Endodesmiadiol, a Friedelane Triterpenoid, and Other Antiplasmodial Compounds from *Endodesmia calophylloides*

Erasmienne Tambou NGOUAMEGNE,[a,b] René Soh FONGANG,[a] Silvère NGOUELA,[a] Fabrice Fekam BOYOM,[c] Michel ROHMER,*[,b] Etienne TSAMO,*[,a] Jiri GUT,[d] and Philip Jon ROSENTHAL[d]

[a] *Laboratoire de Substances Naturelles et Synthèse Organique, Département de Chimie Organique, Faculté des Sciences, Université de Yaoundé I;* [c] *Département de Biochimie, Faculté des Sciences, Université de Yaoundé I; BP 812 Yaoundé, Cameroun:* [b] *Laboratoire de Chimie et Biochimie des Microorganismes, Institut de Chimie, Université Louis Pasteur; CNRS LC3-UMR 7177, 4 rue Blaise Pascal, 67070 Strasbourg, France: and* [d] *Division of Infectious Diseases, Department of Medicine, University of California San Francisco; SFGH, 1001 Potrero Avenue, San Francisco, CA 94100, U.S.A.*
Received September 13, 2007; accepted November 29, 2007; published online December 7, 2007

From the ethyl acetate extract of the stem bark of *Endodesmia calophylloides* (Guttiferae), a novel friedelane triterpenoid named endodesmiadiol (1), as well as the known compounds friedelin (2), canophyllol (3), canophyllal (4), cerin (5), morelloflavone (6), volkensiflavone (7), 8-deoxygartanin (8), 3β-acetoxyoleanolic acid (9) and 1,8-dihydroxy-3-isoprenyloxy-6-methylxanthone (10) have been isolated. The structures of these compounds were established by spectroscopic analysis, and the relative configuration of endodesmiadiol (1) was confirmed by X-ray diffraction. The antiplasmodial activity of the isolated compounds was evaluated against the W2 strain of *Plasmodium falciparum* which is resistant to chloroquine and other antimalarial drugs. All the compounds were found to be active with IC_{50} values ranging from 7.2 to 23.6 μM. The IC_{50} of endodesmiadiol was found to be 11.8 μM.

Key words *Endodesmia calophylloides*; Guttiferae; friedelane triterpenoid; X-ray structure analysis; antimalarial drug

The Guttiferae (Clusiaceae) represent a family almost exclusively tropical in distribution and comprise about 50 genera and 900 species.[1)] This family produces a wide range of secondary metabolites including xanthones,[2—8)] biflavonoids[3,6,9—13)] and triterpenes.[13,14)] The genus *Endodesmia* belonging to the Guttiferae family is an African monotypic genus represented by the sole species *Endodesmia calophylloides* BENTH. This tree is found in Nigeria, Cameroon, Gabon and Angola.[15)] Neither medicinal uses, nor phytochemical studies have been reported for this plant.

The present work describes the isolation and structural elucidation of a novel friedelane triterpene, 2α,28-dihydroxyfriedelan-3-one (**1**), named endodesmiadiol, along with 9 known compounds and their antiplasmodial activity (Fig. 1).

The air-dried and powdered stem bark of *E. calophylloides* was successively extracted with *n*-hexane, EtOAc and MeOH. The three extracts were analysed separately. The *n*-hexane extract was subjected to column chromatography separations and yielded a complex mixture of prenylated aromatic compounds, which is still under investigation. The EtOAc extract was repeatedly fractionated by column chromatography on silica gel and afforded endodesmiadiol (**1**), friedelin (**2**),[16)] canophyllol (**3**), 3β-acetoxyoleanolic acid (**9**),[17)] canophyllal (**4**),[18)] cerin (**5**),[19)] morelloflavone (**6**),[20)] volkensiflavone (**7**),[21)] 8-deoxygartanin (**8**)[2)] and 1,8-dihydroxy-3-isoprenyloxy-6-methylxanthone (**10**).[22)]

Compound **1** was isolated as a colourless crystalline solid (mp 229—230 °C). It responded positively to the Liebermann–Burchard test for triterpenes. The molecular formula of **1** was determined as $C_{30}H_{50}O_3$ by HR-EI-MS, which showed a $[M+Na]^+$ pseudomolecular ion at *m/z* 481.3664 (Calculated *m/z* 481.3645), corresponding to six double-bond equivalents. The presence in **1** of a carbonyl function and two hydroxy groups was indicated by the IR spectrum, which displayed absorption bands at 3525, 3395 (hydroxy groups) and 1703 cm^{-1} (carbonyl group) and was confirmed by the formation of a diacetate (**1a**). The ^{1}H-NMR spectrum of **1** (Table 1) showed singlets due to six tertiary methyl groups at δ 0.97, 0.82, 0.80, 0.75, 0.70 and 0.52, a doublet for one secondary methyl group at δ 0.68 (d, J=6.8 Hz) and a quartet at δ 2.76 (1H, J=6.8 Hz). The additional features of the ^{1}H-NMR spectrum were two signals of protons adjacent to a hydroxy group observed at δ 3.78 (1H, t, J=3.2 Hz) and 3.42 (2H, br s). The ^{13}C-NMR and Distortionless Enbrancement by Polarization Transfer (DEPT) spectra of **1** (Table 1) were in complete agreement with a $C_{30}H_{50}O_3$ molecular formula: it

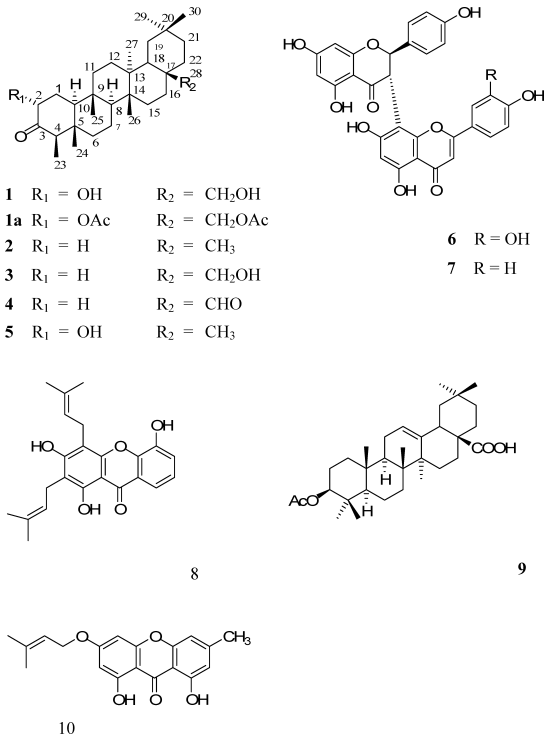

Fig. 1. Structures of Compounds **1**—**10**

* To whom correspondence should be addressed. e-mail: tsamo@cm.refer.org; mirohmer@chimie.u-strasbg.fr

Table 1. NMR Chemical Shift Assignments for Endodesmiadiol (**1**) in $CDCl_3/CD_3OD$ (4 : 1) [^1H (400.1 MHz) and ^{13}C (100.6 MHz)], 2α,28-Diacetoxyfriedelan-3-one (**1a**) and Canophyllol (**3**) in $CDCl_3$ [^1H (300.1 MHz) and ^{13}C (75.4 MHz)]

N°	**1**				**3**	**1a**	
	δ_H [m, *J* (Hz)]	δ_C	HMBC	ROESY	δ_C	δ_H [m, *J* (Hz)]	δ_C
1	1.83 (m); 1.60 (m)	30.0 CH_2	C-2, C-3, C-5, C-9, C-10	H-2 H-2	22.1 CH_2		29.6 CH_2
2	3.78 (t, *J*=3.2)	73.6 CH	C-1, C-3, C-10	H-24, H-1	41.3 CH_2	4.94 (t, *J*=3.3)	76.5 CH
3		214.9 C			212.6 C		208.2 C
4	2.76 (q, *J*=6.8)	52.7 CH	C-3, C-5, C-23, C-24	H-6, H-23	57.8 CH	2.65 (q, *J*=6.9)	54.4 CH
5		43.2 C			41.9 C		43.2 C
6	1.18 (m); 1.55 (m)	41.0 CH_2	C-5, C-10, C-23	H-4, H-23	41.0 CH_2		41.0 CH_2
7	1.32 (m)	18.2 CH_2	C-6, C-14	H-8	18.1 CH_2		18.2 CH_2
8	1.31 (m)	52.4 CH	C-6, C-25	H-7, H-10	52.2 CH		52.4 CH
9		36.8 C			37.3 C		36.9 C
10	1.80 (dd, *J*=3.0; 9.4)	52.1 CH	C-1, C-2, C-4, C-5, C-9, C-24, C-25	H-8	59.1 CH		53.3 CH
11	1.17 (m)	35.1 CH_2	C-9, C-10		35.3 CH_2		35.3 CH_2
12	1.22 (m)	30.2 CH_2	C-9, C-26		29.9 CH_2		30.0 CH_2
13		39.4 C			39.1 C		39.4 C
14		38.1 C			38.0 C		39.2 C
15	1.14 (m)	31.3 CH_2	C-17, C-27, C-28		31.3 CH_2		31.2 CH_2
16	1.70 (m)	29.0 CH_2	C-14, C-15, C-17, C-28		29.0 CH_2		28.2 CH_2
17		35.1 C			35.1 C		38.1 C
18	1.10 (m)	39.5 CH	C-13, C-7, C-20, C-23, C-26, C-28		39.2 CH		39.2 CH
19	1.30 (m)	34.4 CH_2	C-18, C-20		34.4 CH_2		34.3 CH_2
20		28.0 C			27.9 C		28.0 C
21	1.22 (m)	31.1 CH_2	C-18		31.4 CH_2		32.2 CH_2
22	1.10 (m); 1.23 (m)	33.2 CH_2	C-18, C-20, C-28		33.2 CH_2		33.1 CH_2
23	0.68 (d, *J*=6.8)	6.3 CH_3	C-3, C-4, C-5		6.7 CH_3	0.88 (d, *J*=6.6)	6.5 CH_3
24	0.52 (s)	13.9 CH_3	C-4, C-5, C-6, C-10		14.5 CH_3	0.70 (s)	14.1 CH_3
25	0.70 (s)	17.9 CH_3	C-10		18.0 CH_3	0.84 (s)	18.0 CH_3
26	0.97 (s)	18.9 CH_3	C-12, C-13, C-14		18.9 CH_3	1.14 (s)	19.0 CH_3
27	0.75 (s)	19.2 CH_3	C-8, C-13, C-14		19.1 CH_3	0.93 (s)	19.2 CH_3
28	3.42 (br s)	67.3 CH_2	C-16, C-17, C-18		67.0 CH_2	3.99 (d, *J*=10.8) 4.12 (d, *J*=10.8)	69.2 CH_2
29	0.80 (s)	32.7 CH_3	C-20, C-30		32.9 CH_3	0.97 (s)	32.8 CH_3
30	0.82 (s)	34.1 CH_3	C-20, C-29		34.2 CH_3	0.98 (s)	34.2 CH_3
2CH_3CO						2.05 (s), 2.12 (s)	21.1; 21.2 CH_3
2CO							169.7; 168.1 C

exhibited 30 carbon atom signals shared between six quaternary carbon atoms and one carbonyl group (δ 214.9), five methine groups with an oxygenated one (δ 73.6), eleven methylene groups including an oxygenated one (δ 67.3), one secondary methyl (δ 6.3) and six tertiary methyl groups (δ 34.1, 32.7, 19.2, 18.9, 17.9, 13.9). These ^1H- and ^{13}C-NMR data suggested a triterpene skeleton for **1**. The presence in the MS spectrum of **1** of characteristic fragments with *m*/*z* 317, 289, 203, 151 and 109 were in accord with a friedelane type triterpene,[23,24] with the carbonyl and one hydroxy group in the ring A or B and the second in the ring E. Higher plant triterpenoids being derived from oxidosqualene cyclization,[25,26] an oxygenated function has been assigned to C-3. The carbonyl function has been located at C-3. This was in accord with the chemical shift of H-4 (δ 2.76) in α position of a carbonyl group and with the multiplicity (quartet) of the signal of this proton only coupled with those of the C-23 methyl group. The location of the two hydroxy groups at C-2 and C-28 on the friedelane skeleton comes from correlations observed with the methine and methylene protons at δ 3.78 (1H, t, *J*=3.2 Hz) and 3.42 (2H, br s), respectively, on the HMBC spectrum of **1** (Table 1). This was further confirmed by the mass spectrum fragmentation pattern and by comparison of ^{13}C-NMR chemical shifts of **1** with those of canophyllol,[17] which shares with **1** identical BCDE ring moieties.

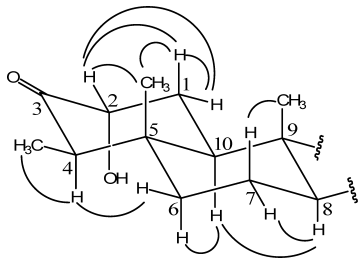

Fig. 2. Selected Roesy Correlations in A and B-Rings of Endodesmiadiol (**1**)

The stereochemistry at C-2 was elucidated by the analysis of the ROESY NMR data. The observation of rOe associations between H-2/Me-5 (H-24) suggested that the methyl

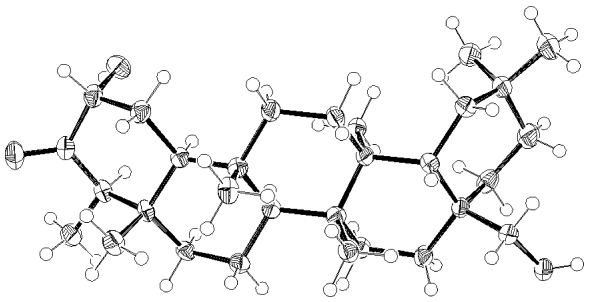

Fig. 3. ORTEP Diagram of the Crystal Structure of Endodesmiadiol (**1**)

Table 2. Antiplasmodial Activity of Extracts and Compounds **1**—**6**, **8**, **9**

	IC_{50} [a)]	S.D.	Erythrocyte susceptibility
Extracts	(μg/ml)		
Hexane extract	9.3	1.0	
EtOAc extract	7.4	0.6	>1 mg/ml
MeOH extract	12.8	1.0	
Compounds	(μM)		
Endodesmiadiol **1**	13.0	0.1	
Friedelin **2**	7.2	0.5	
Canophyllol **3**	15.0	0.1	
Canophyllal **4**	18.2	1.0	
Cerin **5**	14.1	1.0	>20 mM
Morelloflavone **6**	23.6	1.8	
8-Deoxygartanin **8**	11.8	0.4	
3β-Acetyloleanolic acid **9**	13.1	1.0	

a) IC_{50}: concentrations that killed 50% of parasites relative to negative control. S.D.=standard deviation of results; compounds and extracts were tested in triplicate.

group at C-5 and the proton at C-2 are on the β-side of **1**, and those between H-4/H-8/H-10 that the protons at C-4, C-8 and C-10 are on the α-side (Fig. 2). The assigned relative configuration of the stereogenic centres, especially in ring A, was confirmed by X-ray diffraction analysis, affording the tridimensional structure of endodesmiadiol (**1**) (Fig. 3), which is thus 2α,28-dihydroxyfriedelan-3-one. The complete assignment of signals arising from the ring system carbons (B, C, D and E rings) was made by comparison with those of the literature data for canophyllol (**3**).[17)]

Extracts and compounds showed toxicity to erythrocytes at concentrations respectively above 1 mg/ml and 20 mM, many orders of magnitude above concentrations with antiplasmodial activity.

Compounds **1**—**6**, **8**, **9** were tested for antiplasmodial activity against the W2 strain of *Plasmodium falciparum*, which is resistant to chloroquine and other antimalarial drugs (Table 2). They were found to exhibit antiplasmodial activity *in vitro* with IC_{50} values of 13.0; 7.2; 15.0; 18.2; 14.1; 23.6; 11.8; and 13.1 μM respectively for compounds **1**—**6**, **8**, **9**. The related crude extracts obtained with hexane, EtOAc, and MeOH showed antiplasmodial activities with IC_{50} values of 9.3, 7.4 and 12.8 μg/ml respectively. The activity of compound **2** (friedelin) corroborates the result previously published by Lenta *et al.*,[27)] who obtained an IC_{50} value of 7.7 μM with friedelan-3-one, with the same parasite strain. In addition, the novel friedelane triterpenoid named endodesmiadiol (**1**) showed potency with an IC_{50} value of 11.8 μM. From these results, we could anticipate arguing that friedelane derivatives might be interesting sources of potential antimalarial leads.

Experimental

General Melting points were determined on Büchi melting point apparatus B-545. IR spectra were obtained with a Perkin-Elmer 881 Infrared spectrophotometer or a Nicolet Avatar 320 FT-IR with KBr discs and $[\alpha]_D$ with a Perkin-Elmer 341 polarimeter. NMR spectra were recorded on a Bruker AC300 instrument equipped with a 5 mm ^{1}H and ^{13}C probe operating at 300 and 75 MHz respectively with $CHCl_3$ (δ=7.26) and $CDCl_3$ (δ=77.0) as internal standards. 2D experiments were performed using Bruker WP400 spectrometer. Silica gel 230—400 mesh (Merck) and silica gel 70—230 mesh (Merck) were used for flash and column chromatography, while precoated aluminium silica gel 60 F_{254} sheets were used for TLC. Spots were visualised under UV lamps (254, 365 nm) or by treatment with an ethanolic solution of phosphomolybdic acid (20% Aldrich) or diluted sulphuric acid (50%) and heating at 100 °C.

Plant Material Stem bark of *E. calophylloides* was collected in August 2004 at Ntui in the Centre province of Cameroon. The plant was identified by Mr. Victor Nana, botanist at the National Herbarium of Cameroon where a voucher specimen (HNC 29528) has been deposited.

Extraction and Isolation The air-dried powdered stem bark of *E. calophylloides* (3 kg) was extracted successively with *n*-hexane (5 l), EtOAc (5 l) and MeOH (5 l) at room temperature for 24 h. The filtrates were concentrated to dryness under reduced pressure to give viscous extracts from hexane (20 g), EtOAc (15 g) and MeOH (25.0 g). The EtOAc extract (12 g) was subjected to repeated flash column chromatography over silica gel (70—230 mesh) as stationary phase eluted with *n*-hexane and *n*-hexane/EtOAc mixtures of increasing polarity, yielding friedelin (**2**, 180 mg), canophyllal (**4**, 12 mg), 1,8-dihydroxy-3-isoprenyloxy-6-methylxanthone (**10**, 4.0 mg), canophyllol (**3**, 14 mg), cerin (**5**, 9 mg), 8-deoxygartanin (**8**, 22 mg), 3β-acetoxyoleanolic acid (**9**, 14 mg), endodesmiadiol (**1**, 235 mg), volkensiflavone (**7**, 21 mg) and morelloflavone (**6**, 446 mg).

Evaluation of the Biological Activities During the preparation of stock solutions, extracts and some compounds showed limited solubility in dimethyl sulfoxide (DMSO). Therefore, saturated solutions of extracts and referred compounds were warmed up to 35 °C until complete dissolution, and evaluated for biological activities. This evaluation consisted in two steps 1) a preliminary test of erythrocytes susceptibility to compounds and extracts, and 2) the screening for antiplasmodial activity.

Evaluation of Erythrocyte Susceptibility to Compounds *in Vitro* A preliminary toxicological assessment was carried out to determine the highest drug concentrations that can be incubated with erythrocytes without any significant damage. This was done according to the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide/phenazine methosulfate (MTT/PMS) colorimetric assay described by Cedillo-Rivera *et al.*,[29)] with some modifications. The drugs were serially diluted in 96 well culture plates, and each concentration incubated in triplicate with erythrocytes (2% hematocrit) in a final 100 μl culture volume (at 37 °C, in a 3% O_2, 5% CO_2 and 91% N_2 atmosphere, in the presence of RPMI 1640, 25 mM HEPES, pH 7.4 for 48 h). At the end of the incubation period, the cultures were transferred into polypropylene microcentrifuge tubes and centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. 1.5 ml MTT solution with 250 mg PMS were added to the pellets. Controls contained no erythrocytes. The tubes were thereafter incubated for 45 min at 37 °C, then centrifuged, and the supernatant was discarded. The pellets were re-suspended in of 0.04 M HCl in isopropanol (0.75 ml) to extract and dissolve the dye (formazan) from the cells. After 5 min, the tubes were vigorously mixed and centrifuged, and the absorbance of the supernatant was determined at 570 nm.

Evaluation of Antiplasmodial Activity *Plasmodium falciparum* strain W2, which is resistant to chloroquine and other antimalarial drugs,[30)] was cultured in sealed flasks at 37 °C, in a 3% O_2, 5% CO_2 and 91% N_2 atmosphere in RPMI 1640, 25 mM HEPES, pH 7.4, supplemented with heat inactivated 10% human serum and human erythrocytes to achieve a 2% hematocrit. Parasites were synchronized in the ring stage by serial treatment with 5% sorbitol (Sigma)[31)] and studied at 1% parasitemia.

Compounds were prepared as 10 mM stock solutions in DMSO, diluted as needed for individual experiments, and tested in triplicate. The stock solutions were diluted in supplemented RPMI 1640 medium so as to have at most 0.2% DMSO in the final reaction medium. An equal volume of 1% parasitemia, 4% hematocrit culture was thereafter added and gently mixed thoroughly. Negative controls contained equal concentrations of DMSO. Positive controls contained 1 μM chloroquine phosphate (Sigma). Cultures were incubated at 37 °C for 48 h (1 parasite erythrocytic life cycle). Parasites

at ring stage were thereafter fixed by replacing the serum medium by an equal volume of 1% formaldehyde in PBS. Aliquots (50 μl) of each culture were then added to 5 ml round-bottom polystyrene tubes containing 0.5 ml 0.1% Triton X-100 and 1 nM YOYO nuclear dye (Molecular Probes) in PBS. Parasitemias of treated and control cultures were compared using a Becton-Dickinson FACSort flow cytometer to count nucleated (parasitized) erythrocytes. Data acquisition was performed using CellQuest software. These data were normalized to percent control activity and 50% inhibitory concentrations (IC_{50}) calculated using Prism 3.0 software (GraphPad) with data fitted by non linear regression to the variable slope sigmoidal dose-response formula $y=100/[1+10^{((\log IC_{50}-x)H}]$, where H is the hill coefficient or slope factor.[30]

Endodesmiadiol (**1**): *Rf* 0.49 (cyclohexane–EtOAc 6 : 4), colourless crystalline solid, mp 229—230 °C, $[\alpha]_D^{20}$ −61.8° (c=1, $CHCl_3$); IR ν_{max} cm^{-1} (KBr) 3525, 3395, 1703, 1389, 1052; HR-ESI-MS *m/z*: Found 481.3664 $[M+Na]^+$, required for $C_{30}H_{50}NaO_3$ 481.3645; EI-MS *m/z* (rel. int.): 427 (94), 317 (32), 289 (35), 273 (9), 203 (18), 151 (17), 137 (100), 109 (49); ^1H ($CDCl_3$/CD_3OD 4 : 1) and ^{13}C ($CDCl_3$/CD_3OD 4 : 1) NMR data: see Table 1.

2α,28-Diacetoxyfriedelan-3-one (**1a**): Endodesmiadiol (**1**, 8 mg) was dissolved in pyridine (0.5 ml) and treated with Ac_2O (0.5 ml) overnight at room temperature. After removing the solvents under nitrogen, the reaction mixture was purified by preparative TLC using cyclohexane–EtOAc (85 : 15) as eluent to obtain the acetylated derivative (**1a**, 6 mg, *Rf*=0.37).

mp: 213—214 °C; $[\alpha]_D^{20}$ −37.0° (c=0.3, $CHCl_3$); IR ν_{max} cm^{-1} ($CHCl_3$) 1720, 1725, 1732; ^1H (300 MHz, $CDCl_3$) and ^{13}C (75 MHz, $CDCl_3$) NMR data: see Table 1.

X-Ray Crystallography of 1 Colourless crystal collected at room temperature from MeOH/cyclohexane (crystal dimensions 0.30×0.25×0.20 mm), orthorhombic space group $P2_12_12_1$, a=7.01590(10), b=12.7038(2), c=28.9748(5) Å, V=2582.48(7) $Å^3$, Z=4, D_c=1.180 mg m^{-3}. Data were collected on a Nonius MACH-3 diffractometer using graphite monochromated Mo$K\alpha$ radiation (λ=0.71073 Å). The structures were refined by full-matrix least-squares on F^2 using Bruker SHELXL-97.[28] The final R and R_w were 0.0450 and 0.1107 respectively. Crystallographic data for endodesmiadiol (**1**) have been deposited with the Cambridge Crystallographic Data Centre as supplementary Publication No. CCDC-649531.

Copies of the data can be obtained free of charge *via* www.ccdc.cam.ac.uk/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgements The authors wish to thank the "Agence Universitaire de la Francophonie (AUF)" for the Doctoral fellowship awarded to Erasmienne NGOUAMEGNE; Michel ROHMER was supported by a grant from "Institut Universitaire de France" and Phillip Rosenthal by a grant from the National Institute of Health. They also thank Maurice Coppe, Jean-Daniel Sauer for NMR analyses, Dr. Raymonde Baltenweck for running MS spectra, Dr. Lydia Brelot for the X-ray analysis and Victor Nana for the identification of the plant.

References

1) Troupin G., "Flore du Rwanda," Vol. 1, Institut National de Recherche Scientifique, Butare (République Rwandaise), 1978, pp. 297—307.
2) Govindachari T. R., Kalyanaraman P. S., Muthukumaraswamy N., Pai B. R., *Tetrahedron*, **27**, 3919—3926 (1971).
3) Waterman P. G., Crichton E. G., *Phytochemistry*, **19**, 2723—2726 (1980).
4) Tanaka N., Takaishi Y., Shikishima Y., Nakanishi Y., Bastow K., Lee K., Honda G., Ito M., Tajeda Y., Kodzhimatov O. K., Ashurmetov O., *J. Nat. Prod.*, **67**, 1870—1875 (2004).
5) Tanaka N., Takaishi Y., *Phytochemistry*, **67**, 2146—2151 (2006).
6) Mbwambo Z. H., Kapingu M. C., Moshi M. J., Machumi F., Apers S., Cos P., Ferreira D., Marais J. P. J., Berghe D. V., Maes L., Vlietinck A., Pieters L., *J. Nat. Prod.*, **69**, 369—372 (2006).
7) Ngouela S., Zelefack F., Lenta B. N., Ngouamegne E. T., Tchamo D. N., Tsamo E., Connolly J. D., *Nat. Prod. Res.*, **19**, 685—688 (2005).
8) Ngouela S., Lenta B. N., Noungoue D. T., Ngoupayo J., Boyom F. F., Tsamo E., Gut J., Rosenthal P. J., Connolly J. D., *Phytochemistry*, **67**, 302—306 (2006).
9) Karanjgaokar C. G., Radhakrishnan P. V., Venkataraman K., *Tetrahedron Lett.*, **33**, 3195—3198 (1967).
10) Herbin G. A., Jackson B., Locksley H. D., Scheinmann F., Wolstenholme W. A., *Phytochemistry*, **9**, 221—226 (1970).
11) Joshi B. S., Kamat V. N., Viswanathan N., *Phytochemistry*, **9**, 881—888 (1970).
12) Chen F. C., Lin Y. M., Hung J. G., *Phytochemistry*, **14**, 300—303 (1975).
13) Gunatilaka A. A. L., De Silva A. M. Y. J., Sotheeswaran S., Balasubramaniam S., Wazeer M. I. M., *Phytochemistry*, **23**, 323—328 (1984).
14) Govindachari T. R., Viswanathan N., Pai B. R., Rao R., Srinivasan M., *Tetrahedron*, **23**, 1901—1910 (1967).
15) Hutchinson L. L., Dalziel J. M., "Flora of West Tropical Africa," 2nd ed., Oxford University Press, London, 1954, pp. 286—287.
16) Betancor C., Freire R., Gonzalez A. G., Salazar J. A., Pascard C., Prange T., *Phytochemistry*, **19**, 1989—1993 (1980).
17) Mahato S. B., Kundu A. P., *Phytochemistry*, **37**, 1517—1575 (1994).
18) Nozaki H., Suzuki H., Hirayama T., Kasai R., Wu R.-Y., Lee K.-H., *Phytochemistry*, **25**, 479—485 (1986).
19) Zhong S. M., Waterman P. G., Jeffreys J. A. D., *Phytochemistry*, **23**, 1067—1072 (1984).
20) Li X.-C., Joshi A. S., Tan B., Elsohly H. N., Walker L. A., Zjawiony J. K., Ferreira D., *Tetrahedron*, **58**, 8709—8717 (2002).
21) Chari V. M., Ilyas M., Wagner H., Neszmelyi A., Chen F. C., Chen L. K., Lin Y. C., Lin Y. M., *Phytochemistry*, **16**, 1273—1278 (1977).
22) Billia A. R., Yusuf A. W., Braca A., Keita A., Morelli I., *J. Nat. Prod.*, **63**, 16—21 (2000).
23) Budzikiewicz H., Wilson J. M., Djerasi C., *J. Am. Chem. Soc.*, **85**, 3688—3699 (1963).
24) Shannon J. S., Macdonald C. G., *Tetrahedron Lett.*, **4**, 173—179 (1963).
25) Rohmer M., Bouvier P., Ourisson G., *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 847—851 (1979).
26) Ourisson G., Rohmer M., Anton R., "Recent Advances in Phytochemistry," Vol. 13, ed. by Swain T., Waller G. R., Plenum Press, New York, 1979, pp. 131—162.
27) Lenta N. B., Ngouela S., Boyom F. F., Tantangmo F., Feuya Tchouya G. R., Tsamo E., Gut J., Rosenthal P. J., Connolly J. D., *Chem. Pharm. Bull.*, **55**, 464—467 (2007).
28) Sheldrick G. M., "SHELXL97, Program for the refinement of crystal structures," University of Göttingen, Germany, 1997.
29) Cedillo-Rivera R., Ramfrez A., Munoz O., *Arch. Med. Res.*, **23**, 59—61 (1992).
30) Singh A., Rosenthal P. J., *Antimicrob. Agents Chemother.*, **45**, 949—951 (2001).
31) Lambros C., Vanderberg J. P., *J. Parasitol.*, **65**, 418—420 (1979).