Two 2,6-Dioxabicyclo[3.3.1]nonan-3-ones from *Phragmanthera capitata* (SPRENG.) BALLE (Loranthaceae)

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Phytochemical investigation of the leaves of *Phragmanthera capitata* collected on *Cassia spectabilis* tree led to the isolation of two natural lactones, *rel*-(1*R*,5*S*,7*S*)-7-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one (**1**) and 4-{2-[*rel*-(1*R*,3*R*,5*S*)-7-oxo-2,6-dioxabicyclo[3.3.1]non-3-yl]ethyl}phenyl 3,4,5-trihydroxybenzoate (**2**) together with the known compounds betulinic acid (**3**), dodoneine (**4**), quercetin 3-*O*-α-L-rhamnopyranoside (**5**), quercetin 3-*O*-α-L-arabinofuranoside (**6**), quercetin (**7**), betulin (**8**), lupeol (**9**), and sitosterol (**10**). Their structures were established by means of modern spectroscopic techniques, and the relative configuration of compound **1** was confirmed by X-ray analysis. Compounds **1** and **2** were tested *in vitro* for their antiplasmodial activity against the *Plasmodium falciparum* chloroquine sensitive-strains NF54 and 3D7. Compound **2** exhibited good antiplasmodial activity against both strains with IC_{50} of 2.4 and 4.9 μM, respectively, while compound **1** was inactive.

Introduction. – *Phragmanthera capitata* (SPRENG.) BALLE is a mistletoe plant of Loranthaceae family having woody shrub, pendent branches, and is often found with ants' nests [1]. The degree of damages caused by this parasitic plant is becoming more and more considerable regarding crop quality and yield. The parasitic plant is abundant in West African tropical countries like Nigeria, in West-Central Africa like Cameroon, and only in Angola in South Tropical Africa [2]. Infusion of the plant leaves is used in African traditional medicine to treat fever, diabetes, abdominal pains, parasitic diseases, and urinary tract infections [2][3]. Previous phytochemical investigations of plants of the Loranthaceae family revealed the presence of flavonoids [4][5], 5,6-dihydropyran-2-ones [6], peptides, carbohydrates [7], steroids, and terpenoids [8]. Other studies reported positive reactions of various plant parts for alkaloids and tannins, but have not been substantiated by isolation and proper identification [8]. There is, therefore, a lot more to be done to fully explore the chemical constituent

profile of the African mistletoes and to evaluate their antiparasitic potency. In a continuing search for bioactive compounds from Cameroonian medicinal plants, we have investigated the MeOH extract of the leaves of *P. capitata*, which was found to be active *in vitro* against *Plasmodium falciparum*. We report here the isolation and the structure elucidation of two naturally occurring lactones and the evaluation of their antiplasmodial activity against *P. falciparum* strains NF 54 and 3D7.

Results and Discussion. – The dried leaves of *P. capitata* were extracted at room temperature with MeOH. Fractionation and purification of the extract was carried out by column chromatography over silica gel and yielded two new natural compounds, *rel*-(1*R*,5*S*,7*S*)-7-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one (**1**) and *rel*-(1*R*,5*S*,7*S*)-7-[2-(4-*O*-galloylphenyl)ethyl]-2,6-dioxabicyclo-[3.3.1]nonan-3-one (=4-{2-[*rel*-(1*R*,3*R*,5*S*)-7-oxo-2,6-dioxabicyclo[3.3.1]non-3-yl]ethyl}phenyl 3,4,5-trihydroxybenzoate, **2**), together with the known compounds betulinic acid (**3**) [9], dodoneine (**4**) [6], quercetin 3-*O*-*α*-rhamnopyranoside (**5**) [4], quercetin 3-*O*-*α*-L-arabinofuranoside (**6**) [10], quercetin (**7**) [10], betulin (**8**) [9], lupeol (**9**) [9], and sitosterol (**10**) [11] (*Fig. 1*).

Compound **1** was obtained as yellow crystals. It gave a positive $FeCl_3$ test, indicating the presence of a phenol moiety. The molecular formula was deduced as $C_{15}H_{18}O_4$ from

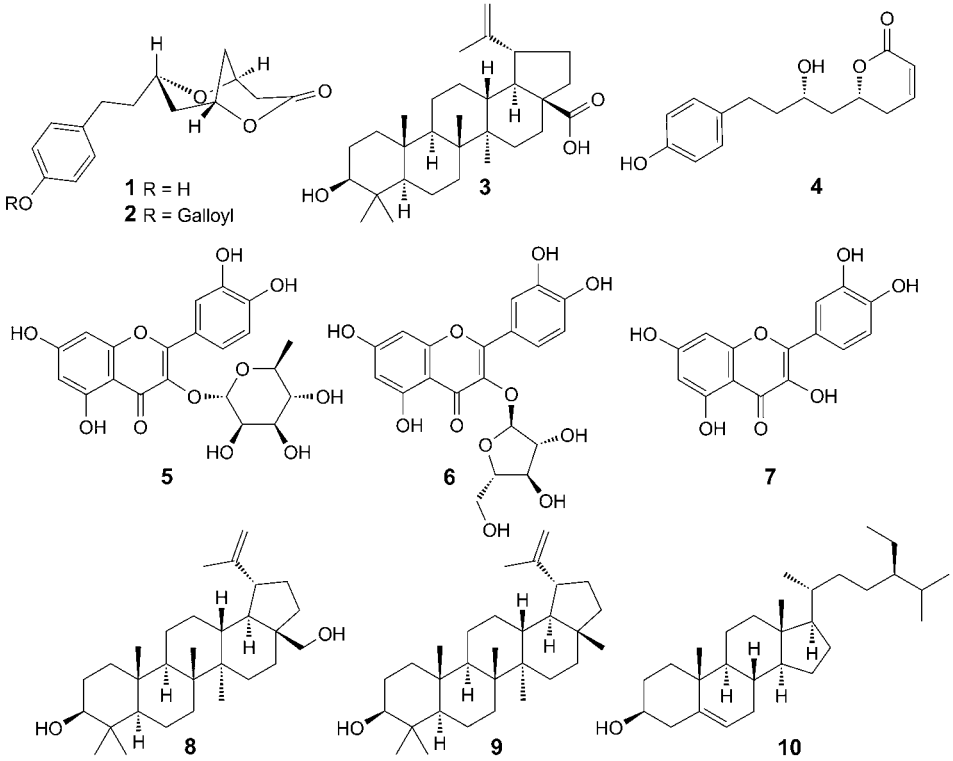

Fig. 1. *Structures of the isolated compounds* **1**–**10**

HR-ESI-MS (*m/z* 285.1095 ($[M+Na]^+$, $C_{15}H_{18}NaO_4^+$; calc. 285.1097)) corresponding to seven C=C bond equivalents. The IR spectrum displayed bands at 3337 (OH), 2948 (C–H), 1705 and 1613 (C=O), 1513 (C=C), and 1077 (C–O–C) cm^{-1}. All 15 C-atoms were resolved in the ^{13}C-NMR, DEPT, and HSQC spectra (*Table 1*) as signals corresponding to five CH_2 groups (δ(C) 28.9, 29.9, 36.0, 36.4, 37.4), seven CH groups (among them three aliphatic O–CH groups (δ(C) 64.0, 65.3, and 72.6), as well as four aromatic C-atoms (δ(C) 115.0 (2 C) and 129.1 (2 C))), and three quaternary C-atoms (among them a C-atom of an ester group (δ(C) 169.4)). The ^{1}H-NMR spectrum of compound **1** (*Table 1*) exhibited signals for a *para*-substituted benzene ring (δ(H) 6.67 (*d*, *J* = 8.5, 2 H) and δ(H) 6.97 (*d*, *J* = 8.5, 2 H)). The same spectrum also showed signals of three O–CH H-atoms (δ(H) 4.81 (br. *d*, *J* = 1.7), 4.26 (br. *s*), and 3.52–3.55 (*m*)) and ten other aliphatic H-atoms between δ(H) 1.50 and 2.95. Correlations observed in the COSY spectrum in combination with those of the HMBC spectrum indicated that the ten aliphatic H-atoms belonged to five CH_2 groups. In fact, the COSY spectrum of **1** exhibited couplings between the CH_2 H-atoms (δ(H) 2.40–2.59 (*m*, CH_2(11))) and those at δ(H) 1.59–1.68 (*m*, CH_2(10)) and suggested their vicinal position. The HMBC spectrum of compound **1** (*Fig. 2*) exhibited correlations between the CH_2(4) group (δ(H) 2.57–2.58 (*m*) and 2.87 (*dd*, *J* = 5.3, 19.0)) and the C-atoms C(3) (δ(C) 169.4), C(5) (δ(C) 65.3), and C(9) (δ(C) 28.9), as well as between CH_2(8) (δ(H) 1.50–1.56 (*m*) and 1.82–1.87 (*m*)) and the C-atoms C(1) (δ(C) 72.6), C(9), and C(10) (δ(C) 37.4). The same spectrum also showed cross-peaks between H–C(5)

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of* **1** *and* **2** *in (D_6)DMSO.* δ in ppm, *J* in Hz.

Position[a])	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	4.81 (br. *d*, *J* = 1.7)	72.6	4.84 (br. *d*, *J* = 1.5)	73.1
3	–	169.4	–	169.9
4	2.57–2.58 (*m*), 2.87 (*dd*, *J* = 5.3, 19.0)	36.0	2.58–2.62 (*m*), 2.90 (*dd*, *J* = 5.4, 19.0)	36.5
5	4.26 (br. *s*)	65.3	4.29 (br. *s*)	65.8
7	3.52–3.55 (*m*)	64.0	3.58–3.60 (*m*)	64.8
8	1.50–1.56 (*m*), 1.82–1.87 (*m*)	36.4	1.54–1.60 (*m*), 1.85–1.91 (*m*)	36.9
9	1.82–1.91 (*m*, 2 H)	28.9	1.85–1.90 (*m*, 2 H)	29.4
10	1.59–1.68 (*m*, 2 H)	37.4	1.70–1.75 (*m*, 2 H)	37.6
11	2.40–2.59 (*m*, 2 H)	29.9	2.58–2.64, 2.68–2.74 (2*m*, 2 H)	30.7
12	–	131.6	–	139.7
13, 13′	6.67 (*d*, *J* = 8.5, 2 H)	115.0	7.10 (*d*, *J* = 8.1, 2 H)	129.7
14, 14′	6.97 (*d*, *J* = 8.5, 2 H)	129.1	7.25 (*d*, *J* = 8.1, 2 H)	122.2
15	–	155.3	–	149.3
1″			–	165.1
2″			–	118.8
3″, 3‴			7.10 (*s*, 2 H)	109.5
4″, 4‴			–	146.2
5″			–	139.6

[a]) Atom numbering as indicated in *Figs. 2* and *4*.

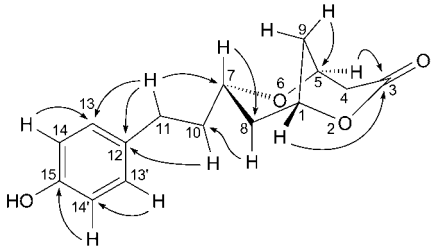

Fig. 2. *Selected HMBCs of compound* **1**

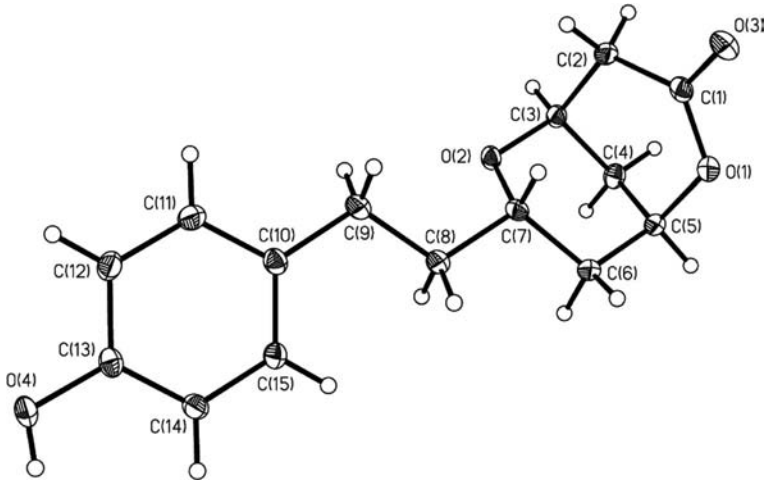

Fig. 3. *ORTEP Plot of the molecular structure of compound* **1** (50% probability ellipsoids, arbitrary atom numbering)

(δ(H) 4.26) and C(1), C(7) (δ(C) 64.0), and the ester C-atom C(3); between H–C(1) (δ(H) 4.81) and C(7) and C(3); between H–C(7) (δ(H) 3.52 – 3.55) and the C-atoms C(1), C(5), C(10), and C(11) (δ(C) 29.9). All these data suggested the presence of a 2,6-dioxabicyclo[3.3.1]nonan-3-one moiety. In addition, correlations observed between the vicinal CH_2 H-atoms CH_2(10) and CH_2(11) and the aromatic C-atom C(12) suggested the presence of a phenylethane moiety. The junction between the two moieties was deduced from the HMBC spectrum, where correlations between H–C(7) and C(10) and C(11) were observed. Thus, the structure of compound **1** was assigned as 7-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one. The relative configuration of compound **1** was deduced from the NOESY spectrum, where no correlation was observed between H–C(1), H–C(5), and H–C(7) and confirmed by the X-ray crystallographic analysis (*Fig. 3*). Thus, compound **1** was determined to be *rel*-(1*R*,5*S*,7*S*)-7-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one, a secondary metabolite isolated for the first time from a natural source and previously synthesized from dodoneine (**4**) *via* a *Michael* addition of the OH group at C(7) onto

the unsaturated lactone, under basic conditions [6]. It has also been obtained as a byproduct during the synthesis of dodoneine [12].

Compound **2** was obtained as white powder, which reacted positively to the $FeCl_3$ test, indicating its phenolic nature. The molecular formula was deduced as $C_{22}H_{22}O_8$ from the HR-ESI-MS (*m/z* 437.1205 ($[M+Na]^+$, $C_{22}H_{22}NaO_8^+$; calc. 437.1207)) corresponding to twelve C=C bond equivalents. The value of the observed mass peak is 152 units higher than that for **1**, suggesting the presence of an additional $C_7H_4O_4$ fragment in compound **2**. The IR spectrum exhibited bands at 3363 (OH), 2953 (C–H), 1714 and 1603 (C=O), and 1189 and 1163 (C–O) cm^{-1}. The broad-band decoupled ^{13}C-NMR spectrum of compound **2** (*Table 1*) displayed 22 C-atom signals, which were sorted using DEPT and HSQC spectra into nine CH groups (with three O-bearing CH groups (δ(C) 64.8 (C(7)), 65.8 (C(5)), and 73.1 (C(1)))), five CH_2, and seven quaternary C-atoms of which are two ester C-atoms (δ(C) 165.1 (C(1″)) and 169.9 (C(3))). The ^{1}H-NMR spectrum of compound **2** (*Table 1*) exhibited signals for a *para*-substituted benzene ring (δ(H) 7.10 (*d*, *J* = 8.1, 2 H) and 7.25 (*d*, *J* = 8.1, 2 H)) and two aromatic, equivalent H-atoms (δ(H) 7.10 (*s*, 2 H). Additional signals of three O-bearing CH groups (δ(H) 4.84 (br. *d*, *J* = 1.5), 3.58 – 3.60 (*m*), and 4.29 (br. *s*)), and ten aliphatic H-atoms between δ(H) 1.50 and 2.95 were observed. Couplings observed in the COSY spectrum in combination with correlations of the HMBC spectrum (*Fig. 4*) indicated that the ten aliphatic H-atoms belonged to five CH_2 groups which were analogously present as in compound **1**. The spectroscopic data of compound **2** pointed to a close similarity with the structure of **1**. The major difference between **1** and **2** is the appearance of additional signals for a galloyl moiety in compound **2** at δ(H) 7.10 (*s*, 2 H), as well as δ(C) 109.5 (C(3″,3‴), 118.8 (C(2″)), 139.6 (C(5″)), 146.2 (C(4″,4‴)), and 165.1 (C(1″)). The attachment of the gallic acid at C(15) was deduced from the HMBC spectrum. In fact, in the HMBC spectrum of compound **2**, the aromatic H–C(3″) gave no cross-peak with the bicyclo[3.3.1]nonane moiety. These data suggested that the HO–C_6H_4 group observed in compound **1** was esterified by gallic acid in compound **2**. The relative configuration of compound **2** was deduced from the NOESY spectrum in which no correlations were observed between H–C(1), H–C(5), and H–C(7) in analogy to compound **1**. Thus, the structure of **2** was established as 4-{2-[*rel*-(1*R*,3*R*,5*S*)-7-oxo-2,6-dioxabicyclo[3.3.1]non-3-yl]ethyl}phenyl 3,4,5-trihydroxybenzoate.

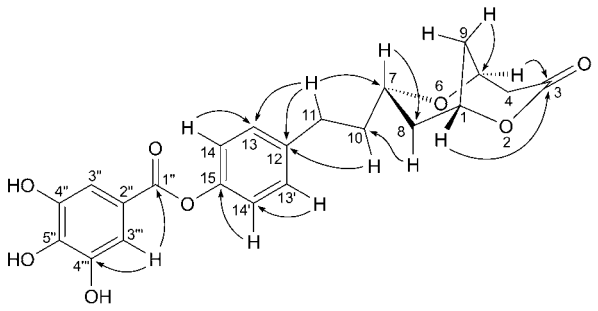

Fig. 4. *Selected HMBCs of compound* **2**

TLC Analysis revealed the presence of compound **1** and **2** in a crude extract prepared from fresh leaves after collection, which confirms their genuine nature.

Compounds **1** and **2** were tested *in vitro* for their antiplasmodial activity against the chloroquine-sensititive strains of *Plasmodium falciparum* strains NF 54 and 3D7 (*Table 2*). Compound **2** exhibited good antiplasmodial activity against both strains with IC_{50} values of 2.4 ± 0.5 μM and 4.9 ± 3.3 μM, respectively, while compound **1** was inactive. Chloroquine was used as the positive control. Thus, the substitution at C(15) with a galloyl moiety seems to be important for the activity of compound **2**.

Table 2. In vitro *Antiplasmodial Activity of the Crude MeOH Extract of the Leaves of* P. capitata *and Compounds* **1** *and* **2** *against* P. falciparum *NF54 and 3D7 Strains*

Tested samples	$IC_{50} \pm$ SD[a])	
	NF 54 strain	3D7 strain
MeOH extract	4.75	–
1	> 100	> 100
2	2.4 ± 0.5	4.9 ± 3.3
Chloroquine[b])	0.03	0.045

[a]) Extract in μg/ml and pure compounds in μM. [b]) Positive control.

Experimental Part

General. All reagents were of anal. grade. TLC: Silica gel *60* F_{254} (SiO_2; *Merck*); visualization by using ceric sulfate spray reagent. Column chromatography (CC): SiO_2 (70–230 mesh; *Merck*). FCC: SiO_2 (230–400 mesh, *Merck*). Optical rotations: *JASCO DIP-3600* polarimeter. UV Spectra: *JASCO V-630* spectrophotometer. IR spectra: *JASCO FT/IR-410* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C-NMR, DEPT, COSY, HSQC, and HMBC spectra: *Bruker AMX 500* instrument (at 500 and 125 MHz, resp.); in (D_6)DMSO; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *Finnigan MAT 95* mass spectrometer (70 eV) with perfluorokerosene as reference substance for HR-EI-MS; in *m*/*z*. HR-ESI-MS: *APEX III* (*Bruker Daltonik*) *7 Tesla* (ESI-FT-ICR-MS); in *m*/*z*.

Plant Material. The leaves of *P. capitata* were collected on *Cassia spectabilis* tree in the campus of the Higher Teacher Training College at the University of Yaoundé 1 in Cameroon in April 2013. The plant was identfied by Mr. *Nana Victor*, botanist at the National Herbarium, Yaounde, Cameroon, where a voucher specimen (Nr. 5068/SRF/Cam) was deposited.

Extraction and Isolation. The grounded dried leaves of *P. capiata* (2.8 kg) were extracted with MeOH at r.t. for 72 h. The solvent was evaporated under reduce pressure to yield 67 g of MeOH extract. This extract was fractionated by FCC (SiO_2) eluting with mixtures of hexane/AcOEt and AcOEt /MeOH of increasing polarity, resulting in the collection of 165 subfractions of 200 ml each, which were combined on the basis of TLC analysis to yield three main fractions, *F1–F3*.

Fraction *F1* (10.5 g) was subjected to CC (SiO_2), eluting with gradient mixtures of hexane/AcOEt (1:0 to 7:3), which resulted in 95 subfractions of 100 ml each. They were combined on the basis of TLC analysis. CC (SiO_2) of the subfractions *F1-20–F1-69* eluting with gradient mixtures of hexane/AcOEt (1:0 to 7:3) afforded betulinic acid (**3**, 12 mg), betulin (**8**, 6 mg), lupeol (**9**, 8 mg), and sitosterol (**10**, 22 mg).

Fraction *F2* (6.3 g) was subjected to CC (SiO_2), eluting with gradient mixtures of hexane/AcOEt (7:3 to 1:1), which resulted in 116 subfractions of 100 ml each. They were combined on the basis of TLC analysis. Subfractions *F2-12–F2-25* were complex mixtures containing mostly chlorophyll and were thus not studied. Subfractions *F2-27–F2-46* were subjected to CC (SiO_2) and eluted with a gradient of increasing polarity of mixtures of hexane/AcOEt (4:1 to 7:3) and yielded dodoneine (**4**, 7 mg).

Fraction *F3* (20.7 g) was subjected to CC (SiO_2). Elution with gradient mixtures of CH_2Cl_2/MeOH (1:0 to 8:2) resulted in 125 subfractions of 100 ml each, which were combined on the basis of TLC analysis. Subfractions *F3-1 – F3-40* were subjected to successive CC (SiO_2); eluting with CH_2Cl_2/MeOH (9:1) yielded more dodoneine (**4**, 12 mg), *rel*-(1*R*,5*S*,7*S*)-7-[2-(4-hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one (**1**, 205 mg), and 4-{2-[*rel*-(1*R*,3*R*,5*S*)-7-oxo-2,6-dioxabicyclo[3.3.1]non-3-yl]ethyl}phenyl 3,4,5-trihydroxybenzoate (**2**, 210 mg), and quercetin (**7**, 11 mg). Subfractions *F3-68 – F3-110* were subjected to successive CC (SiO_2) eluting with CH_2Cl_2/MeOH (9:2 to 9:3), which yielded quercetin 3-*O*-*α*-rhamnopyranoside (**5**, 11 mg) and quercetin 3-*O*-*α*-L-arabinofuranoside (**6**, 9 mg).

rel-*(1*R*,5*S*,7*S*)-7-[2-(4-Hydroxyphenyl)ethyl]-2,6-dioxabicyclo[3.3.1]nonan-3-one* (**1**). Yellow crystals. M.p. 174–175°. $[\alpha]_D^{20} = -37$ ($c = 0.5$, MeOH). UV (MeOH): 279 (2.9), 221 (3.4). IR (neat): 3337 (OH), 2948 (C–H), 1705 (C=O), 1613 (C=O), 1513 (C=C), 1077 (C–O–C). ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 285.1095 ($[M + Na]^+$, $C_{15}H_{18}NaO_4^+$; calc. 285.1097).

*4-{2-[*rel*-(1*R*,3*R*,5*S*)-7-Oxo-2,6-dioxabicyclo[3.3.1]non-3-yl]ethyl}phenyl 3,4,5-Trihydroxybenzoate* (**2**). White powder. $[\alpha]_D^{20} = -22$ ($c = 0.5$, MeOH). UV (MeOH): 282 (2.8), 218 (3.2). IR (neat): 3363 (OH), 2953 (C–H), 1714 and 1603 (C=O), 1189 and 1163 (C–O). ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 316 (20.1), 302 (19.6), 273 (34.6), 205 (38.3), 191 (52.6), 147 (38.0), 109 (82.0), 95 (100). HR-ESI-MS: 437.1205 ($[M + Na]^+$, $C_{22}H_{22}NaO_8^+$; calc. 437.1207).

X-Ray Crystal-Structure Determination of **1**[1]. A single crystal of **1** was selected under paratone oil and transferred on a *SuperNova*, Single source at offset, *Eos* diffractometer. The crystal was kept at 100.0(1) K during data collection. Using Olex2 [13], the structure was solved with the Superflip [14] structure solution program using Charge Flipping and refined with the ShelXL-97 [15] refinement package using Least Squares minimization. Sum formula $C_{15}H_{18}O_4$, $M = 262.29$ g/mol, monoclinic, space group $P2_1$ (no. 4), $a = 6.19705(13)$ Å, $b = 7.59281(17)$ Å, $c = 13.7251(3)$ Å, $\beta = 102.096(2)°$, $V = 631.47(2)$ Å^3, $Z = 2$, $\mu(CuK_\alpha) = 0.099$ mm^{-1}, $D_{calc} = 1.379$ g/cm^3, 36893 reflections measured ($6.1° \leq 2\theta \leq 60.0°$), 3689 unique ($R_{int} = 0.0638$) which were used in all calculations. The final R_1 was 0.0373 ($I > 2\sigma(I)$) and wR_2 was 0.0985 (all data).

Antiplasmodial Activity against P. falciparum *3D7 Strain. In vitro* antimalarial activity was evaluated by the *Plasmodium* lactate dehydrogenase (pLDH) immunodetection assay against *P. falciparum* 3D7 chloroquine sensitive strain, with a commercially available sandwich enzyme immunosorbent assay (*Advanced Practical Diagnostics BVBA*, Turnhout, Belgium), as reported previously [16]. Assays were performed in a 96-well culture plate with cultures mostly at ring stages at 1% parasitaemia (haematocrit, 2%). Parasite culture was incubated with increasing concentrations of the tested compound (0.001 to 100 μg/ml, six concentrations) for 96 h at 37° under reduced oxygen condition (Candle jar). Healthy erythrocytes were used as a negative control and the positive control consisted of chloroquine diphosphate (*Sigma–Aldrich*, Saint Quentin Fallavier, France), within the same range of concentrations. Each experiment was performed three times in duplicate.

Antiplasmodial Activity against P. falciparum *NF54 Strain*. Extracts and compounds were tested for their effects against the chloroquine-sensitive *P. falciparum* strain NF54, using the Malstat assay, which measures pLDH activity [17][18]. Briefly, parasites were cultivated in *RPMI 1640* medium (*Gibco*) supplemented with hypoxanthine (*Sigma–Aldrich*), A^+ human serum, and gentamicin (*Invitrogen*), and cultures were maintained at 37° in an atmosphere of 5% O_2, 5% CO_2, 90% N_2. Cultures were synchronized by repeated sorbitol treatment as described previously [19]. The assay was performed with synchronized ring stage parasites, plated in triplicate in 96-well plates at a parasitemia of 1% and 3% haematocrit. The pure compounds (dissolved in DMSO) were 2-fold serially diluted and screened at concentrations range between 100 μM – 780 nM. Extracts dissolved in DMSO were 2-fold serially diluted and tested at concentrations ranging from 125 mg/ml to 976 μg/ml. Chloroquine (dissolved in double-dist. H_2O) was used as internal control, and 0.5% DMSO was used as negative control. The cultures were incubated for 72 h at 37° under the above mentioned conditions. After incubation, 20 μl of culture from each well were transferred to a new 96-well plate and 100 μl of the *Malstat* reagent was added in each

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[1]) CCDC-1016061 contains the supplementary crystallographic data for this article. These data can be obtained free of charge from *The Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

well. The assessment of pLDH activity was obtained by adding 20 µl of a mixture of NBT (Nitro Blue Tetrazolium)-diaphorase (1:1; 1 mg/ml stock each) to the *Malstat* reaction, and optical densities were measured at 630 nm (*Infinite M200*, *Tecan*). Two to three independent tests were performed. The *OD* values were plotted against logarithmic concentrations of the compounds and a dose-response curve was automatically generated.

IC_{50} Values were calculated using GraphPad Prism Software (GraphPad Prism version 5).

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