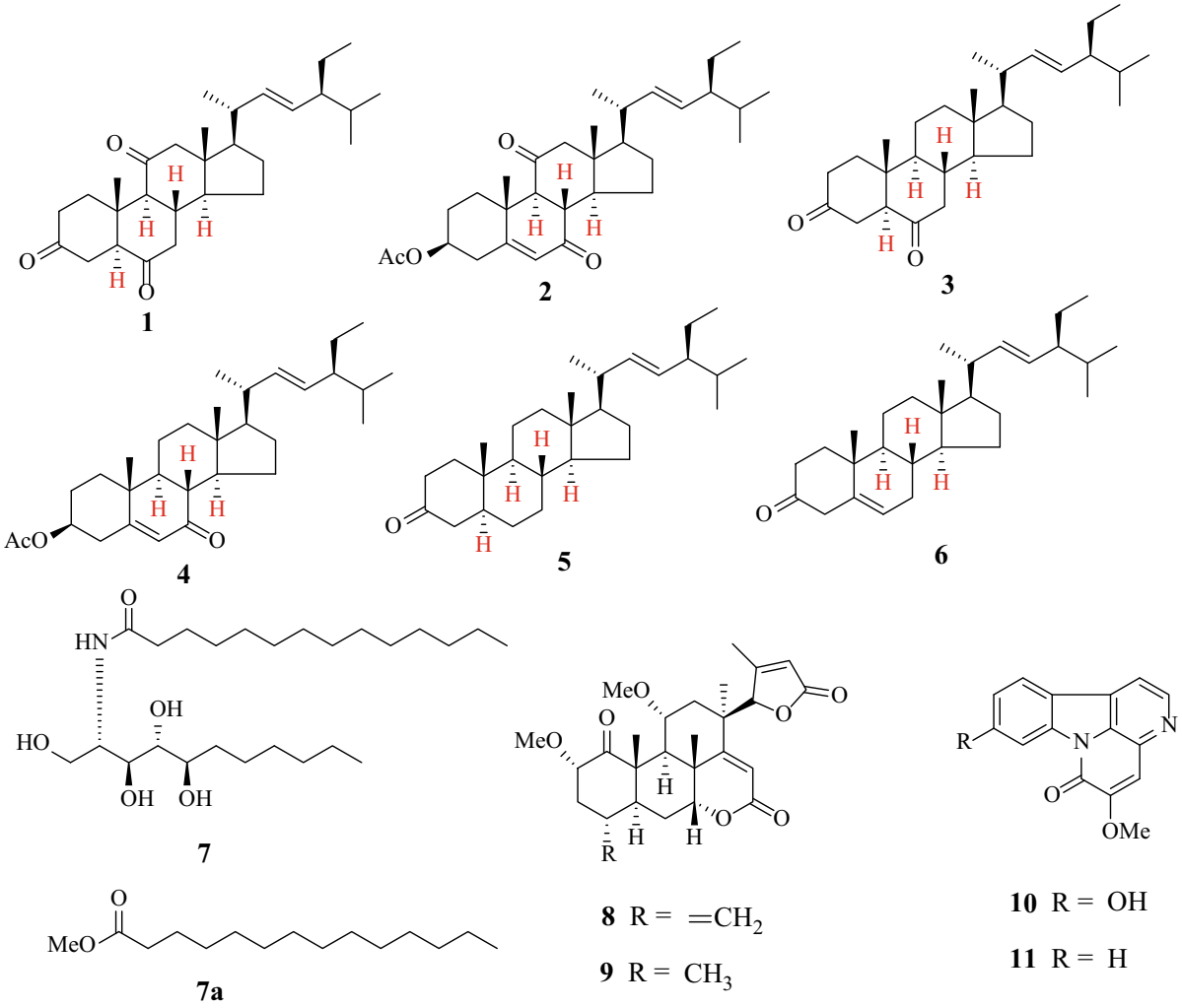


Fig. 1. Structures of the isolated compounds.

methoxycanthin-6-one (**10**), 5-methoxycanthin-6-one (**11**), tirucalla-7,24-dien-3-one, oleanolic acid, and 3-acetyloleanolic acid. The structures were confirmed by spectra comparison with authentic and published values [7 – 10].

Compound **1** was obtained as a colorless powder. The molecular composition was found to be $C_{29}H_{44}O_3$ by HR-EI-MS ($[M]^+$ at $m/z = 440.3249$, calcd. 440.3290). The presence of the carbonyl functions was revealed by strong absorptions at 1695 $cm^{-1}$ in the IR spectrum. Its $^1H$ NMR spectrum (Table 1) showed two *trans*-olefinic protons at $\delta = 5.18$ (dd, $J = 15.1$; 8.2 Hz, H-22) and 5.07 ppm (dd, $J = 15.1$; 8.8 Hz, H-23) as well as three methyl doublets at $\delta =$ 0.80 (d, $J = 6.3$ Hz, H-26), 0.97 (d, $J = 6.3$ Hz, H-27), and 1.03 ppm (d, $J = 6.3$ Hz, H-21), a methyl triplet at $\delta = 0.84$ (t, $J = 6.3$ Hz, H-29) and two methyl singlets at $\delta = 0.79$ and 1.19 ppm. A comparison with the stigmast-22-ene-3,6-dione suggested that both compounds had the same side chain [11]. The $^{13}C$ NMR spectrum (Table 1) confirmed the presence of a carbon-carbon double bond with signals at $\delta = 137.9$ (C-22), 129.7 ppm (C-23) and three carbonyl functions with signals at $\delta = 211.4$ (C-6), 209.2 (C-3), 209.2 ppm (C-11). Furthermore, the $^1H$ NMR spectrum showed a doublet at $\delta = 2.59$ ppm (d, $J = 8.8$ Hz, H-9) and an AB system of two protons at $\delta = 2.43$ (d, $J = 10.7$ Hz, H-12a) and 2.37 ppm (d, $J = 10.7$ Hz, H-12b) due to

Table 1. $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR assignments for **1** and **2** in $CDCl_3$[a].

| Attribution | **1** | | **2** | |
|---|---|---|---|---|
| | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ |
| 1 | 38.1 | | 37.1 | |
| 2 | 39.3 | 2.09 (m) | 28.2 | |
| | | 2.09 (m) | | |
| 3 | 209.2 | – | 73.3 | 4.47 (brs) |
| 4 | 37.0 | 2.61 (brs) | 38.5 | 2.03 (m) |
| | | 2.40 (brs) | | 2.06 (m) |
| 5 | 57.5 | 2.61(brs) | 168.4 | – |
| 6 | 211.4 | | 126.3 | 5.83 (s) |
| 7 | 46.6 | 2.41 (dd, $J = 13.2, 10.7$) | 200.5 | – |
| | | 2.37 (dd, $J = 10.7, 8.8$) | | |
| 8 | 31.8 | 2.10 (m) | 45.8 | 2.54 (dd, $J = 5.2, 5.2$) |
| 9 | 57.5 | 2.59 (d, $J = 8.8$) | 56.0 | 2.51 (d, $J = 5.2$) |
| 10 | 41.3 | – | 37.9 | – |
| 11 | 209.2 | – | 207.1 | – |
| 12 | 56.6 | 2.43 (d, $J = 10.7$) | 53.6 | 2.42 (brs) |
| | | 2.37 (d, $J = 10.7$) | | 2.20 (brs) |
| 13 | 43.0 | – | 40.5 | – |
| 14 | 55.8 | – | 51.2 | – |
| 15 | 25.4 | – | 23.0 | – |
| 16 | 28.4 | – | 28.8 | – |
| 17 | 56.7 | – | 55.9 | – |
| 18 | 11.9 | 0.79 (s) | 12.0 | 0.76 (s) |
| 19 | 18.9 | 1.19 (s) | 19.0 | 0.78 (s) |
| 20 | 40.4 | 2.09 (m) | 39.6 | 2.00 (m) |
| 21 | 12.3 | 1.03 (d, $J = 6.3$) | 12.2 | 1.05 (d, $J = 6.9$) |
| 22 | 137.9 | 5.18 (dd, $J = 15.1, 8.2$) | 138.1 | 5.17 (dd, $J = 15.1, 8.2$) |
| 23 | 129.7 | 5.07 (dd, $J = 15.1, 8.8$) | 129.5 | 5.05 (dd, $J = 15.1, 8.8$) |
| 24 | 51.2 | 1.56 (m) | 51.2 | 1.57 (m) |
| 25 | 29.1 | 1.57 (m) | 31.8 | 1.57 (m) |
| 26 | 18.7 | 0.80 (d, $J = 6.3$) | 20.9 | 0.87 (d, $J = 5.6$) |
| 27 | 21.1 | 0.97 (d, $J = 6.3$) | 19.8 | 0.95 (d, $J = 5.6$) |
| 28 | 24.0 | 1.40 (m) | 24.1 | 1.39 (m) |
| 29 | 12.0 | 0.84 (d, $J = 6.3$) | 12.3 | 0.83 (d, $J = 6.3$) |
| $1'$ | | | 168.4 | – |
| $2'$ | | | 21.1 | 2.19 |

[a] Assignments were based on HMQC, HMBC and NOESY experiments. Multiplicities and coupling constants in Hz in parentheses.

the presence of the protons adjacent to the third carbonyl group. The positions of the three carbonyl functions were determined by the HMBC and EI-MS spectra. In the HMBC spectrum, correlations between H-8 ($\delta = 2.10$) and C-6 ($\delta = 211.4$), C-11 ($\delta = 209.2$), C-13 ($\delta = 43.0$) and C-10 ($\delta = 41.3$) and between H-5 ($\delta = 2.61$) and C-6 ($\delta = 211.4$), C-3 ($\delta = 209.2$), C-7 ($\delta = 46.6$) and C-10 ($\delta = 41.3$) indicate the position of the three carbonyl groups at C-3, C-6 and C-11. This assignment was in agreement with the EI-MS, which showed two prominent fragment ion peaks at $m/z = 260$ ($C_{16}H_{20}O_3$) and 257 ($C_{16}H_{17}O_3$) resulting from the loss of the side chain and D-ring carbons. These ion peaks indicate that the three carbonyl functions are located on the rings A, B or C. The fragment ion peak at $m/z = 124$ ($C_8H_{12}O$) arises from the loss of the side chain and the cleavage mechanisms (bonds C-9/C-11 and C-8/C-14) involving transfer of hydrogen from C-9 to the charge-retaining fragment, confirming the position C-11 for the third carbonyl [12]. Furthermore, the molecular fragment at $m/z = 177$ ($C_{11}H_{13}O_2$) resulted from the hydrogen transfer from C-7 to C-9 and from C-9 to C-14, followed by the cleavage of the bond C-9/C-11, indicating that the second carbonyl group is located at C-6 [12]. Finally, according to biogenetic considerations of steroid and ion fragments at $m/z = 55$ ($C_3H_3O$), 69 ($C_4H_6O$) and 81 ($C_5H_6O$) observed in the EI-MS, the first carbonyl function is located at C-3 [13, 14]. From the above spectroscopic studies, the structure of compound **1** was determined as 22*E*,24*R*-stigmast-22-ene-3,6,11-trione.

Compound **2** was obtained as a colorless powder. The molecular composition was found to be $C_{31}H_{46}O_3$ by HR-EI-MS ($[M]^+$ at $m/z = 482.3390$, calcd. 482.3396). The presence of the carbonyl and enone functions was revealed by strong absorptions at 1735 and 1655 $cm^{-1}$ in the IR spectrum and confirmed by a strong absorption at 245 nm in the UV spectrum [15]. Its $^1H$ NMR spectrum (Table 1) showed three olefinic protons at $\delta = 5.83$ (s, H-6), 5.17 (dd, $J = 15.1$; 8.2 Hz, H-22) and 5.05 ppm (dd, $J = 15.1$; 8.8 Hz, H-23) as well as three methyl doublets at $\delta = 0.87$ (d, $J = 5.6$ Hz, H-26), 0.95 (d, $J = 5.6$ Hz, H-27) and 1.05 ppm (d, $J = 6.9$ Hz, H-21), a methyl triplet at $\delta = 0.83$ ppm (t, $J = 6.3$ Hz, H-29) and two methyl singlets at $\delta = 0.76$ and 0.78 ppm. A comparison with the spectrum of stigmasterol suggested that both compounds possess the same side chain [13]. The $^{13}C$ NMR spectrum (Table 1) confirmed the presence of an enone group with signals at $\delta = 168.4$ (C-5) and 126.3 ppm (C-6), a carbon-carbon double-bond at $\delta = 138.1$ (C-22) and 129.5 ppm (C-23), two carbonyl functions at $\delta = 200.5$ (C-7) and 207.1 ppm (C-11), one oxymethine at $\delta = 73.3$ ppm (C-3), and an acetate group at $\delta = 168.4$ (C-1′) and 21.1 ppm (C-2′). Furthermore, the $^1H$ NMR spectrum showed four signals at $\delta = 2.54$ (dd, $J = 5.2$; 5.2 Hz, H-8), 2.51 (d, $J = 5.2$ Hz, H-9), 2.42 (brs, H-12a), and 2.20 ppm (brs, H-12b) due to protons adjacent to the carbonyl groups and one additional methyl group at $\delta = 2.19$ ppm (s) confirming the presence of an acetate function. In addition, it showed a signal at $\delta = 4.37$ ppm (brs) attributed to H-3, confirming the location of the acetate group at C-3 position, in agreement with the reported data [15]. The positions of the two carbonyl functions were determined by HMBC and EI-MS spectra. The HMBC spectrum reveals correlations between H-8 ($\delta = 2.54$) and C-11 ($\delta = 207.1$), C-6 ($\delta = 126.3$), C-5 ($\delta = 168.4$) and C-10 ($\delta = 37.9$), and between H-9 ($\delta = 2.51$) and C-11 ($\delta = 207.1$), C-7 ($\delta$200.5), C-5 ($\delta = 168.4$), C-12 ($\delta = 53.6$) and C-14 ($\delta = 51.2$), indicating the position of the two carbonyl groups at C-7 and C-11. This assignment is in agreement with the EI-MS, which showed two prominent fragment ion peaks at $m/z = 302$ ($C_{18}H_{22}O_4$) and 300 ($C_{18}H_{20}O_4$) resulting from the loss of the side chain and D-ring carbons. These ion peaks indicated that the two carbonyl functions are located on the rings A, B or C. The fragment ion peak at $m/z = 124$ ($C_8H_{12}O$) arises from the loss of the side chain and the cleavage mechanisms (bonds C-9/C-11 and C-8/C-14) involving transfer of hydrogen from C-9 to the charge-retaining fragment, confirming the position C-11 for the third carbonyl [12]. Furthermore, the molecular fragments at $m/z = 194$ ($C_{11}H_{14}O_3$) and 152 ($C_9H_{12}O_2$), arise from the RDA fragmentation and the loss of an acetyl group, suggesting that the second carbonyl is placed at C-7 [16]. The spectroscopic studies identify the structure of compound **2** as 22*E*,24*R*-3-acetylstigmasta-5,22-diene-7,11-dione.

Compound **7** was obtained as a colorless amorphous powder. The IR spectrum showed an absorption band at 3450 $cm^{-1}$ due to OH functions, a strong absorption band at 1660 $cm^{-1}$ indicating the presence of a secondary amide group [17], and additional absorption bands at 2950, 2910 and 1507 $cm^{-1}$ (aliphatic) suggesting the compound to be a fatty acid amide. The molecular composition was found to be $C_{25}H_{52}NO_5$ by ESI-MS ($m/z = 446$; $[M+H]^+$) and HR-ESI-MS ($[M+H]^+$ at $m/z = 446.3840$, calcd. 446.3845).

The $^1H$ NMR spectrum of **7** displayed a downfield doublet at $\delta = 7.21$ ppm (d, $J = 8.8$ Hz, NH), a very strong aliphatic methylene signal at $\delta = 1.15 - 1.33$ ppm, as well as signals of two methyl protons at $\delta = 0.76$ (t, $J = 6.9$ Hz, H-11 and H-14′) and five methines at $\delta = 3.80$ (m, H-2), 3.74 (dd, $J = 6.9$; 4.7 Hz, H-3), 3.48 (dd, $J = 10.7$; 5.2 Hz, H-1a), 3.44 (dd, $J = 10.7$; 5.2 Hz, H-1b), 3.27 (m, H-4), and 3.25 ppm (m, H-5). The $^{13}C$ NMR and DEPT spectra data of **7** supported the above analysis, showing a carbonyl group at $\delta = 173.9$ ppm (C-1′), four oxygenated carbons at $\delta = 75.1$ (C-4), 71.6 (C-3), 71.6 (C-5), and 61.1 ppm (C-1), one methane carbon at $\delta = 51.9$ ppm (C-2), aliphatic methylenes in the region $\delta = 22.4 - 32.4$ ppm, and two methyls at $\delta = 14.3$ ppm (C-11 and C-14′) [18]. The correlations of $\delta = 7.21$ (NH) with $\delta = 173.9$ (C-1′), 71.6 (C-3) and 51.9 (C-2) observed in the HMBC spectrum confirmed the presence of an amide function and suggested compound **7** to be a ceramide [19].

In order to determine the lengths of the sphingosine and fatty acid chains and the absolute configuration of **7**, the acid methanolysis method of Gaver and Sweeley was applied [19]. Fatty acid methyl ester **7a** was obtained by methanolysis of compound **7**. The length of the fatty acid chain in **7a** was determined by EI-MS, which showed significant ion peaks at $m/z = 242$ $[CH_3(CH_2)_{12}COOCH_3]^+$ and 211 $[CH_3(CH_2)_{12}CO]^+$. Cross peaks in the $^1H$-$^1H$ COSY

spectrum were observed between an amide proton ($\delta =$ 7.21) and H-2 ($\delta = 3.80$), which, in turn, was coupled to three protons at $\delta = 3.48$ (H-1a), $\delta = 3.44$ (H-1b) and $\delta = 3.74$ (H-3). Furthermore, H-3 showed correlations with H-2 and H-4, as well as H-4 with H-5 ($\delta = 3.25$) suggesting that the hydroxyl groups are present at C-1, C-3, C-4 and C-5 of the sphingosine chain. On the basis of this evidence, the structure of compound **7** was determined to be *N*-(1,3,4,5-tetrahydroxyundecan-2-yl)tetradecanamide.

The stereochemistry at the stereogenic centers C-2 to C-5 has already been established in basalamine A [20]. Comparison of the optical rotation value of **7** $\{[\alpha]_D^{28} = +25.5$ ($c = 0.20$, MeOH)$\}$ with the published value of basalamine A revealed the absolute configuration of compound **7** to be 2*S*, 3*S*, 4*R*, and 5*R*. Thus, the structure of compound **7** was assigned as (2*S*,3*S*,4*R*,5*R*)-*N*-(1,3,4,5-tetrahydroxyundecan-2-yl)tetradecanamide.

Tests of the pure compounds on paper disk diffusion agar against the bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, the fungi *Mucor miehei* and *Candida albicans* and the plant pathogen oomycetes *Aphanomyces cochlioides*, *Pythium ultimum* and *Rhizoctonia solani* resulted in missing or low activities, the latter with MIC values $> 1$ mg mL$^{-1}$.

However, alkaloids **10** and **11** displayed moderate cytotoxic activity against the human lung adenocarcinoma cell line A 549 ($IC_{50}$ 2.5 and 4.5 $\mu$m).

## Experimental Section

*General experimental procedures*

Ultraviolet spectra were recorded on a Hitachi UV 3200 spectrophotometer in MeOH. Infrared spectra were recorded on a Jasco 302-A spectrophotometer. ESI-HR mass spectra were recorded on a Bruker FTICR 4.7 T mass spectrometer. EI-MS were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for HR-EI-MS. The $^1$H and $^{13}$C NMR spectra were recorded at 500 MHz and 125 MHz, respectively, on Bruker DRX 500 NMR spectrometers. Methyl, methylene and methine carbons were distinguished by DEPT experiments. Homonuclear $^1$H connectivities were determined by using the COSY experiment. One-bond $^1$H-$^{13}$C connectivities were determined with HMQC gradient pulse factor selection. Two- and three-bond $^1$H-$^{13}$C connectivities were determined by HMBC experiments. Chemical shifts are reported in $\delta$ (ppm) using TMS as internal standard and coupling constants (*J*) were measured in Hz. Column chromatography was carried out on silica gel (70 – 230 mesh, Merck). TLC was performed on Merck precoated silica gel 60 $F_{254}$ aluminum foil, and spots were detected using ceric sulfate spray reagent. Phenolic compounds were detected using $FeCl_3$ reagent. The purity of the compounds was investigated by means of $^1$H NMR and ESI-MS. The degree of purity of all tested compounds was $> 95$ %, and of the positive control (Doxorubicin) 99.9 %. All other substances, if not specified ortherwise, were purchased from Sigma-Aldrich (Germany). All reagents used were of analytical grade.

*Plant material*

The stem bark of *O. gabonensis* was collected at the Mbalmayo locality in Central Cameroon in August 2007 and identified by Mr. Nana Victor of the National Herbarium, Yaoundé, Cameroon, where a voucher specimen (ref. 9861 SRF/CAM) was deposited.

*Extraction and isolation*

The air-dried and powdered stem bark (3.5 kg) of *O. gabonensis* was extracted with $CH_2Cl_2$ at room temperature for 72 h. After evaporation under reduced pressure, 46.5 g of crude extract was obtained. The $CH_2Cl_2$ extract was purified by column chromatography over silica gel 60 (230 – 400 mesh) and preparative TLC using a gradient system of petroleum ether, $CH_2Cl_2$, acetone and MeOH. 200 sub-fractions (*ca.* 250 mL each) were collected and pooled on the basis of TLC analysis leading to five main fractions (A–E).

Fraction A (10.2 g, combined from sub-fractions 1 – 50) was chromatographed over a silica gel 60C column with a petroleum ether-$CH_2Cl_2$ gradient. A total of 30 fractions of *ca.* 100 mL each was collected and combined on the basis of TLC. Fractions 5 – 10 were further chromatographed on silica gel 60H with a mixture of petroleum ether-$CH_2Cl_2$ (4 : 1) for elution to yield tirucalla-7,24-dien-3-one (10.5 mg), stigmasterone (**6**) (8.5 mg) and $\beta$-sitosterone (**5**) (4.5 mg). Fractions 11 – 25 were chromatographed on silica gel 60H with a mixture of petroleum ether-$CH_2Cl_2$ (4 : 1) for elution to yield 22*E*,24*R*-stigmast-22-ene-3,6-dione (**3**) (8.4 mg) and 22*E*,24*R*-3-acetylstigmasta-5,22-diene-7-one (**4**) (11.5 mg). Fraction B (7.0 g, combined from sub-fractions 51 – 70) was chromatographed over a silica gel 60C column with a petroleum ether-$CH_2Cl_2$ gradient. A total of 20 fractions of *ca.* 100 mL each was collected and combined on the basis of TLC. Fractions 7 – 15 were further chromatographed over a silica gel 60H with a mixture of petroleum ether-$CH_2Cl_2$ (5 : 2) to yield 22*E*,24*R*-stigmast-22-ene-3,6,11-trione (**1**) (15.2 mg) and 22*E*,24*R*-3-acetylstigmasta-5,22-diene-7,11-dione (**2**) (7.5 mg). Fraction C (6.5 g, combined from sub-fractions 71 – 100) was chromatographed on a silica gel 60C column

with a petroleum ether-$CH_2Cl_2$ gradient. 25 fractions of *ca*. 100 mL each were collected and combined on the basis of TLC. Fractions 16 – 25 were further chromatographed over silica gel 60H with petroleum ether-$CH_2Cl_2$ (1 : 1) to yield (–)-odyendene (**8**, 60.0 mg) and (–)-odyendane (**9**, 7.8 mg). Fraction D (9.5 g, combined from sub-fractions 101 – 130) was chromatographed on a silica gel 60C column with a petroleum ether-$CH_2Cl_2$ (1 : 3) and $CH_2Cl_2$-aceton (3 : 1) gradient. As a result, 40 fractions of *ca*. 100 mL each were collected and combined on the basis of TLC. Fractions 1 – 15 were further chromatographed over silica gel 60H with petroleum ether-$CH_2Cl_2$ (1 : 4) to yield 9-hydroxy-5-methoxycanthin-6-one (**10**, 5.5 mg) and 5-methoxycanthin-6-one (**11**, 7.5 mg). Fractions 16 – 40 were further chromatographed over silica gel 60H with a mixture of $CH_2Cl_2$-aceton (1 : 2) to yield 3-acetyloleanolic acid (10.0 mg), oleanolic acid (12.0 mg) and (2*S*,3*S*,4*R*,5*R*)-*N*-(1,3,4,5-tetrahydroxyundecan-2-yl)tetradecanamide (**7**, 16.5 mg). Fraction E (10.5 g) was combined from sub-fractions 131 – 200 and eluted with $CH_2Cl_2$-acetone (4 : 1) and acetone-MeOH (5 : 1).

*Chemical derivatives*

*Methanolysis of compound **7***

Compound **7** (6.5 mg) was added to a mixture of HCl (3.5 mL, N) and dry MeOH (6.0 mL), and refluxed for 16 h with magnetic stirring. 10.0 mL $H_2O$ was added, and the refluxed mixture was extracted with *n*-hexane (3 × 10 mL). The fatty acid methyl ester (**7a**, 2.0 mg) was obtained after purification of the *n*-hexane extract over a silica gel column with *n*-hexane-$CH_2Cl_2$ (9 : 1) as solvent. The MeOH/$H_2O$ phase was evaporated under reduced pressure.

*22E,24R-Stigmast-22-ene-3,6,11-trione (**1**)*

Colorless powder ($CHCl_3$); m. p. 180 – 183 °C; $R_f = 0.75$, silica gel 60 $F_{254}$, petroleum ether-$CH_2Cl_2$ (6 : 1). – IR (KBr): $\nu$ = 3005, 1700, 1640, 1300, 1045 $cm^{-1}$. – $^1$H NMR and $^{13}$C NMR data, see Table 1. – MS ((+)-EI): $m/z(\%)$ = 440 (8) $[M]^+$, 410 (30), 260 (15), 257 (40), 177 (25), 124 (45), 109 (31), 69 (75), 55 (68). – HRMS ((+)-EI): $m/z$ = 440.3249 (calcd. for $C_{29}H_{44}O_3$, 440.3290, $[M]^+$).

*22E,24R-3-Acetylstigmasta-5,22-diene-7,11-dione (**2**)*

Colorless powder ($CHCl_3$); m. p. 198 – 200 °C; $R_f = 0.75$, silica gel 60 $F_{254}$, petroleum ether-$CH_2Cl_2$ (6 : 1). – UV (MeOH): $\lambda_{max}(\log\varepsilon)$ = 245(3.45) nm. – IR (KBr): $\nu$ = 3350, 1735, 1655, 1560, 1300, 1062, 845 $cm^{-1}$. – $^1$H NMR and $^{13}$C NMR data, see Table 1. – MS ((+)-EI): $m/z(\%)$ = 482 (20) $[M]^+$, 439 (15), 302 (18), 300 (36), 287 (38), 245 (44), 194 (33), 151 (25), 124 (34), 55 (100). – HRMS ((+)-EI): $m/z$ = 482.3390 (calcd. 482.3396 for $C_{31}H_{46}O_3$, $[M]^+$).

*(2S,3S,4R,5R)-N-(1,3,4,5-tetrahydroxyundecan-2-yl)-tetradecanamide (**7**)*

Colorless amorphous powder; $R_f$ = 0.60, silica gel 60 $F_{254}$, $CH_2Cl_2$-MeOH (9 : 1). – $[\alpha]_D^{28}$ = +25.5 ($c$ = 0.20, MeOH). – IR ($CH_3OH$): $\nu$ = 3450, 2950, 2910, 1660, 1507 $cm^{-1}$. – $^1$H NMR (500 MHz, [$D_6$]DMSO ): $\delta$ = 0.76 (t, $J$ = 6.9 Hz, H-11 and H-14′), 1.15 – 1.31 (brs, H-6–H10, H-4′–H12′), 1.50 (m, H-2′), 1.54 (m, H-6 and H-3′), 3.25 (m, H-5), 3.27 (m, H-4), 3.44 (dd, $J$ = 5.2; 10.7 Hz, H-1b), 3.48 (dd, $J$ = 5.2; 10.7 Hz, H-1a), 3.74 (dd, $J$ = 4.7; 6.9 Hz, H-3′), 3.80 (m, H-2), 7.21 (d, $J$ = 8.8 Hz, -NH). – $^{13}$C NMR (125 MHz, [$D_6$]DMSO ): $\delta$ = 14.2 (C-11 and C-14′), 22.4 (C-10 and C-13′), 25.8 (C-3′), 31.7 – 32.4 (methylenes), 51.9 (C-2), 61.1 (C-1), 71.6 (C-5), 71.6 (C-3), 75.1 (C-4), 173.9 (C-1′). – MS ((+)-ESI): $m/z$ = 446 $[M+H]^+$. – HRMS ((+)-ESI): $m/z$ = 446.3840 (calcd. 446.3845 for $C_{25}H_{52}NO_5$, $[M+H]^+$).

*Fatty acid methyl ester **7a***

Colorless oil. – $^1$H NMR (500 MHz, $CHCl_3$): $\delta$ = 3.75 (3H, OMe), 1.67 – 1.80 (m, H-3), 1.27 – 1.24 (methylene band), 0.87 (t, $J$ = 7.0 Hz, H-14).

*Biological activities*

*Antimicrobial assays*

The studies on agar diffusion test plates with the bacteria *Bacillus subtilis* and *Escherichia coli* (on peptone agar), *Staphylococcus aureus* (Bacto nutrient agar) and the fungi *Mucor miehei* and *Candida albicans* (Sabouraud agar) as test strains were performed as previously described [21]. For the plant pathogen oomycetes *Aphanomyces cochlioides*, *Pythium ultimum* and *Rhizoctonia solani*, squares of 0.5 × 0.5 cm were cut with a microbiological hook from the growth margins of mycelial mats grown on PDA plates, inoculated onto the centers of fresh plates and cultivated for 24 h at 28 °C to initiate radial growth.

The compounds were dissolved in $CH_2Cl_2$-MeOH (9 : 1), and paper disks (∅ 9 mm) were each impregnated with 40.0 µg, dried for 1 h under sterile conditions and arranged evenly on the pre-made agar test plates, wheras for oomycetes, the disks were placed around the mycelial squares at a distance of 30 mm. Bacteria and fungi plates were kept in an incubator at 37 °C for 15 h, oomycetes at 28 °C for 48 h. The diameter of inhibition zones (in mm) was measured directly or calculated from the radius. Nystatin (Maneesh Pharmaceutic PVT. Ltd., Govandi, Mumbai, 400 043, India) was used as positive control for fungi and gentamycin (Jinling Pharmaceutic (Group) corp., Zhejang Tieng Feng Pharmaceutic Factory, No. 11 Chezhan Road, Huzhou City, Zhejang, China) for bacteria.

*Microdilution broth assay*

The MICs of compounds were measured by the microdilution broth susceptibility assay [22]. The inocula of bacterial and fungal strains were prepared from 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The compounds were dissolved in 10 % DMSO and serial two-fold diluted in 96-well microtiter plates in duplicate, using BHI broth for bacterial and sabouraud dextrose broth for fungal tests. Standardized inocula of test strains were added and incubated at 37 °C for 24 h on a rotary shaker at 200 rpm. The MIC is read as the lowest concentration that inhibits test strain growth.

*Cytotoxicity assay*

Cytotoxic activities of the compounds were evaluated against the human lung carcinoma cell line A 549 (#CCL-185) by the MTT method according to a reported protocol [23]. Freshly trypsinized cell suspensions were seeded into 96-well microtiter plates at densities of $1 \times 10^4$ cells per well, and test compounds were added from DMSO-diluted stock. After 3 days, the attached cells were incubated with MTT and subsequently solubilized in DMSO. The absorbance at 550 nm was measured by using a microplate reader. The $IC_{50}$ is the concentration of agent that reduced cell growth under experimental conditions to 50 %, with doxorubicin as positive control ($IC_{50}$ 1.5 $\mu$m).

*Acknowledgement*

We wish to acknowledge the Alexander von Humboldt (AvH) Foundation for postdoctoral fellowships to J. D. Wansi. We also thank the International Foundation for Sciences (IFS, Sweden) for providing financial support (F/3978-2).

[1] H. G. A. Engler, *Pflanzenfamilien* **1896**, *3*, 215.
[2] A. Aubreville, *Flore du Gabon*, Paris **1962**, pp. 3.
[3] A. Bouquet, *Féticheurs et médecines traditionnelles du Congo, (Brazzaville), mémoires O.R.S.T.O.M.*, N° 36, Paris **1969**, pp. 229.
[4] P. Forgacs, J. Provost, A. Touche, D. Guenard, C. Thal, J. Guilhem, *Tetrahedron Lett.* **1985**, *26*, 3457 – 3460.
[5] P. Forgacs, J. Provost, A. Touche, *Planta Med.* **1982**, *46*, 187 – 189.
[6] P. G. Waterman, S. A. Ampofo, *Planta Med.* **1984**, *50*, 261 – 263.
[7] M. S. M. Donkwe, E. N. Happi, J. D. Wansi, N. B. Lenta, K. P. Devkota, N. Sewald, *Planta Med.* **2012**, *78*, 1949 – 1956.
[8] K. Mitsunaga, K. Kioke, T. Tanaka, Y. Ohkawa, Y. Kobayashi, T. Sawaguchi, T. Ohmoto, *Phytochemistry* **1994**, *35*, 799 – 802.
[9] E. Kotani, T. Takeya, H. Egawa, S. Tobinaga, *Chem. Pharm. Bull.* **1997**, *45*, 750 – 752.
[10] S. N. J. Grosvenor, K. Mascoll, S. McLean, W. F. Reynolds, W. F. Tinto, *J. Nat. Prod.* **2006**, *69*, 1315 – 1318.
[11] E. M. M. Gaspar, H. J. C. Das Neves, *Phytochemistry* **1993**, *34*, 523 – 527.
[12] D. H. Williams, J. M. Wilson, H. Budzikiewicz, C. Djerassi, *J. Am. Chem. Soc.* **1963**, *85*, 2091 – 2105.
[13] M. A. Khuroo, M. A. Qureshi, T. K. Razdan, P. Nichols, *Phytochemistry* **1988**, *27*, 3541 – 3544.
[14] S. Gupta, M. Ali, M. S. Alan, M. Niwa, T. Sakai, *Phytochemistry* **1992**, *31*, 2558 – 2560.
[15] J. D. Wansi, J. Wandji, W. A. F. Kamdem, J. C. Ndom, E. N. Happi, D. D. Chiozem, M. I. Choudhary, N. Tsabang, F. Tillequin, Z. T. Fomum, *Nat. Prod. Res.* **2006**, *20*, 586 – 592.
[16] M. Della Greca, P. Monaco, L. Previtera, *J. Nat. Prod.* **1990**, *53*, 1430 – 1435.
[17] U. A. Viqar, J. Hussain, H. Hussain, U. Farooq, E. Akber, S. A. Nawaz, M. I. Choudhary, *Z. Naturforsch.* **2004**, *59b*, 329 – 333.
[18] Y. B. Meffo, K. Krohn, H. Hussain, E. Dongo, B. Schulz, H. Qunxiu, *Z. Naturforsch.* **2006**, *61b*, 78 – 82.
[19] B. N. Su, R. Misico, E. J. Park, B. D. Santarsiero, D. A. Mesecar, H. H. S. Fong, J. M. Pezzuto, D. Kinghorn, *Tetrahedron* **2002**, *58*, 3453 – 3466.
[20] J. D. Wansi, B. L. J. Djouaka, E. N. Happi, K. P. Devkota, B. N. Lenta, M. A. Mesaik, M. I. Choudhary, Z. T. Fomum, N. Sewald, *Z. Naturforsch.* **2009**, *64b*, 452 – 458.
[21] R. P. Maskey, R. N. Asolkar, E. Kapaun, I. Wagner-Döbler, H. Laatsch, *J. Antibiot.* **2002**, *55*, 643 – 649.
[22] *Performance standards for antimicrobial susceptibility testing*, 19th ed., CLSI/NCCLS M 100-S18, Clinical and Laboratory Standards Institute/NCCLS, Wayne, PA (USA) **2008**.
[23] Q. Zhao, Q. Chen, X. J. Hao, J. Han, Y. G. Zuo, C. Zuo, G. L. Xu, *Chem. Pharm. Bull.* **2008**, *56*, 210 – 212.