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Diastereomeric Stilbenoid Glucoside Dimers from the Rhizomes of *Gnetum africanum*

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ABSTRACT:

In a continuing search for neuroprotective stilbenoids, two uncommon diastereomeric stilbenoid glucosides, africanoside A (**1**) and B (**2**) as well as fifteen known stilbenes, *E*-resveratrol, *E*-gnetifolin E, *E*-piceid, *E*-resveratrolside, *E*-gnetifolin K, *E*-gnetol, *E*-isorhapontigenin, *E*-isorhapontin, *E*-isorhapontigenin-4'-*O*-glucopyranoside, *E*-gnetin C, *E*-bisisorhapontigenin B, *E*-gnemonoside A, *E*-gnemonoside C, *E*-gnemonoside D, and *E*-gnetin E, were isolated from rhizomes of *Gnetum africanum*, using a combination of centrifugal partition chromatography and preparative HPLC. The structure of these two stilbenoids was investigated by NMR, vibrational circular dichroism (VCD), and electronic circular dichroism (ECD) experiments. The absolute configurations of **1**, and **2** were established by VCD as (7*aS*,8*aS*) and (7*aR*,8*aR*), respectively. Compound **1** significantly chelated Fe(II) at 100, 250 and 500 μ M. The ability of stilbenoids to chelate ferrous ions which are implicated in physiopathological hallmark of Alzheimer's disease provides useful data for potential treatment.

Keywords: *Gnetum africanum*, Gnetaceae, stilbenoids, polyphenols, absolute configuration, iron chelation

1. Introduction

The genus *Gnetum* is the only one of the Gnetaceae family. It consists of 40 species, mostly lianas, distributed in the tropical and subtropical regions of Asia, South America and Central Africa. In Africa, four different species of *Gnetum*, *G. africanum*, *G. bucholzianum*, *G. interruptum*, and *G. latispicum* have been described on the basis of morphologies features (Biye et al., 2014). The african gnetums are distributed in the humid tropical forests, which extend from Nigeria to Angola, via Cameroon, the Central African Republic, Gabon and the Democratic Republic of Congo (Biye et al., 2014). The African species are dioecious lianas. In Cameroon, gnetum also called “Eru”, is one of the most important native vegetable with a high economic values. Market studies indicate that more than 3600 tons of gnetum leaves are annually exported from Cameroon (Kanmegne et al., 2007). *G. africanum* and *G. bucholzianum* are used as vegetables, eaten raw or cooked in soups and salads. The aerial parts are used traditionally to treat diabetes, arterial hypertension; as a medicine against sore throat; and as a purgative (“*Gnetum africanum* (PROTA),”). Many studies have shown that the leaves of African species such as *G. africanum* and *G. bucholzianum* give a significant nutritional and antioxidant activity (Ali et al., 2011; Mvitu-Muaka et al., 2012; Ogonnaya and Chinedum, 2013). The compounds isolated from the plants of *Gnetum* genus included C-glycosylflavones, specially from the leaves of *G. africanum* and *G. bucholzianum* (Ouabonzi et al., 1983); flavonostilbenes (Iliya et al., 2003b); and stilbenoids, specially from lianas of *G. hainanense* (Huang et al., 2000), *G. montanum* (Li et al., 2004), *G. parvifolium* (Tanaka et al., 2001), stem of *G. gnemonoides* (Iliya et al., 2001), bark of *G. cuspidatum* (Shimokawa et al., 2012), seeds and roots of *G. gnemon* (Iliya et al., 2003a; Kato et al., 2009), and roots of *G. macrostachyum* (Sri-in et al., 2011). In our recent work, two diastereoisomers of gnemonoside A were isolated from the rhizomes of *G. africanum* and the absolute configuration was established by vibrational circular dichroism (VCD) spectroscopy (Buffeteau et al., 2014). As a part of our continuing research for plant-derived neuroprotective agents, a fractionation of a

G. africanum rhizome extract resulted in the isolation of two novel dimeric stilbenes glucosides, africanoside A (**1**) and B (**2**), which were purified along with fifteen known stilbenes, using solvent extraction and a combination of HPLC and centrifugal partition chromatography. The present work deals with the structure elucidation and iron chelating activity of compounds **1** and **2** (Figure 1).

2. Results and Discussion

Dried and powdered rhizomes of *G. africanum* were defatted with toluene. The defatted powder was extracted with aqueous acetone. The hydroacetonic extract was concentrated at 35 °C under reduced pressure. To enhance the stilbenoid content, aqueous residue was prepurified on Amberlite XAD-7 (H₂O and acetone) to provide a semipurified enriched extract (GA). GA was further partitioned between EtOAc and H₂O. The organic and aqueous extracts were concentrated and freeze-dried. The organic extract (GAE) was purified by a combination of centrifugal partition chromatography and preparative HPLC over C₁₈ reversed phase. From these extract, the known compounds were identified as *E*-resveratrol, *E*-gnetol, *E*-piceid, *E*-isorhapontigenin, *E*-isorhapontin, *E*-gnetifolin E, *E*-gnemonoside C, *E*-gnemonoside D, *E*-gnetin C, *E*-bisisorhapontigenin B, and *E*-gnetin E, by comparison of physicochemical data with those reported in the literature (Boralle et al., 1993; Buffeteau et al., 2014; Fernández-Marín et al., 2012; Iliya et al., 2002; Lins et al., 1982; Xu and Lin, 1997). The aqueous extract (GAA) was purified by preparative HPLC over C₁₈ reversed phase to afford two new compounds **1** and **2** along with six known stilbenes, *E*-resveratrol-3,4'-*O*-diglucopyranoside, *E*-gnetifolin K, *E*-resveratroloside, *E*-isorhapontin, *E*-gnemonoside A. The spectroscopic data of these known stilbenes were found to be similar with those reported in the literature (Buffeteau et al., 2014; Larronde et al., 2005; Waffo Teguo et al., 1998). Compound **1** gave a [M – H][–] HRESIMS quasi-molecular ion peak at *m/z* 807.2512, consistent with the molecular formula C₄₁H₄₄O₁₇. The presence of ions at *m/z* 645.1983 and

90 m/z 483.1436, corresponding to a neutral loss of two $C_6H_{10}O_5$, suggested that compound **1**
 91 contains two hexosyl groups. Moreover, the empirical formula of the ion at 483.1436
 92 corresponded to a dimer of resveratrol with an additional methoxy group.

93 Assignments of all ^1H and ^{13}C NMR signals for compound **1** were achieved by the analysis of
 94 ^1H - ^1H COSY, ^1H - ^{13}C HSQC, ^1H - ^{13}C HMBC data (**Table 1**). The ^1H NMR spectrum showed
 95 the presence of two regions of signals with a characteristic resonance. The former, between
 96 δ_H 7.2 and 6, consisted of an ABC-spin system [δ_H 7.20 (H-2b), δ_H 7.16 (H-5b), and δ_H 7.08
 97 (H-6b)], an AA'BB'-spin system [δ_H 7.26 (H-2a and H-6a), δ_H 7.10 (H-3a and H-5a)], an
 98 AB₂-spin system [δ_H 6.13 (H-10a and H-14a), δ_H 6.16 (H-12a)], a 1,3,4,5-tetra substituted
 99 phenyl ring [δ_H 6.54 (H-10b), δ_H 6.68 (H-14b)], and two coupled doublets at δ_H 7.05 (H-7b)
 100 and δ_H 6.99 (H-8b) with a large coupling constant ($J = 16.3$ Hz) for a *E*-olefinic proton
 101 system. The second region between δ_H 6 and 3, was characterized by two doublets at δ_H 5.40
 102 (H-7a) and δ_H 4.33 (H-8a) for a dihydrobenzofuran moiety, a singlet at δ_H 3.92 (3b-OMe),
 103 and two overlapped doublets at δ_H 4.92 (H-1' and H-1'') for the proton at the anomeric center
 104 of the two hexose units. The complete assignments of each glycosidic proton spin system
 105 were achieved by analysis of COSY and TOCSY experiments. Also, the ^{13}C NMR spectrum
 106 showed a completed overlapping of six signals at δ_C 101.2, 73.6, 76.8, 70.1, 76.8, and 61.3
 107 ppm which are characteristic for a glucose unit (Waffo Teguo et al., 1998). The proton at
 108 anomeric center at δ_H 4.92 (1H, *d*, $J = 7.6$ Hz) indicated the existence of β -glucosyl residues.
 109 Therefore, compound **1** could be proposed as a diglucopyranoside of gnetin C-*O*-methylated.

110 The position of glucosyl residues in compound **1** was determined by HMBC experiment,
 111 which showed long-range correlations between the H-1' and H-1'' of glucose at δ_H 4.92 and
 112 C-4a and C-4b at δ_C 157.6 and 146.4, respectively. The position of the methoxyl group was
 113 also confirmed by a long-range correlation between the proton of methoxyl at δ_H 3.92 and C-
 114 3b at δ_C 150. The absence of a cross-peak between the protons H-7a (δ_H 5.4) and H-8a

115 (δ_{H} 4.33) in the ROESY spectrum (Supporting Information, Figure S3), indicated a *trans*-
116 orientation of these two protons. The structure of the new compound **1** was assigned as
117 gnemonoside A-3b-methoxyl and named africanoside A.

118 Compound **2** gave a $[\text{M} - \text{H}]^-$ HRESIMS quasi-molecular ion peak at m/z 807.2514 indicating
119 a molecular mass of 808 amu in agreement with the molecular formula $\text{C}_{41}\text{H}_{44}\text{O}_{17}$. The
120 presence of ions at m/z 645.1984 and m/z 483.1440, corresponding to a neutral loss of two
121 $\text{C}_6\text{H}_{10}\text{O}_5$, suggesting that compound **2** contains two hexosyl group. Moreover, the empirical
122 formula of the ion at 483.1440 corresponded to a dimer of resveratrol with an additional
123 methoxy group. The ^1H and ^{13}C NMR spectra (**Table 1**) of compounds **1** and **2** were
124 superimposable, suggesting that the structures of both compounds were very similar. Thus,
125 the structure of **2** was concluded to be a gnemonoside A-3b-methoxyl isomer and named
126 africanoside B.

127 The relative configurations of the chiral centers (C-7a and C-8a) of compounds **1** and **2** were
128 deduced in the same manner as for the two diastereomers of (–)-*E*-gnemonoside A (Buffeteau
129 et al., 2014), using vibrational circular dichroism (VCD). VCD spectra of **1** and **2** were
130 recorded in $\text{DMSO}-d_6$ at a concentration of 50 mM and are reported in **Figure 2a**. The two
131 spectra are not perfectly opposite with respect to the baseline, revealing that the two
132 molecules are not enantiomers, but diastereomers. Indeed, since the glucopyranosyl (Glc)
133 groups have the same chirality (β -D-Glc) for the two molecules, **1** and **2** are diastereoisomers.

134 Nevertheless, the VCD spectra of compounds **1** and **2** are quasi identical to those published
135 for the two diastereomers of (–)-*E*-gnemonoside A (Buffeteau et al., 2014). The VCD spectra
136 of the aglycone of the two diastereomers of (–)-*E*-gnemonoside A (ca (+)-*E*-gnetin C and (–)-
137 *E*-gnetin C) associated with DFT calculations allowed us to determine the absolute
138 configuration of these two enantiomers as (–)-(7a*S*,8a*S*)-*E*-gnetin C and (+)-(7a*R*,8a*R*)-*E*-
139 gnetin C. In this study, the VCD spectrum of compound **1** is well reproduced by the one
140 published for (7a*S*,8a*S*)-*E*-gnemonoside A, and in turn, VCD spectrum of compound **2** is well

reproduced by the one published for (7a*R*,8a*R*)-*E*-gnemonoside A.

As electronic circular dichroism (ECD) experiments are currently used in the structural studies of stilbenes, ECD spectra of **1** and **2** are reported in **Figure 2b**. The ECD spectrum of **1** exhibits two positive bands at 222 and 252 nm and a broad negative band between 262 and 350 nm. A similar ECD spectrum has been measured for (–)-*E*-gnetin C compound (Supporting Information, **Figure S19**) for which the (7a*S*,8a*S*) configuration has been determined (Buffeteau et al., 2014).

Compounds **1** and **2**, as well as eight known stilbenes isolated from *G. africanum* organic and aqueous extracts (*E*-isorhapontigenin, *E*-gnetol, *E*-resveratrol, *E*-isorhapontigenin-4'-*O*-glucoside, *E*-gnetin C, *E*-gnemonoside A, *E*-gnemonoside C, *E*-gnemonoside D) were evaluated for their iron-chelating activity. As presented in **Figure 3**, the compound **1** significantly chelated Fe(II) at 38.5%, 61.3% and 73.1% at 100, 250 and 500 μM, respectively. *E*-Isorhapontigenin-4'-*O*-glucoside also exhibited notable chelation at 40.6% and 67.0% at 250 and 500 μM, respectively. The other tested compounds from *G. africanum* were less active as chelators of Fe(II) giving rise values inferior to 40% at 500 μM which was similar to the data reported for *E*-resveratrol whose concentration for 50% of chelation of Fe(II) was 746 μM (Kantham et al., 2017). These findings may afford beneficial effects into Alzheimer's disease (AD) treatment. The physiopathological hallmark of AD is an abnormal fibrillation and accumulation of amyloid beta peptide (Aβ) forming amyloid plaques in brain. This process leads to neurotoxic effects such as cell membrane perturbation and oxidative stress among others (Hornedo-Ortega et al., 2018). Otherwise, the presence of metal binding sites and the large amount of iron in the amyloid plaque can accelerate the fibril formation modulating the morphology of Aβ oligomers and improving the toxicity of Aβ cores (Kantham et al., 2017). In addition, the strong presence of ferrous ions could be a source of reactive oxygen species (ROS) as Fe(II) is the catalyst of Fenton reaction arousing oxidative stress and neurotoxic effects (Ayton et al., 2017). As a consequence, the ability of compounds

1 to chelate ferrous ions provides useful data for potential AD treatment. Furthermore, our findings revealed different biological activities between the two new diastereoisomer forms **1** and **2**. Even if the chemical structure is very similar, other authors have confirmed that most of isomers show notable differences in several biological domains related to pharmacology, bioavailability, toxicology, and metabolism process (McConathy and Owens, 2003; Nguyen et al., 2006). In fact, stereoisomers of miyabenol C, a trimeric stilbene, have already exhibited different inhibition on fibrillation of A β (Papastamoulis et al., 2015). As a consequence, the chiral separation followed by the determination of absolute configuration of stereoisomers should be promoted in order to discriminate their biological activities and to provide accurate treatment.

3. Conclusions

The structural investigation of compounds depends widely on the choice of analytical tools. In the present work, different techniques were applied to assure the orthogonality of the data. The absolute configuration of two novel diastereomeric stilbenoid glucosides was successfully elucidated by using NMR, mass spectrometry, VCD, and ECD. (7aS,8aS)-Africanoside A (**1**) and (7aR,8aR)-africanoside B (**2**) were determined as dimers of resveratrol with an additional methoxy group and two hexosyl group. These two compounds as well as others stilbenoids from *G. africanum* exhibited a wide range of iron-chelating activities. These findings allowed to gain into the knowledge for plant-derived neuroprotective agents. In addition, the different activity between the two diastereoisomers africanoside A and B promote the chiral separation to ensure a specific treatment.

4. Experimental

4.1. General experimental procedures.

¹H and ¹³C NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer and analyzed with Bruker Topspin software. Compounds were measured in 3 mm NMR tubes, using CD₃OD and acetone-d₆ as the solvents. The specific optical rotations were

determined in methanol at 20 °C on a JASCO P-2000 polarimeter using the sodium emission wavelength ($\lambda = 589$ nm). The centrifugal partition chromatography (CPC) used in this study (FCPC1000®) was provided by Kromaton Technologies (Sainte-Gemmes-sur-Loire, France). The solvents were pumped by an ECOM IOTA S100 2-way binary high-pressure Isocratic pump. The samples were introduced into the CPC column via a high pressure injection valve equipped with a 50 mL sample loop. The CPC fractions were monitored with an ECOM FLASH 06 DAD detector, at wavelengths of 306 and 280 nm. HPLC preparative separations were carried out using a Varian Prostar 345 UV-visible detector and a binary pump with a Phenomenex Kinetex XB-C18 AXIA packed (5 μ m, 100 Å, 250 $\times$ 21.4 mm) column. The mobile phase was composed of two solvents: (A) 0.025% TFA in H₂O and (B) MeCN. HRESIMS data were acquired using a Q-Exactive Plus mass spectrometer (Thermo Scientific, Bremen, Germany) fitted with a heated electrospray ionization (H-ESI II) probe.

4.2. Plant material.

The rhizomes of *Gnetum africanum* were collected in Yaoundé (Centre Province, Cameroon) in November 2013. A voucher specimen (accession number R-GA/2013) has been deposited in the Department of the Molécules d'Intérêt Biologique (MIB) at ISVV. The identification was confirmed by Mr. Victor Nana, Botanist of the Cameroon National Herbarium.

4.3. Extraction and isolation.

Dried and finely powdered rhizomes of *G. africanum* (600 g) were defatted twice 1.5 L of toluene. The extraction was carried out as follows: The defatted powder is moistened with 60% aqueous acetone and placed in the percolator. The percolator was filled with 60% aqueous acetone and covered up. The bottom outlet was opened until get a regular dripping and then closed and left macerated for 24 h. After this time, the outlet bottom was opened and left dripping slowly. The maceration-percolation extraction was repeated 2 times and the hydroacetic extract was concentrated at 35 °C under reduced pressure. To enhance the stilbenoid content, aqueous residue was prepurified on amberlite XAD-7 (H₂O and acetone),

using the previously published protocol to provide a semi-purified extract enriched (GA, 51.43 g) (Buffeteau et al., 2014). 50 g of GA was further partitioned between EtOAc and H₂O. The organic and aqueous extracts were concentrated and freeze-dried to yield 28.87 g (EtOAc, GAE) and 17.6 g (H₂O, GAA), respectively.

1 and **2** were purified from 1 g of GAA by preparative HPLC using a Varian Prostar 345 UV-visible detector and a binary pump with a Phenomenex Kinetex 100-5 XB-C₁₈ (5 μ m, 250 $\times$ 21.4 mm) column with a security Guard. The mobile phase was composed of two solvents: (A) 0.025% TFA in H₂O and (B) MeCN and the elution program at 21 mL/min was 10% B (0-2 min), 10-30% B (2-22 min), 30-38% B (22-37 min), 38-100% B (37-45 min), 100% B (45-50 min), 100-10% B (50-51 min), 10% B (51-60 min).

Preparative centrifugal partition chromatography separation of EtOAc extract (GAE) was performed in quaternary Arizona-L system consisting of n-heptane/ethyl acetate/methanol/water (2:3:2:3). Four Arizona solvent systems (J, K, L, and M) were tested in order to select the optimal system L. Three batches of 5 g were submitted to CPC separation in descending mode. In our experiment, the rotor was filled by injecting the organic lighter stationary phase at 50 mL/min and 500 rpm. Then, the rotation speed was increased to 1000 rpm. The mobile phase was pumped into the column at 15 mL/min until the equilibrium was reached and the sample solution containing 5 g of dry GAE dissolved in 40 mL of a mixture consisting of upper and lower phase (1:1, v/v) was injected through a 50 mL loop. The CPC fractions were constituted according to the elution profile. The collected fraction were evaporated and freeze-dried. A total of six fractions numbered from Fr 1 to Fr 6 were obtained. Fr 1 (1 g) was subjected to preparative HPLC [(solvent A: 0.025% TFA in H₂O and B: MeCN; the elution program at 21 mL/min was 10% B (0-2 min), 10- 20% B (2-22 min), 20% B (22-35 min), 20- 40% B (35-36 min), 40% B (36-40 min), 40% B (36-40 min)] to yield: *E*-resveratrolside (41.6 mg), *E*-isorhapontigenin-4'-*O*-glucoside (18.7 mg), *E*-gnemonoside A (16.8 mg), *E*-piceid (28.5 mg), *E*-isorhapontin (12.3 mg), *E*-gnetol (10 mg),

E-gnemonoside C (107.6 mg), and *E*-gnemonoside D (34.7 mg). The purification of the Fr 5 (750 mg) was performed by preparative HPLC [the same mobile phases as above were used and the elution program at 21 mL/min was 17% B (0-2 min), 17-30% B (2-22 min), 30-38% B (22-37 min), 38-100% B (37-45 min), 100% B (45-50 min), 100-17% B (50-51 min), 17% B (51-60 min)] to provide: *E*-resveratrol (157 mg), *E*-isorhapontigenin (59 mg), *E*-gnetin C (182 mg), *E*-Bisisorhapontigenin B (46 mg), and *E*-gnetin E (5 mg).

Compound 1: white amorphous powder; $[\alpha]_D^{20} = -58.2$ ($c = 0.086$, MeOH); ^1H and ^{13}C NMR spectroscopic data, (see Table 1); HRESIMS $[\text{M}-\text{H}]^- m/z$ 807.2519.

Compound 2: white amorphous powder; $[\alpha]_D^{20} = +22.6$ ($c = 0.102$, MeOH); ^1H and ^{13}C NMR spectroscopic data, (see Table 1); HRESIMS $[\text{M}-\text{H}]^- m/z$ 807.2517.

4.4. UV-vis and ECD Measurements.

ECD spectra of compounds **1** and **2** were recorded in EtOH at 20 °C with a 1 cm path length quartz cell. The concentration of the two compounds was taken in the range $0.7 \cdot 10^{-4} - 1 \cdot 10^{-4}$ M. Spectra were recorded in the 210 – 400 nm wavelength range with a 0.5 nm increment and a 1s integration time. Spectra were processed with standard spectrometer software, baseline corrected and slightly smoothed by using a third order least square polynomial fit. Spectral units were expressed in difference of molar extinction coefficients ($\Delta\epsilon$ in L/mol/cm).

4.5. VCD measurements.

IR and VCD spectra were recorded with a ThermoNicolet Nexus 670 FTIR spectrometer equipped with a VCD optical bench (Buffeteau et al., 2005). In this optical bench, the light beam was focused by a BaF₂ lens (191 mm focal length) to the sample, passing an optical filter, a BaF₂ wire grid polarizer (Specac), and a ZnSe photoelastic modulator (Hinds Instruments, Type II/ZS50). The light was then focused by a ZnSe lens (38.1 mm focal length) onto a 1x1 mm² HgCdTe (ThermoNicolet, MCTA* E6032) detector. IR and VCD spectra were recorded at a resolution of 4 cm⁻¹ by co-adding 50 scans and 36000 scans (12h acquisition

time), respectively. The sample was held in a fixed path length (100 μm) cell with BaF_2 windows. IR and VCD spectra of compounds **1** and **2** were measured in $\text{DMSO-}d_6$ at a concentration of 50 mM. Baseline correction of the VCD spectra was performed by subtracting the VCD spectrum of $\text{DMSO-}d_6$ solvent. In all experiments, the photoelastic modulator was adjusted for a maximum efficiency at 1400 cm^{-1} . Calculations were done with the standard ThermoNicolet software, using Happ and Genzel apodization, de-Haseth phase-correction and a zero-filling factor of one. Calibration spectra were recorded using a birefringent plate (CdSe) and a second BaF_2 wire grid polarizer. Finally, the solvent absorption was subtracted out in the presented IR spectra.

4.6. Iron chelating activity.

The ferrozine assay protocol was used to estimate the concentration of free ferrous ions by measuring the absorbance of the Fe(II) -ferrozine complex at 560 nm (Chan et al., 2016). The decrease of absorbance allowed to evaluate the binding of Fe(II) to the tested compounds. The assay was performed according to previously published protocols with slight modifications (Chan et al., 2016; Dinis et al., 1994). Tests were carried out in 96 well plates and solutions were prepared in HEPES buffer (15 mM, pH 6.8). Briefly, 50 μL of a Fe(II) solution at 200 μM was incubated with 50 μL of pure compounds (*E*-gnetol, *E*-isorhapontigenin, *E*-isorhapontigenin-4'-*O*-glucoside, *E*-resveratrol, *E*-gnetin C, *E*-gnemonoside A, *E*-gnemonoside C, *E*-gnemonoside D, africanoside A and africanoside B) to reach the following final concentrations: 0, 25, 50, 100, 250, 500 μM . To maximize the solubility of pure compounds, MeOH was used as co-solvent at final concentration of 1% (v/v). Gallic acid was used as positive control. After 30 min of incubation, ferrozine at 1 mM (50 μL) was added leading to the formation of purple complex. The reaction reached the equilibrium after 10 min of incubation. At this moment, absorbance at 560 nm was measured by an automated plate reader (Fluostar Omega; BMG Labtech, Offenburg, Germany). The chelation of Fe(II) values (%) by compounds was calculated as follows (Kantham et al., 2017):

296 $Chelation\ (\%) = (1 - (A_{Fe(II) + compound + ferrozine} - A_{Fe + compound}) / (A_{Fe(II) + ferrozine} - A_{Fe(II)})) \times 100$

297 All assays were repeated at least in triplicate and performed in quadruplicate.

298 **4.7. Statistical analysis.**

299 Statistical analysis was carried out with one-way ANOVA followed by Newman-Keuls
300 multiple comparison *post hoc* tests. GraphPad Prism software v5.03 (La Jolla, CA) was used.
301 Significant differences between Fe(II) chelation percentage of pure compounds are
302 represented by different letters.

303

304 **Declaration of Competing Interest**

305 The authors declare no competing information.

306

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311

312 **Appendix A. Supplementary data**

313 **Supporting Information:** ¹H NMR spectra of known stilbenoids in methanol-d₄ or acetone-
314 d₆. ¹H, ¹H-¹H COSY, ¹H-¹H ROESY, HSQC and HMBC spectra of **1**. ECD spectra of (–)-*E*-
315 gnetin C and (+)-*E*-gnetin C compounds.

316

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FIGURES CAPTIONS

Figure 1. Chemical structures of compound **1** and **2** in CD₃OD.

Figure 2. a) Experimental VCD spectra of **1** and **2** in DMSO-*d*₆ solution (50 mM, 100 μm path length). b) Experimental ECD spectra of **1** and **2** in EtOH solution (7.7 10⁻⁵ M, 1 cm path length).

Figure 3. Fe(II) chelation (%) activities of pure compounds from *Gnetum africanum* at different concentrations (μM). Statistical analysis was carried out with one-way ANOVA followed by Newman-Keuls multiple comparison post hoc tests. Different letters means significant differences.

*Gallic acid was used as positive control.

Table 1. ^1H and ^{13}C NMR data of compounds **1** and **2** in methanol- d_4 at 600 MHz for ^1H and 150 MHz for ^{13}C

position	1 or 2	
	δ_{C} , type	δ_{H} (J in Hz)
1a	136.1, C	
2a/6a	126.6, CH	7.26 <i>d</i> (8.5)
3a/5a	116.5, CH	7.10 <i>d</i> (8.5)
4a	157.6, C	
7a	92.9, CH	5.4 <i>d</i> (5.1)
8a	55.4, CH	4.33 <i>d</i> (5.1)
9a	145.3, C	
10a/14a	105.6, CH	6.13 <i>d</i> (2.2)
11a/13a	158.3, C	
12a	100.8, CH	6.16 <i>t</i> (2.2)
1b	132.8, C	
2b	110.3, CH	7.20 <i>d</i> (2.0)
3b	150, C	
4b	146.4, C	
5b	116.8, CH	7.16 <i>d</i> (8.5)
6b	119.6, CH	7.08 <i>dd</i> (8.5, 2.0)
7b	127.6, CH	7.05 <i>d</i> (16.3)
8b	127.6, CH	6.99 <i>d</i> (16.3)
9b	140, C	
10b	107.1, CH	6.54 <i>brd</i>
11b	154.5, C	
12b	114.2, C	
13b	162, C	
14b	98.5, CH	6.68 <i>brd</i>
3b-OMe	55.7, CH	3.92 <i>s</i>
4a- <i>O</i> -Glc		
1'	101.2, CH	4.92 <i>d</i> (7.6)
2'	73.6, CH	3.46 <i>m</i>
3'	76.8, CH	3.47 <i>m</i>
4'	70.1, CH	3.40 <i>m</i>
5'	76.8, CH	3.43 <i>m</i>
6'a	61.3, CH ₂	3.70 <i>m</i>
6'b		3.89 <i>m</i>
4b- <i>O</i> -Glc		
1''	101.2, CH	4.92 <i>d</i> (7.4)
2''	73.6, CH	3.46 <i>m</i>
3''	76.8, CH	3.47 <i>m</i>
4''	70.1, CH	3.40 <i>m</i>
5''	76.8, CH	3.43 <i>m</i>
6''a	61.3, CH ₂	3.70 <i>m</i>
6''b		3.89 <i>m</i>