A new procyanidin B from *Campylospermum zenkeri* (Ochnaceae) and antiplasmodial activity of two derivatives of (±)-serotobenine

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**ABSTRACT**

Phytochemical investigation of the stem bark of *Campylospermum zenkeri* led to the isolation of five known compounds: (Z,Z)-9,12-octadecadienoic acid (1), serotobenine (2), agathisflavone (3), lophirone A (4) and lophirone F (5), together with a new derivative of procyanidin B, a catechin dimer named zenkerinol (6). Serotobenine (2) is structurally related to decursivine which shows moderate activity against D6 and W2 strains of *Plasmodium falciparum*. For a better understanding of structure-activity relationships, three new semi-synthetic derivatives of serotobenine (2) have been prepared. These are: serotobenine monopropionate (2a), serotobenine monopivalate (2b) and serotobenine cyclohexyl ether (2c) respectively. Two of them (2a) and (2b), were evaluated for their antiplasmodial activity against *P. falciparum* 3D7 strain in a parasite lactate-dehydrogenase (pLDH) assay. Compound 2b was more active than compound 2a based on their IC50 values (36.6 and 123 μM, respectively).

**KEYWORDS**

*Campylospermum zenkeri*; Ochnaceae; zenkerinol; flavonoids; serotobenine; hemisynthesis; antiplasmodial activity

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1. Introduction

Malaria is the most prevalent disease in the world, killing about 1–2 million people each year. It is a threat to approximately 3.4 billion people in about 209 countries and territories (Diarra et al. 2015). In 2012, there were an estimated 207 million cases of malaria with over 627,000 deaths (World Health Organization 2014). Sub-Saharan Africa carries the biggest burden of this disease, where about 90% of cases and death occur particularly among children and pregnant women (Chinsembu 2015) due to poverty and debilitation. Increasingly, malarial parasites, particularly *P. falciparum*, are developing resistance to the most frequently used antimalarial drugs (White 2004). The challenge in malaria chemotherapy is to find novel molecular targets in light of the drug resistance crisis (Frederich et al. 2008). In this context, the search for new compounds from Cameroonian medicinal plants could provide new leads to antimalarial drugs. Plants of the genus *Campylospermum* (Ochnaceae) are widely distributed throughout the tropical zone of Africa Continent. Several species belonging to this genus are reported in folk medicine in the treatment of various diseases such as gastric pains, gonorrhoea, icterus, whitlow, malaria and aphrodisiac matter (Bouquet 1969; Ngono et al. 2011, 2015). Previous reports indicated that the genus and many others belonging to Ochnaceae family are a rich source of compounds with wide variety of structures (Abouem à Zintchem et al. 2008; Abouem et al. 2014; Bayiha Ba Njock et al. 2013; Ghogomu et al. 1990; Ndongo et al. 2010, 2015; Ngo Mbing et al. 2014; Ngono Bikobo et al. 2011, 2014, 2015). Hence, this study was carried out to isolate and elucidate the structure of constituents from stem bark extract of *C. zenkeri*, then to perform few chemical transformations on an isolated one serotobenine (which is the marker of the
genus) in order to evaluate the potent bioactive derivative for antiplasmodial activity. Serotobenine was chosen for derivatisation because of its structural similarity to decursivine, which has shown antimalarial properties in previous studies (Zhang et al. 2002; Qin et al. 2011).

2. Results and discussion

The methanolic extract of the stem bark of C. zenkeri was fractionated and purified by a silica gel column chromatography, affording five known compounds: \((Z,Z)\)-9,12-octadecadienoic acid (1) (Park et al. 2011), racemic serotobenine (2) (Ngono Bikobo et al. 2015; Sato et al. 1985), agathisflavone (3) (Mashima et al. 1970; Bayiha Ba Njock et al. 2011), lophirone A (4) (Ghogomu et al. 1987) and lophirone F (5) (Ghogomu et al. 1990) together with one newly described compound, zenkerinol (6, Figure 1). In addition to these compounds, three new serotobenine derivatives were prepared by using racemic serotobenine (2) as starting material (Scheme 1). They are: serotobenine monopropionate (2a), serotobenine monopivalate (2b) and serotobenine cyclohexyl ether (2c). While in decursivine, two phenolic hydroxyl groups are part of a methylenedioxy-acetal substructure, it seemed reasonable to protect the free hydroxyl group of serotobenine (2) in order to explore the importance of the acetal for the antiplasmodial activity. Therefore, the free phenolic group was covered as ester structures (Figure S1 and S2).

Compound 6 was obtained as an orange powder, soluble in methanol. The molecular formula of this compound was inferred to be C\(_{30}\)H\(_{22}\)O\(_{11}\) based on its molecular ion at m/z 557.1089 [M − H]\(^{-}\) in negative ion mode HR-ESI-MS (calcld. 557.1131). The \(^{13}\)C NMR spectral data of 6 (Table S2) revealed that this compound is a procyanidin derivative with two condensed flavonoids. That presence of two flavonoid units was indicated by \(^{13}\)C NMR resonances at \(\delta_c\) 140.0 (C-2), 133.1 (C-3), 48.8 (C-4) (ring C) which corresponds to deprotonated catechin and at \(\delta_c\) 78.3 (C-2\(\prime\)), 65.1 (C-3\(\prime\)), 28.5 (C-4\(\prime\)) (ring F), arising from flavanyl and flavenyl heterocyclic rings, respectively. These signals provide a distinct key entry point into the two-dimensional spectra (Ragab et al. 2013). Along with the absence of H-8\(\prime\) of D-ring in the flavan unit (sub-structure II, Figure S3), the above-mentioned signals indicated that this compound is a condensed flavonoid of procyanidin B series (Cádiz-Gurrea et al. 2014; Klika et al. 2015).

The attachment of proton signals at \(\delta_h\) 3.18 (H-4), in sub-structure I to the carbon atom at \(\delta_c\) 48.8 (C-4) ppm was noted in the HSQC spectrum (Figure S13). The same observations are found for signals at \(\delta_h\) 4.65 (H-2\(\prime\)), 3.91 (H-3\(\prime\)), 2.66 and 2.46 (H-4\(\prime\)) with carbons at \(\delta_c\) 78.3 (C-2\(\prime\)), 65.1 (C-3\(\prime\)) and 28.5 (C-4\(\prime\)), respectively in sub-structure II, which were consistent with the terminal unit of a catechin moiety (Figure S3) (Mello de et al. 1999; Wang et al. 2015). In the \(^1H\) NMR spectrum, the set of meta-coupled protons at \(\delta_h\) 5.88 (1H, d, \(J = 2.3\) Hz) and 5.71 (1H, d, \(J = 2.3\) Hz) was, respectively, assigned to H-6 and H-8 protons of the A-ring of the sub-structure I, while a residual aromatic proton singlet appeared at \(\delta_h\) 6.18 (s, H-6\(\prime\)) from D ring. The HMBC spectrum (Figure S14) of compound 6 revealed correlation of the signal at \(\delta_h\) 5.88 (H-6) and carbons at \(\delta_c\) 156.5 (C-7), 99.9 (C-4a) 94.4 (C-8) and 156.0 (C-5) (Figure S3). The C-8\(\prime\) involvement in the interflavan lineage was construed from the HMBC correlations, which enabled us to assign the C-8\(\prime\) carbon atom. From the HMBC spectrum, correlations were observed between H-4 (\(\delta = 3.18\)) with C-8\(\prime\) (\(\delta\) 102.4), C-7\(\prime\) (\(\delta\) 156.0) and C-3 (\(\delta\) 133.1) confirming the C-4 → C-8\(\prime\) bond. Moreover, comparison of the high chemical shift values of C-4a and C-8\(\prime\) at \(\delta_c\) 99.9 and 102.4 with literature data (Malan et al. 1996; Messanga et al. 2015).
Figure 1. Structures of natural products 1–6 isolated from the stem bark of *Campylospermum zenkeri* and antiplasmodial template decursivine.

Scheme 1. Synthesis of three serotobine derivatives 2a, 2b and 2c.
were less potent than decursivine. For decursivine, an antimalarial activity of 3.9 μM (D6-*falciparum*) characterized based on the spectroscopic data mentioned in supplementary material. By any correlation in the NOESY spectrum (Figure S17) between neither H-2 and H-4 and H-3 and H-6 (δH 5.88) of the cyclohexyl units indicated, however clearly the flav-C-5 (δC 156.5) in another side. Moreover, this linkage was strengthened by the analysis of NOESY spectrum (Figure S17) through cross-peaks observed between H-6’ (δH 6.18) and H-6 (δH 5.88). Additional data from the HMBC spectrum, enabled us to locate C-2 at δC 140.0, C-1’ at δC 120.2 (through correlations between H-6’ (δH 6.89) and olefinic carbons at δC 140.0 (C-2) and C-1’ (120.2), respectively) and C-3 (δC 133.1) involved in a relationship with H-4 (δH 3.18); these data were consistent with a flav-2-en-3-ol type flavonoid (Fukami et al. 2013).

Consequently, all those various details emphasize the presence of another flavonoid linkage for this molecule. It was noted that the flav-2-en-3-ol moiety was found as intermediate in the synthesis of anthocyanin (Fukami et al. 2013). In addition, IR spectrum (Figure S10) at 1595 cm⁻¹ also confirmed the presence of a conjugated olefinic double bond in this molecule (Marković et al. 2013). Signals for ABX coupling system [δH 6.89 (1H, d, J = 1.7 Hz, H-2’); δH 6.66 (1H, dd, J = 7.2; 1.7 Hz, H-6’) and δH 6.65 (1H, d, J = 7.2 Hz, H-5’)] of sub-structures I and II of the terminal unit (due to rings B and E) were observed (Table S2). These findings indicated that compound 6 was a B-type procyandin dimer derivative consisting of a flav-2-en-3-ol and flavan-3-ol units. Further evidence to support the proposed structure could be seen in HMBC spectrum (Figure S14), some observations were made between hydroxyl protons OH-3’ at δH 8.80 with C-3’ (δC 144.8) and OH-4’ (δH 8.74) with C-4’ (δC 144.7), indicating an ortho position of these sub-structures in the molecule (Figure S3). These finding confirmed that cyanidin derivative and catechin are the basic skeleton of this molecule with loosing of a H2O molecule to form a supplementary pyranic cycle as described above. The coupling constants of protons of the cyclohexyl units indicated, however, clearly the trans-relationships between H-2” (δH 4.65; J = 9.5 Hz) and H-3” (δH 3.91; J = 9.5; 4.2 Hz) (Table S2). All these results are confirmed by any correlation in the NOESY spectrum (Figure S17) between neither H-2” and H-3” nor H-4 and H-3”, respectively. These data corroborated the relative stereochemistry found for other analogues described in the literature (Messanga et al. 1998; Mello de et al. 1999; Cádiz-Gurrea et al. 2014; Klika et al. 2015). The additional data emphasized on the double linkage between the two flavonoid units. Based on these spectral evidences and comparison with reported data, the structure of 6 was therefore determined as (4S,2R*,3’S*)-cyanidin-(4a-8)-[5-O-7”]-catechin, named zenkerinol. To our knowledge, this is the first paper reporting the isolation of a procyandin dimer in the *Campylospermum* genus.

Serotobenine monopropionate (2a), serotobenine monopivalate (2b) and serotobenine cyclohexyl ether (2c) were prepared according to Scheme 1, and their structures were characterized based on the spectroscopic data mentioned in supplementary material.

Serotobenine derivatives 2a and 2b were tested for their antimalarial activity against *P. falciparum* strain D7 in a parasite lactate dehydrogenase (pLDH)-Assay. Both derivatives were less potent than decursivine. For decursivine, an antimalarial activity of 3.9 μM (D6-*P. falciparum* clone sensitive to chloroquine) has been reported (Zhang et al. 2002). Compound 2b has an IC₅₀ value of 36.6 μM and is about 10 times less active than decursivine, but is approximately 3.37-fold better than compound 2a (123 μM). The bulky tert-butyl moiety leads to a slight increase of the antimalarial activity. Moreover, according to the Basco et al.
(1994) criteria, the activity against *P. falciparum* can be considered moderate for compound 2b and low for compound 2a (Table S3).

3. Experimental section

3.1. General procedures

Melting points were uncorrected and were measured on a Mettler Toledo instrument. IR spectra were recorded on an Alpha FT-IR Spectrometer from Bruker, while 1D and 2D NMR spectra were obtained on a Bruker DRX 500 (500 MHz for 1H and 125 MHz for 13C spectra) spectrometer (Bruker, Rheinstetten, Germany) with chemical shifts reported in δ (ppm) using TMS (δH) as an internal standard. The HR-ESI-MS were obtained on LTQ-FT instrument (Thermo Scientific). LC-MS were measured with Shimadzu LC-MS system. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. Silica gel 60 (230–400 mesh E. Merck, Darmstadt, Germany) was employed for column chromatography, the solvent mixing systems for elution were mainly CH2Cl2/MeOH for the phytochemical study and CHCl3/MeOH for the purity of compounds from hemisynthesis reactions with increasing polarity each. All reactions sensitive to oxygen or moisture were conducted under argon atmosphere.

3.2. Plant material

Stem barks of *C. zenkeri* were collected at Pala Mount near Kribi in the South Region of Cameroon in April 2015 and identified by a botanist. A voucher specimen (N° 24093 SFRCAM) was deposited at the National Herbarium in Yaoundé, Cameroon.

3.3. Extraction and isolation

Dried and powered stem bark of *C. zenkeri* (315 g) were extracted for 48 h with MeOH (3 × 1L) at room temperature. After filtration and evaporation of solvent, the crude MeOH extract (20 g) was subjected to CC (SiO2, eluting with a gradient solvent system (CH2Cl2/MeOH)) giving five main fractions: I (3 g), II (4 g), III (4 g), IV (4 g), V (5 g). Fraction III (4 g) was submitted to CC (SiO2) using solvent system pentane/ethyl acetate (40/1) to give compound 1 (120 mg). Fraction IV (4 g) was submitted to CC (SiO2) using solvent system CH2Cl2/MeOH (60/1 to 5/1) to give three sub-fractions (IVa, IVb and IVc). Sub-fraction IVb (0.8 g) was chromatographed (SiO2) using CH2Cl2/MeOH (40/1) to afford compound 2 (35 mg; [α]D 25° = 0 (c 0.15, MeOH).

Using the same process, fraction V (5 g) gave 4 sub-fractions (Va, Vb, Vc and Vd). Sub-fraction Va (1.3 g) was further chromatographed on a silica gel column using CH2Cl2/MeOH (10/1) to afford compound 3 (13 mg). Sub-fraction Vb (1.8 g) was purified by repeated CC on silica gel with the solvent system CH2Cl2/MeOH (10/1–5/1) to provide compound 4 (7 mg) and compound 5 (6 mg). Sub-fraction Vc (0.85 g) was subjected to CC (SiO2) with the system CH2Cl2/MeOH (5/1–3/1) and compound 6 (5 mg; [α]D 25° = −79° (c 0.3, MeOH) was obtained.

3.4. Structural characterisation of compounds

3.4.1. Serotobenine monopropionate (2a)

White solid, (5.5 mg, 95%) (Scheme 1). Rf = 0.44 (CHCl3/MeOH :15:1); m.p. 250–252 ºC; UV/Vis (MeOH): 336 (log e): 206 (4.50), 204 (3.44), 201 (4.50) nm; IR cm⁻¹ 3333, 2940, 1755,
3.4.2. Serotobenine monopivalate (2b)
White solid, (5.8 mg, 93%) (Scheme 1). Rf = 0.38 (CHCl₃/MeOH: 30:1); m.p. 269–272 °C; UV/Vis (MeOH): λ max (log ε): 206 (4.50), 204 (3.44), 201 (4.05) nm; IR cm⁻¹: 3427, 3344, 3212, 2963, 2904, 1738, 1658, 1579, 1509; ¹H NMR (acetone-d₆, 500 MHz); δ H: 10.21 (1H, br, H-1), 8.03 (1H, br, H-10), 7.29 (1H, dd, J = 9.3 Hz, H-6’), 7.25 (1H, d, H-2’), 7.19 (1H, s, H-2), 7.10 (1H, d, J = 7.0 Hz, H-7), 7.08 (1H, d, J = 7.0 Hz, H-6); 6.74 (1H, d, J = 9.3 Hz, H-5’), 6.41 (1H, d, J = 10.5 Hz, H-7’), 4.78 (1H, d, J = 10.5 Hz, H-8’), 4.17–4.12 (1H, m, H-3b), 3.83 (3H, s, CH₃O-), 3.60–3.57 (1H, m, H-9a), 3.16–3.15 (1H, m, H-8a), 3.12–3.09 (2H, m, H-8b), 1.35 (9H, s, H-3’); ¹³C NMR (acetone-d₆, 125 MHz), δ C: 176.4 (C-1’), 171.3 (C-9’), 153.5 (C-3’), 152.2 (C-5’), 141.6 (C-1’), 140.6 (C-7a), 133.9 (C-4’), 125.7 (C-3a), 125.7 (C-6’), 124.9 (C-2’), 123.4 (C-6), 118.9 (C-7), 114.7 (C-4), 112.2 (C-3), 111.3 (C-2’), 105.4 (C-5’), 85.3 (C-7’), 56.1 (CH₃O-), 55.2 (C-8’), 41.3 (C-9a; 9b), 39.4 (C-2’), 30.5 (C-8a; 8b), 27.3 (C-3’); HR-ESI-MS m/z: 433.4845 [M – H]⁻ (calcd for C₂₅H₂₂N₂O₅ 433.4833).

3.4.3. Serotobenine cyclohexyl ether (2c)
White solid, (2.5 mg, 20%). (Scheme 1). Rf = 0.40 (CHCl₃/MeOH :20:1); ¹H NMR (acetone-d₆, 500 MHz); δ H: 10.28 (1H, br, H-1), 8.02 (1H, br, H-10), 7.26 (1H, dd, J = 9.3 Hz, H-6’), 7.25 (1H, d, H-2’), 7.19 (1H, s, H-2), 7.13 (1H, d, J = 7.0 Hz, H-7), 7.00 (1H, d, J = 7.0 Hz, H-6); 6.69 (1H, d, J = 9.3 Hz, H-5’), 6.34 (1H, d, J = 10.5 Hz, H-7’), 4.78 (1H, d, J = 10.5 Hz, H-8’), 4.16–4.11 (1H, m, H-9b), 3.80 (3H, s, CH₃O-), 3.51–3.49 (1H, m, H-9a), 3.50 (1H, m, H-1’), 3.18–3.14 (1H, m, H-8a), 3.05 (2H, m, H-8b), 1.67–1.61 (4H, m, H-2”), 1.54–1.50 (4H, m, H-3”), 1.45–1.39 (2H, m, H-4’); ¹³C NMR (acetone-d₆, 125 MHz), δ C: 171.5 (C-9’), 153.5 (C-3’), 151.9 (C-5’), 147.8 (C-7a), 136.2 (C-1’), 134.0 (C-4’), 125.8 (C-6’), 125.6 (C-2’), 123.8 (C-3a), 119.6 (C-6), 117.6 (C-7), 115.0 (C-4), 112.2 (C-3), 111.7 (C-2’), 105.4 (C-5’), 85.3 (C-7’), 69.2 (C-1’), 56.2 (CH₃O-), 55.2 (C-8’), 41.4 (C-9a; 9b), 29.0 (C-8a; 8b), 24.0 (C-4”) 23.8 (C-3’); HR-ESI-MS m/z: 455.4987 [M + Na]⁺ (calcd for C₂₆H₂₉N₂O₄Na, 455.5001).

3.4.4. Zenerkolin (6)
Orange solid; [α] D = –79° (c 0.3, MeOH); M.p. 295–297 °C; IR (KBr cm⁻¹): 3267, 2923, 2853, 1595, 1512, 1226, 1159; TLC Rf: 0.42 (CH₃Cl/MeOH: 90:10); ESI-MS m/z: 583.4941 [M + Na]⁺ (calcd for C₃₀H₂₉O₃Na, 583.4937); ¹H-NMR (500 MHz, DMSO-d₆), δ H: 6.89 (2H, d, J = 1.7 Hz, H-2’ and H-4”), 6.66 (2H, d, J = 7.2; 1.7 Hz, H-6’ and 6”), 6.65 (2H, dd, J = 7.2 Hz, H-5’ and H-5”), 6.18 (1H, s, H-6’), 5.88 (1H, d, J = 2.3 Hz, H-6), 5.71 (1H, d, J = 2.3 Hz, H-8), 4.65 (1H, d, J = 9.5 Hz, H-2”), 3.91 (1H, m, J = 9.5; 4.2 Hz, H-3”), 3.18 (1H, s, H-4”), 2.66 (1H, dd, J = 16.7; 5.9 Hz, H-8”), 2.46 (1H, dd, J = 16.7; 4.2 Hz, H-4a”); ¹³C-NMR (125 MHz, DMSO-d₆), δ C: 156.8 (C-5’), 156.5*
3.5. Antiplasmodial activity

Serotobenine derivatives 2a and 2b were tested for their antimalarial activity against *P. falciparum* strain 3D7 in a parasite lactate dehydrogenase (pLDH)-Assay. The parasites were cultured for 72 h in A + human erythrocytes at a haematocrit of 4% in RPMI media (PAA) supplemented with 10% A + human serum, 200 μM hypoxanthine and 20 μg ml⁻¹ gentamycin as described previously (Trager & Jensen 1976) in the presence and absence of the compounds, respectively. Cultures for growth assays were set up at the ring stage of parasites (0.1% parasitemia) in 96-well plates at a hematokrit of 3%. Parasites were cultured in presence or absence of drugs for up to 72 h. Subsequently cells were harvested, washed and frozen at −20 °C until analysis. pLDH was detected and measured as described elsewhere (Makler et al. 1993; Arnot et al. 2008). Growth was calculated as follow:

\[
G = \frac{\text{OD}_{650\text{nm}a} - \text{OD}_{650\text{nm}b}}{\text{OD}_{650\text{nm}c} - \text{OD}_{650\text{nm}b}} \times 100
\]

a = iRBC treated with drugs; b = iRBC treated with cycloheximide; c = non treated iRBC

G = plasmodial growth.

4. Conclusion

The genus *Campylospermum* is a good source of various classes of compounds such as: flavonoids, biflavonoids, ellagic acids, terpenoids, steroids and alkaloids. A large number of antimalarial compounds with a wide variety of structures have been isolated from plants. Among them, several belong to classes mentioned above. Indole alkaloid derivative such as decursivine showed a promising in vitro activity. Structure modification of racemic serotobenine afforded three derivatives nevertheless any of them among the tested compounds do not displayed good activity as the template decursivine.

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Disclosure statement

No potential conflict of interest was reported by the authors.
References


