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

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19

20 **ABSTRACT:**

21 In a continuing search for neuroprotective stilbenoids, two uncommon diastereomeric  
22 stilbenoid glucosides, africanoside A (**1**) and B (**2**) as well as fifteen known stilbenes, *E*-  
23 resveratrol, *E*-gnetifolin E, *E*-piceid, *E*-resveratrolside, *E*-gnetifolin K, *E*-gnetol, *E*-  
24 isorhapontigenin, *E*-isorhapontin, *E*-isorhapontigenin-4'-*O*-glucopyranoside, *E*-gnetin C, *E*-  
25 bisisorhapontigenin B, *E*-gnemonoside A, *E*-gnemonoside C, *E*-gnemonoside D, and *E*-gnetin  
26 E, were isolated from rhizomes of *Gnetum africanum*, using a combination of centrifugal  
27 partition chromatography and preparative HPLC. The structure of these two stilbenoids was  
28 investigated by NMR, vibrational circular dichroism (VCD), and electronic circular dichroism  
29 (ECD) experiments. The absolute configurations of **1**, and **2** were established by VCD as  
30 (7a*S*,8a*S*) and (7a*R*,8a*R*), respectively. Compound **1** significantly chelated Fe(II) at 100, 250  
31 and 500 μM. The ability of stilbenoids to chelate ferrous ions which are implicated in  
32 physiopathological hallmark of Alzheimer's disease provides useful data for potential  
33 treatment.

34

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

37

## 38 **1. Introduction**

39 The genus *Gnetum* is the only one of the Gnetaceae family. It consists of 40 species, mostly  
40 lianas, distributed in the tropical and subtropical regions of Asia, South America and Central  
41 Africa. In Africa, four different species of *Gnetum*, *G. africanum*, *G. bucholzianum*, *G.*  
42 *interruptum*, and *G. latispicum* have been described on the basis of morphologies features  
43 (Biye et al., 2014). The african gnetums are distributed in the humid tropical forests, which  
44 extend from Nigeria to Angola, via Cameroon, the Central African Republic, Gabon and the  
45 Democratic Republic of Congo (Biye et al., 2014). The African species are dioecious lianas.  
46 In Cameroon, gnetum also called “Eru”, is one of the most important native vegetable with a  
47 high economic values. Market studies indicate that more than 3600 tons of gnetum leaves are  
48 annually exported from Cameroon (Kanmegne et al., 2007). *G. africanum* and *G.*  
49 *bucholzianum* are used as vegetables, eaten raw or cooked in soups and salads. The aerial  
50 parts are used traditionally to treat diabetes, arterial hypertension; as a medicine against sore  
51 throat; and as a purgative (“*Gnetum africanum* (PROTA),”). Many studies have shown that  
52 the leaves of African species such as *G. africanum* and *G. bucholzianum* give a significant  
53 nutritional and antioxidant activity (Ali et al., 2011; Mvitu-Muaka et al., 2012; Ogbonnaya  
54 and Chinedum, 2013). The compounds isolated from the plants of *Gnetum* genus included C-  
55 glycosylflavones, specially from the leaves of *G. africanum* and *G. bucholzianum* (Ouabonzi  
56 et al., 1983); flavonostilbenes (Iliya et al., 2003b); and stilbenoids, specially from lianas of *G.*  
57 *hainanense* (Huang et al., 2000), *G. montanum* (Li et al., 2004), *G. parvifolium* (Tanaka et al.,  
58 2001), stem of *G. gnemonoides* (Iliya et al., 2001), bark of *G. cuspidatum* (Shimokawa et al.,  
59 2012), seeds and roots of *G. gnemon* (Iliya et al., 2003a; Kato et al., 2009), and roots of *G.*  
60 *macrostachyum* (Sri-in et al., 2011). In our recent work, two diastereoisomers of gnemonoside  
61 A were isolated from the rhizomes of *G. africanum* and the absolute configuration was  
62 established by vibrational circular dichroism (VCD) spectroscopy (Buffeteau et al., 2014). As  
63 a part of our continuing research for plant-derived neuroprotective agents, a fractionation of a

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

69

## 70 **2. Results and Discussion**

71 Dried and powdered rhizomes of *G. africanum* were defatted with toluene. The defatted  
72 powder was extracted with aqueous acetone. The hydroacetic extract was concentrated at  
73 35 °C under reduced pressure. To enhance the stilbenoid content, aqueous residue was  
74 prepurified on Amberlite XAD-7 (H<sub>2</sub>O and acetone) to provide a semipurified enriched  
75 extract (GA). GA was further partitioned between EtOAc and H<sub>2</sub>O. The organic and aqueous  
76 extracts were concentrated and freeze-dried. The organic extract (GAE) was purified by a  
77 combination of centrifugal partition chromatography and preparative HPLC over C<sub>18</sub> reversed  
78 phase. From these extract, the known compounds were identified as *E*-resveratrol, *E*-gnetol,  
79 *E*-piceid, *E*-isorhapontigenin, *E*-isorhapontin, *E*-gnetifolin E, *E*-gnemonoside C, *E*-  
80 gnemonoside D, *E*-gnetin C, *E*-bisisorhapontigenin B, and *E*-gnetin E, by comparison of  
81 physicochemical data with those reported in the literature (Boralle et al., 1993; Buffeteau et  
82 al., 2014; Fernández-Marín et al., 2012; Iliya et al., 2002; Lins et al., 1982; Xu and Lin,  
83 1997). The aqueous extract (GAA) was purified by preparative HPLC over C<sub>18</sub> reversed phase  
84 to afford two new compounds **1** and **2** along with six known stilbenes, *E*-resveratrol-3,4'-*O*-  
85 diglucopyranoside, *E*-gnetifolin K, *E*-resveratrolside, *E*-isorhapontin, *E*-gnemonoside A. The  
86 spectroscopic data of these known stilbenes were found to be similar with those reported in  
87 the literature (Buffeteau et al., 2014; Larronde et al., 2005; Waffo Tegu et al., 1998).

88 Compound **1** gave a [M – H]<sup>–</sup> HRESIMS quasi-molecular ion peak at *m/z* 807.2512,  
89 consistent with the molecular formula C<sub>41</sub>H<sub>44</sub>O<sub>17</sub>. 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  $\delta_H$  7.2 and 6, consisted of an ABC-spin system [ $\delta_H$  7.20 (H-2b),  $\delta_H$  7.16 (H-5b), and  $\delta_H$  7.08  
97 (H-6b)], an AA'BB'-spin system [ $\delta_H$  7.26 (H-2a and H-6a),  $\delta_H$  7.10 (H-3a and H-5a)], an  
98 AB<sub>2</sub>-spin system [ $\delta_H$  6.13 (H-10a and H-14a),  $\delta_H$  6.16 (H-12a)], a 1,3,4,5-tetra substituted  
99 phenyl ring [ $\delta_H$  6.54 (H-10b),  $\delta_H$  6.68 (H-14b)], and two coupled doublets at  $\delta_H$  7.05 (H-7b)  
100 and  $\delta_H$  6.99 (H-8b) with a large coupling constant ( $J = 16.3$  Hz) for a *E*-olefinic proton  
101 system. The second region between  $\delta_H$  6 and 3, was characterized by two doublets at  $\delta_H$  5.40  
102 (H-7a) and  $\delta_H$  4.33 (H-8a) for a dihydrobenzofuran moiety, a singlet at  $\delta_H$  3.92 (3b-OMe),  
103 and two overlapped doublets at  $\delta_H$  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  $\delta_C$  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  $\delta_H$  4.92 (1H, *d*,  $J = 7.6$  Hz) indicated the existence of  $\beta$ -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  $\delta_H$  4.92 and  
112 C-4a and C-4b at  $\delta_C$  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  $\delta_H$  3.92 and C-  
114 3b at  $\delta_C$  150. The absence of a cross-peak between the protons H-7a ( $\delta_H$  5.4) and H-8a

115 ( $\delta_{\text{H}}$  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  $^1\text{H}$  and  $^{13}\text{C}$  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 ( $\beta$ -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

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

142 As electronic circular dichroism (ECD) experiments are currently used in the structural  
143 studies of stilbenes, ECD spectra of **1** and **2** are reported in **Figure 2b**. The ECD spectrum of  
144 **1** exhibits two positive bands at 222 and 252 nm and a broad negative band between 262 and  
145 350 nm. A similar ECD spectrum has been measured for (-)-*E*-gnetin C compound  
146 (Supporting Information, **Figure S19**) for which the (7a*S*,8a*S*) configuration has been  
147 determined (Buffeteau et al., 2014).

148 Compounds **1** and **2**, as well as eight known stilbenes isolated from *G. africanum* organic and  
149 aqueous extracts (*E*-isorhapontigenin, *E*-gnetol, *E*-resveratrol, *E*-isorhapontigenin-4'-*O*-  
150 glucoside, *E*-gnetin C, *E*-gnemonoside A, *E*-gnemonoside C, *E*-gnemonoside D) were  
151 evaluated for their iron-chelating activity. As presented in **Figure 3**, the compound **1**  
152 significantly chelated Fe(II) at 38.5%, 61.3% and 73.1% at 100, 250 and 500  $\mu$ M,  
153 respectively. *E*-Isorhapontigenin-4'-*O*-glucoside also exhibited notable chelation at 40.6% and  
154 67.0% at 250 and 500  $\mu$ M, respectively. The other tested compounds from *G. africanum* were  
155 less active as chelators of Fe(II) giving rise values inferior to 40% at 500  $\mu$ M which was  
156 similar to the data reported for *E*-resveratrol whose concentration for 50% of chelation of  
157 Fe(II) was 746  $\mu$ M (Kantham et al., 2017). These findings may afford beneficial effects into  
158 Alzheimer's disease (AD) treatment. The physiopathological hallmark of AD is an abnormal  
159 fibrillation and accumulation of amyloid beta peptide (A $\beta$ ) forming amyloid plaques in brain.  
160 This process leads to neurotoxic effects such as cell membrane perturbation and oxidative  
161 stress among others (Hornedo-Ortega et al., 2018). Otherwise, the presence of metal binding  
162 sites and the large amount of iron in the amyloid plaque can accelerate the fibril formation  
163 modulating the morphology of A $\beta$  oligomers and improving the toxicity of A $\beta$  cores  
164 (Kantham et al., 2017). In addition, the strong presence of ferrous ions could be a source of  
165 reactive oxygen species (ROS) as Fe(II) is the catalyst of Fenton reaction arousing oxidative  
166 stress and neurotoxic effects (Ayton et al., 2017). As a consequence, the ability of compounds



167 **1** to chelate ferrous ions provides useful data for potential AD treatment. Furthermore, our  
168 findings revealed different biological activities between the two new diastereoisomer forms **1**  
169 and **2**. Even if the chemical structure is very similar, other authors have confirmed that most  
170 of isomers show notable differences in several biological domains related to pharmacology,  
171 bioavailability, toxicology, and metabolism process (Mc.Conathy and Owens, 2003; Nguyen  
172 et al., 2006). In fact, stereoisomers of miyabenol C, a trimeric stilbene, have already exhibited  
173 different inhibition on fibrillation of A $\beta$  (Papastamoulis et al., 2015). As a consequence, the  
174 chiral separation followed by the determination of absolute configuration of stereoisomers  
175 should be promoted in order to discriminate their biological activities and to provide accurate  
176 treatment.

### 177 **3. Conclusions**

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

### 188 **4. Experimental**

#### 189 **4.1. General experimental procedures.**

190 <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer  
191 and analyzed with Bruker Topspin software. Compounds were measured in 3 mm NMR  
192 tubes, using CD<sub>3</sub>OD and acetone-d<sub>6</sub> as the solvents. The specific optical rotations were

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

#### 205 **4.2. Plant material.**

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

#### 210 **4.3. Extraction and isolation.**

211 Dried and finely powdered rhizomes of *G. africanum* (600 g) were defatted twice 1.5 L of  
212 toluene. The extraction was carried out as follows: The defatted powder is moistened with  
213 60% aqueous acetone and placed in the percolator. The percolator was filled with 60%  
214 aqueous acetone and covered up. The bottom outlet was opened until get a regular dripping  
215 and then closed and left macerated for 24 h. After this time, the outlet bottom was opened and  
216 left dripping slowly. The maceration-percolation extraction was repeated 2 times and the  
217 hydroacetic extract was concentrated at 35 °C under reduced pressure. To enhance the  
218 stilbenoid content, aqueous residue was prepurified on amberlite XAD-7 (H<sub>2</sub>O and acetone),

219 using the previously published protocol to provide a semi-purified extract enriched (GA, 51.43  
220 g) (Buffeteau et al., 2014). 50 g of GA was further partitioned between EtOAc and H<sub>2</sub>O. The  
221 organic and aqueous extracts were concentrated and freeze-dried to yield 28.87 g (EtOAc,  
222 GAE) and 17.6 g (H<sub>2</sub>O, GAA), respectively.

223 **1** and **2** were purified from 1 g of GAA by preparative HPLC using a Varian Prostar 345 UV-  
224 visible detector and a binary pump with a Phenomenex Kinetex 100-5 XB-C<sub>18</sub> (5 μm, 250 ×  
225 21.4 mm) column with a security Guard. The mobile phase was composed of two solvents:  
226 (A) 0.025% TFA in H<sub>2</sub>O and (B) MeCN and the elution program at 21 mL/min was 10% B (0-  
227 2 min), 10-30% B (2-22 min), 30-38% B (22-37 min), 38-100% B (37-45 min), 100% B (45-  
228 50 min), 100-10% B (50-51 min), 10% B (51-60 min).

229 Preparative centrifugal partition chromatography separation of EtOAc extract (GAE) was  
230 performed in quaternary Arizona-L system consisting of n-heptane/ethyl  
231 acetate/methanol/water (2:3:2:3). Four Arizona solvent systems (J, K, L, and M) were tested in  
232 order to select the optimal system L. Three batches of 5 g were submitted to CPC separation in  
233 descending mode. In our experiment, the rotor was filled by injecting the organic lighter  
234 stationary phase at 50 mL/min and 500 rpm. Then, the rotation speed was increased to 1000  
235 rpm. The mobile phase was pumped into the column at 15 mL/min until the equilibrium was  
236 reached and the sample solution containing 5 g of dry GAE dissolved in 40 mL of a mixture  
237 consisting of upper and lower phase (1:1, v/v) was injected through a 50 mL loop. The CPC  
238 fractions were constituted according to the elution profile. The collected fraction were  
239 evaporated and freeze-dried. A total of six fractions numbered from Fr 1 to Fr 6 were  
240 obtained. Fr 1 (1 g) was subjected to preparative HPLC [(solvent A: 0.025% TFA in H<sub>2</sub>O and  
241 B: MeCN; the elution program at 21 mL/min was 10% B (0-2 min), 10- 20% B (2-22 min),  
242 20% B (22-35 min), 20- 40% B (35-36 min), 40% B (36-40 min), 40% B (36-40 min)] to  
243 yield: *E*-resveratrolside (41.6 mg), *E*-isorhapontigenin-4'-*O*-glucoside (18.7 mg), *E*-  
244 gnemonoside A (16.8 mg), *E*-piceid (28.5 mg), *E*-isorhapontin (12.3 mg), *E*-gnetol (10 mg),

245 *E*-gnemonoside C (107.6 mg), and *E*-gnemonoside D (34.7 mg). The purification of the Fr 5  
246 (750 mg) was performed by preparative HPLC [the same mobile phases as above were used  
247 and the elution program at 21 mL/min was 17% B (0-2 min), 17-30% B (2-22 min), 30-38% B  
248 (22-37 min), 38-100% B (37-45 min), 100% B (45-50 min), 100-17% B (50-51 min), 17% B  
249 (51-60 min)] to provide: *E*-resveratrol (157 mg), *E*-isorhapontigenin (59 mg), *E*-gnetin C (182  
250 mg), *E*-Bisisorhapontigenin B (46 mg), and *E*-gnetin E (5 mg).

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

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

#### 255 **4.4. UV-vis and ECD Measurements.**

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

#### 262 **4.5. VCD measurements.**

263 IR and VCD spectra were recorded with a ThermoNicolet Nexus 670 FTIR spectrometer  
264 equipped with a VCD optical bench (Buffeteau et al., 2005). In this optical bench, the light  
265 beam was focused by a BaF<sub>2</sub> lens (191 mm focal length) to the sample, passing an optical  
266 filter, a BaF<sub>2</sub> wire grid polarizer (Specac), and a ZnSe photoelastic modulator (Hinds  
267 Instruments, Type II/ZS50). The light was then focused by a ZnSe lens (38.1 mm focal length)  
268 onto a 1x1 mm<sup>2</sup> HgCdTe (ThermoNicolet, MCTA\* E6032) detector. IR and VCD spectra were  
269 recorded at a resolution of 4 cm<sup>-1</sup> by co-adding 50 scans and 36000 scans (12h acquisition

270 time), respectively. The sample was held in a fixed path length (100  $\mu\text{m}$ ) cell with  $\text{BaF}_2$   
271 windows. IR and VCD spectra of compounds **1** and **2** were measured in  $\text{DMSO-}d_6$  at a  
272 concentration of 50 mM. Baseline correction of the VCD spectra was performed by  
273 subtracting the VCD spectrum of  $\text{DMSO-}d_6$  solvent. In all experiments, the photoelastic  
274 modulator was adjusted for a maximum efficiency at  $1400\text{ cm}^{-1}$ . Calculations were done with  
275 the standard ThermoNicolet software, using Happ and Genzel apodization, de-Haseth phase-  
276 correction and a zero-filling factor of one. Calibration spectra were recorded using a  
277 birefringent plate ( $\text{CdSe}$ ) and a second  $\text{BaF}_2$  wire grid polarizer. Finally, the solvent absorption  
278 was subtracted out in the presented IR spectra.

#### 279 **4.6. Iron chelating activity.**

280 The ferrozine assay protocol was used to estimate the concentration of free ferrous ions by  
281 measuring the absorbance of the  $\text{Fe(II)}$ -ferrozine complex at 560 nm (Chan et al., 2016). The  
282 decrease of absorbance allowed to evaluate the binding of  $\text{Fe(II)}$  to the tested compounds. The  
283 assay was performed according to previously published protocols with slight modifications  
284 (Chan et al., 2016; Dinis et al., 1994). Tests were carried out in 96 well plates and solutions  
285 were prepared in HEPES buffer (15 mM, pH 6.8). Briefly, 50  $\mu\text{L}$  of a  $\text{Fe(II)}$  solution at 200  
286  $\mu\text{M}$  was incubated with 50  $\mu\text{L}$  of pure compounds (*E*-gnetol, *E*-isorhapontigenin, *E*-  
287 isorhapontigenin-4'-*O*-glucoside, *E*-resveratrolside, *E*-gnetin C, *E*-gnemonoside A, *E*-  
288 gnemonoside C, *E*-gnemonoside D, africanoside A and africanoside B) to reach the following  
289 final concentrations: 0, 25, 50, 100, 250, 500  $\mu\text{M}$ . To maximize the solubility of pure  
290 compounds, MeOH was used as co-solvent at final concentration of 1% (v/v). Gallic acid was  
291 used as positive control. After 30 min of incubation, ferrozine at 1 mM (50 $\mu\text{L}$ ) was added  
292 leading to the formation of purple complex. The reaction reached the equilibrium after 10 min  
293 of incubation. At this moment, absorbance at 560 nm was measured by an automated plate  
294 reader (Fluostar Omega; BMG Labtech, Offenburg, Germany). The chelation of  $\text{Fe(II)}$  values  
295 (%) 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:** <sup>1</sup>H NMR spectra of known stilbenoids in methanol-d<sub>4</sub> or acetone-  
314 d<sub>6</sub>. <sup>1</sup>H, <sup>1</sup>H-<sup>1</sup>H COSY, <sup>1</sup>H-<sup>1</sup>H 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 CD<sub>3</sub>OD.

**Figure 2.** a) Experimental VCD spectra of **1** and **2** in DMSO-*d*<sub>6</sub> solution (50 mM, 100 μm path length). b) Experimental ECD spectra of **1** and **2** in EtOH solution (7.7 10<sup>-5</sup> 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.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of compounds **1** and **2** in methanol- $d_4$  at 600 MHz for  $^1\text{H}$  and 150 MHz for  $^{13}\text{C}$ 

position	<b>1 or 2</b>	
	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$ ( <i>J</i> 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-O-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 <sub>2</sub>	3.70 <i>m</i>
6'b		3.89 <i>m</i>
4b-O-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 <sub>2</sub>	3.70 <i>m</i>
6''b		3.89 <i>m</i>