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1 **Isolation and structure elucidation of cyclopeptide alkaloids from the leaves of *Heisteria***
2 ***parvifolia***
3

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23 **Highlights**

- 24 ► Five undescribed cyclopeptide alkaloids were isolated from *Heisteria parvifolia* Sm.
25 ► Their structures were elucidated by 1D-, 2D-NMR and HR-ESI-MS analyses.
26 ► Their cytotoxicity against the chronic myeloid leukemia K562 cells was evaluated.

27

28

29 **ABSTRACT**

30 *Heisteria parvifolia* Sm. is prescribed in traditional medicine against numerous diseases in Côte
31 d'Ivoire. Due to the shortcoming in scientific knowledge of use of this species, our
32 investigations revealed five undescribed cyclopeptide alkaloids added to one known derivative
33 namely anorldianine. These compounds were elucidated by 1D- and 2D-NMR experiments and
34 comparison with literature data, and confirmed by HR-ESI-MS. Cytotoxic activity evaluation
35 of these compounds against the chronic myeloid leukemia (K565) cell line exhibited an
36 antiproliferative activity with cell growth inhibition from 13% to 46%

37

38 **Keyword:** *Heisteria parvifolia*; Olacaceae; cyclopeptide alkaloids; cytotoxic activity; chronic
39 myeloid leukemia (K565) cell line

40

41 1. Introduction

42 Cyclopeptide alkaloids are widespread and occur in several families: Asteraceae, Celastraceae,
43 Euphorbiaceae, Fabaceae, Menispermaceae, Olacaceae, Pandaceae, Rhamnaceae, Rubiaceae,
44 Sterculiaceae, and Urticaceae (El-Seedi et al. 2007, Gournelis et al. 1997, Morel et al. 2009,
45 Tan and Zhou, 2006). Previous studies have reported cyclopeptides alkaloids from *Heisteria*
46 *nitida* (El-Seedi et al. 1999, El-Seedi et al. 2005). The cyclopeptide alkaloids *sensu stricto* were
47 classified according to the number of amino acid constituents outside and the size of the
48 macrocycle (inside) as 4(13); 5(13); 4(14) and 4(15) type of alkaloids (Joullie and Richard,
49 2004, Tan and Zhou, 2006). Several activities of cyclopeptides alkaloids have been reported,
50 such as antimicrobial (Gournelis et al. 1997, Morel et al. 2005), insecticidal (Sugawara et al.
51 1996), sedative (Suh et al. 1997), and antiplasmodial activity (Suksamrarn et al. 2005).

52 The genus *Heisteria* belonging to the *Olacaceae* family comprises about 65 species in tropical
53 America and 3 in Africa; namely *Heisteria parvifolia* Sm., *Heisteria trillesiana* Pierre ex
54 Heckel, and *Heisteria zimmereri* Engl. *Heisteria parvifolia* Sm. is an evergreen shrub or small
55 tree up to 15 (-20) m tall; 40 (-60) cm in diameter (Malaisse et al. 2004). *H. parvifolia* occurs
56 from Senegal and south-western Mali eastward to the Central African Republic and southward
57 DR Congo and northern Angola; possibly also in Uganda and southern Soudan (Louppe et al.
58 2008). In Côte d'Ivoire, is locally abundant on sandy soils. Its wood is used for construction and
59 tool handles. In several areas, the fruits are eaten fresh; the small oil-rich seeds are eaten fresh,
60 roasted or cooked. The twigs are used as chew-sticks. Various *Heisteria* species are used by
61 South-American Indians or in Africa in the treatment of rheumatism, abscesses, headache,
62 throat infections, swellings, nose bleedings, pain in joints and muscles, diarrhea, hepatic
63 infection (Kvist and Holm-Nielsen, 1987, Russo, 1992, Tan and Zhou, 2006). In traditional
64 medicine in Ghana, ground roots of *H. parvifolia* are applied as enema against stomach-ache.
65 In Congo, sap from the root bark is used as dropps into the nose against migraine and into the
66 eye to treat painful, infected eyes. Stem bark is taken in Ghana, in Côte d'Ivoire and DR Congo
67 as cough medicine. In Gabon, bark is applied to circumcision wounds. In Ghana and Côte
68 d'Ivoire, leaf decoctions are taken or applied as a bath to invigorate rachitic children and to treat
69 convulsions. They are also used as analgesic and rubbed onto painful breasts of young mothers,
70 and in Sierra Leone to treat tooth-ache. In Congo, leaf decoctions are administrated against
71 asthma, costal pain, stomach pain, and menstrual problems. Ground seeds are used to stupefy
72 fish. In DR Congo, powdered bark is an ingredient in the preparation of arrow poison (Abbiw
73 1990, Burkill 1997, Malaisse et al. 2004). Chemical investigations of *Heisteria* species have
74 mainly revealed the presence of triterpenes and proanthocyanidines in *H. pallida* (Dirsch et al.

75 1992, Dirsch et al. 1993), cyclopeptide alkaloid in *H. nitida* (El-Seedi et al. 1999, El-Seedi et
 76 al. 2005), scopolamine in *H. olivae* (Cairo-Valera et al. 1977), and acetylenic fatty acids in *H.*
 77 *accuminata* (Kraus et al. 1998). Up to date, only the composition of the seeds oil of *H. parvifolia*
 78 has been reported as mainly long-chain saturated fatty acids, oleic acid and other mono and di
 79 enoic fatty acids (Malaisse et al. 2004).

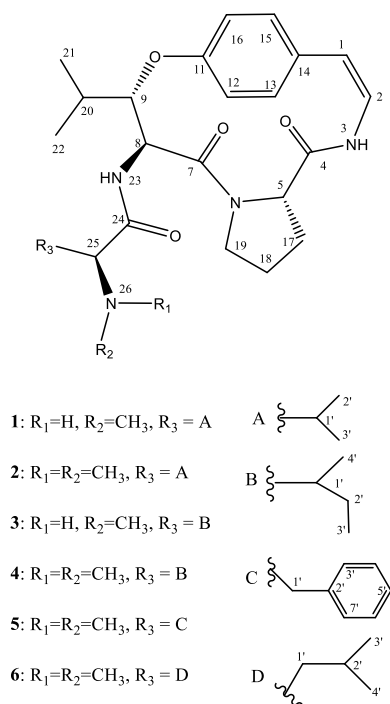
80 As a part of a continuing study for the discovery of medicinal Côte d'Ivoire species, five
 81 undescribed cyclopeptide alkaloids (**1-5**), together with one known compound (**6**), have been
 82 isolated and characterized from the leaves of *H. parvifolia*. Their cytotoxicity against the
 83 chronic myeloid leukemia K562 cells was evaluated.

84

85 2. Results and discussion

86 The crude alkaloid extract prepared with an acid-base method of air-dried and pulverized leaves
 87 of *H. parvifolia* was subjected to silica gel flash chromatography, eluted with increasingly polar
 88 mixtures of CHCl₃/MeOH. Further purification was performed using semi-preparative HPLC.
 89 As a result, five undescribed cyclopeptide alkaloids (**1-5**) were isolated and chemically
 90 characterized, together with one known cyclopeptide alkaloids, anorldianine (**6**) (El-Seedi et al.
 91 1999). Their structures (Fig. 1) were elucidated by 1D- and 2D-NMR experiments and
 92 comparison with literature data, and confirmed by HR-ESI-MS.

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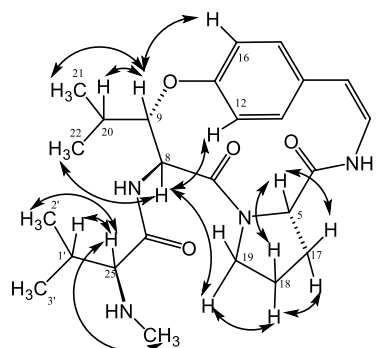
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95 Fig. 1. Chemical structures of compounds **1-6**.

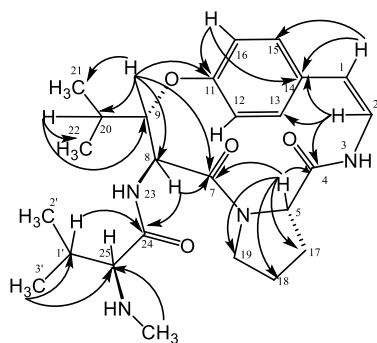
96 The UV spectra of compounds **1-5** showed absorptions at 222-224 and 274-282 nm,
97 wavelengths commonly assigned to peptide bonds and aromatic residues (Dongo et al. 1989;
98 Kang et al. 2015, Schwing et al. 2011), while their IR spectra displayed bands at 3395 and 1682
99 cm^{-1} , which are typical of amide groups (Dongo et al. 1989, Schwing et al. 2011).

100 Compound **1** was obtained as a white powder. Analysis of **1** by high-resolution
101 electrospray ionization mass spectrometry (HR-ESI-MS) identified a pseudo-molecular ion $[\text{M}$
102 $+ \text{H}]^+$ at m/z 457.2807, corresponding to the molecular formula $\text{C}_{25}\text{H}_{36}\text{N}_4\text{O}_4$ (calcd for
103 $\text{C}_{25}\text{H}_{37}\text{N}_4\text{O}_4$, 457.2815), in combination with analysis of NMR data. The ^{13}C NMR (Table 1)
104 and HSQC spectra of **1** showed 25 carbon resonances for four methyls (δ_{C} 13.9, 16.7, 17.5, and
105 19.5), one *N*-methyl (δ_{C} 31.8), three methylenes (δ_{C} 23.3, 28.2, and 46.7), twelve methines (two
106 of which were olefinic carbons at δ_{C} 116.7 and 124.9 and four were aromatic carbons sp^2 at δ_{C}
107 120.9, 121.9, 131.0, and 131.4), two quaternary aromatic carbons (δ_{C} 157.2 and 131.6), and
108 three carbonyl carbons (δ_{C} 171.1, 167.5, and 165.2). The ^1H NMR spectrum (Table 1) displayed
109 signals for two olefinic protons at δ_{H} 6.53 and 6.65, a singlet *N*-methyl (δ_{H} 2.68), four methyl
110 doublets, four aromatic protons, and several methine and methylene protons. The NMR data of
111 compound **1** (Table 1) showed great similarity with the NMR data previously reported for
112 anorldianine (compound **6**) possessing a 14-membered ring type comprising a *p*-oxygenated *Z*-
113 styrylamine group (Dongo et al. 1989, El-Seedi et al. 1999, El-Seedi et al. 2005). The presence
114 of the *p*-oxygenated *Z*-styrylamine group was indicated by two doublets at δ_{H} 6.53 and 6.65
115 (each 1H, $J = 7.7$ Hz; H-1 and H-2, respectively) corresponding to the *Z*-double bond and four
116 aromatic protons appearing as doublets of doublets with *J*-values typical for an *o,m*-coupling
117 pattern (H-12, 13, 15, and 16). The protons H-13 and H-15 (δ_{H} 7.11, dd, $J = 8.7, 2.4$ Hz) showed
118 correlation with C-1 (δ_{C} 116.7) and H-12 and H-16 (δ_{H} 7.27, dd, $J = 8.7, 2.4$ Hz) with C-14 (δ_{C}
119 131.6) in HMBC spectrum. In cyclopeptide alkaloids, the H-9 (β -H of the β -hydroxy-amino
120 acid moiety) chemical shift value (between 5.00 and 5.50 ppm) is characteristic. In this case, a
121 doublets of doublets was present at δ_{H} 4.92 ($J = 8.3, 1.5$ Hz). In the COSY spectrum, two cross
122 peaks were observed for H-9 (δ_{H} 4.92), more specifically with H-8 (δ_{H} 5.01, d, $J = 8.3$ Hz) and
123 H-20 (δ_{H} 2.13, sept, $J = 6.9$ Hz). The proton signal of the CH-group in position 20 also showed
124 cross peaks to H₃-21 (δ_{H} 1.32, d, $J = 6.7$ Hz) and H₃-22 (δ_{H} 1.05, d, $J = 6.7$ Hz). In the HMBC
125 spectrum, correlations between C-9 (δ_{C} 83.4) and H-21 and H-22 were observed (Fig. 2). These
126 data agreed with previously reported data for β -hydroxyleucine (anorldianine). The methine
127 and the methylene protons of proline were observed in the ^1H NMR spectrum: H-5 (δ_{H} 4.16,
128 dd, $J = 7.5, 1.9$ Hz), H₂-17 (δ_{H} 1.65, m; 2.21, dd, $J = 11.5-4.5$ Hz), H₂-18 (δ_{H} 1.75, m; 1.95, m),

129 and H₂-19 (δ_{H} 3.55, brt, $J = 9.8$ Hz; 3.85, m). In the COSY spectrum, cross peaks were observed
 130 between H-5/H-17, H-17/H-18, and H-18/H-19. In the HMBC spectrum, the H-5 exhibited
 131 correlations with C-19 (δ_{C} 46.7) and with the carbonyl C-4 (δ_{C} 167.5) (Fig. 2). The methyl
 132 doublets of *N*-methyl-valine (H-2' and H-3') appeared at δ_{H} 0.95 ($J = 6.7$ Hz) and 0.96 ($J = 6.7$
 133 Hz) and the methine proton (H-1') of this moiety appeared as septuplet at δ_{H} 2.13 ($J = 6.9$ Hz).
 134 The HMBC experiment showed correlations between C-24 (δ 165.2) and H-25 (δ_{H} 3.57, d, $J =$
 135 5.4 Hz) and H-1', and between the *N*-methyl carbon (δ_{C} 31.8) and the H-25 for this moiety. The
 136 combined use of 1D (¹H and ¹³C NMR) and 2D (COSY, HSQC, and HMBC) spectra allowed
 137 an unambiguous assignment of all protons and carbons of the amino acids units (β -
 138 hydroxyleucine, proline, *N*-methyl-valine residues) and the *p*-oxygenated *Z*-styrylamine group
 139 (Table 1). Moreover, the connectivity between the constitutive parts of the molecule were
 140 ascertained by the HMBC correlations between the carbonyl C-4/H-2, the carbonyl C-7/H-5,
 141 the carbonyl C-24/H-8, and C-11/H-9 (Fig. 2). Moreover, the NOE relationships H-8/H-25, H-
 142 8/ H-12 and H-9/H-16 agreed with the β -hydroxyleucine connection with the *N*-methyl-valine
 143 and the *p*-oxygenated *Z*-styrylamine moieties whereas the NOE effects between H-8/H-19
 144 agreed with the connection of β -hydroxyleucine with proline (Fig. 2). Compound **1** was named
 145 cycloheisterin A after its plant origin (Fig. 1).



↔ NOESY



↔ HMBC

146

147 Fig. 2. Selected key HMBC and NOESY interactions for compound **1**.

148 Compound **2** displayed an $[M + H]^+$ ion peak at m/z 471.2979 in the positive HR-ESI-
149 MS, corresponding to the molecular formula $C_{26}H_{38}N_4O_4$, suggesting an additional methyl
150 group compared to **1**. The NMR spectroscopic data of **2** were almost identical with those of **1**
151 except for one additional methyl group (Table 1). The detailed analysis of the 2D-NMR spectra
152 led to the identification, as in **1**, of the amino acids units (β -hydroxy-leucine, proline, and valine
153 residues) and the *p*-oxygenated *Z*-styrylamine group (Table 1). The HMBC cross-peaks of the
154 methyl signals at δ_H 2.92 (6H, s) to C-25 (δ_C 72.7) of the valine residue indicated that the
155 terminal amino acid in **2** is *N,N*-dimethyl-valine. Compound **2** was named cycloheisterin B.

156 Compound **3** displayed an $[M + Na]^+$ ion peak at m/z 493.2785 in the positive HR-ESI-
157 MS, corresponding to the molecular formula $C_{26}H_{38}N_4O_4$, suggesting an additional methylene
158 group compared to **1**. The 1H and ^{13}C NMR values of **3** were almost superimposable on those
159 of **1** (Table 1) excepting those of the *N*-methyl-valine residue in **1**. Instead, an *N*-methyl-
160 isoleucine residue was identified as summarized in Table 1 (Tuenter et al. 2017). 1H - 1H COSY
161 analysis confirmed the presence of an isoleucine residue. The HMBC cross-peak of H-8 of
162 hydroxy-leucine (δ_{H-8} 5.01) to C-24 of isoleucine residue (δ_{C-24} 165.3) and H-25 (δ_{H-25} 3.62) to
163 C-24 and the *N*-methyl carbon (δ_C 31.8) to H-25 for this moiety confirmed that the terminal
164 amino acid is *N*-methyl-isoleucine. Compound **3** was named cycloheisterin C (Fig. 1).

165 Cycloheisterin D (**4**) displayed an $[M + H]^+$ ion peak at m/z 485.3138 in the positive
166 HR-ESI-MS, corresponding to the molecular formula $C_{27}H_{40}N_4O_4$, suggesting an additional
167 methyl group compared to **3**. Comparing the NMR data of **4** with those of **3** (Table 1) and the
168 analysis of the 2D-NMR spectra led to the identification, as in **3**, of the amino acids units (β -
169 hydroxy-leucine, proline and isoleucine residues) and the *p*-oxygenated *Z*-styrylamine group
170 (Table 1). The HMBC cross-peaks of the methyl signals at δ_H 2.91 (6H, s) to C-25 (δ_C 72.2) of
171 the isoleucine residue suggested that the terminal amino acid in **4** is *N,N*-dimethyl-isoleucine.
172 Compound **4** was named cycloheisterin D (Fig. 1).

173 Cycloheisterin E (**5**) exhibited an $[M + Na]^+$ ion peak at m/z 541.2799 in the HR-ESI-
174 MS spectrum, consistent with the molecular formula of $C_{30}H_{38}N_4O_4$. Comparing the NMR data
175 of **5** with those of **1-4** (Table 1) and detailed analysis of the 2D-NMR spectra showed that it
176 had the same macrocycle (inside) composed of the amino acids units (β -hydroxy-leucine and
177 proline) and the *p*-oxygenated *Z*-styrylamine group (Table 1). The 1H and ^{13}C -NMR spectra of
178 **5** exhibited signals corresponding to an aromatic amino acid [δ_H 7.20-7.30, 5H]. Extensive 2D-
179 NMR analysis enabled the full assignments of the *N,N*-dimethyl phenylalanine. (Tuenter et al.
180 2017). The presence of the *N,N*-dimethyl groups was confirmed by the HMBC correlation

181 between the methyl signals at δ_{H} 2.97 (6H, s) and C-25 (δ_{C} 68.1) of the phenylalanine residue.
 182 The HMBC correlation between H-8 (δ_{H} 4.82) of the β -hydroxyleucine and the C-24 (δ_{C} 165.0)
 183 of the *N,N*-dimethyl phenylalanine confirmed it to be the terminal amino acid moiety.
 184 Compound **5** was named cycloheisterin E (Fig. 1).

185 **Table 1.** ^{13}C NMR spectroscopic data for compounds **1-5** (500 MHz, CD_3OD).

	1		2		3		4		5	
	δ_{H} m (J in Hz)	δ_{C}	δ_{H} m (J in Hz)	δ_{C}	δ_{H} m (J in Hz)	δ_{C}	δ_{H} m (J in Hz)	δ_{C}	δ_{H} m (J in Hz)	δ_{C}
1	6.53, d (7.7)	116.7	6.53, d (7.7)	116.8	6.53, d (7.7)	116.3	6.53, d (7.7)	116.8	6.50, d (7.7)	116.8
2	6.65, d (7.7)	124.9	6.65, d (7.7)	125.0	6.66, d (7.7)	124.9	6.65, d (7.7)	124.9	6.60, d (7.7)	125.0
4	-	167.5	-	167.5	-	167.5	-	167.4	-	167.9
5	4.16, dd (7.5, 1.9)	62.5	4.14, d (7.5)	62.6	4.15, d (7.8)	62.5	4.12, d (8.1)	62.6	3.72, d (8.0)	62.4
7	-	171.1	-	171.0	-	171.1	-	171.0	-	170.7
8	5.01, d (8.3)	53.1	5.01, d (8.5)	52.0	5.01, d (8.3)	53.1	5.01, d (8.5)	53.0	4.82, (overlapped)	53.0
9	4.92, dd (8.3, 1.5)	83.4	4.93, dd (8.5, 1.9)	83.2	4.91, dd (8.3, 1.2)	83.4	4.92, dd (8.3, 1.3)	83.2	4.82, (overlapped)	82.7
11	-	157.2	-	157.2	-	157.2	-	157.2	-	157.1
12	7.27, dd (8.7, 2.4)	120.9	7.27, d (8.5)	120.8	7.27, d (8.1)	120.9	7.27, d (8.9)	120.8	7.20, m	119.6
13	7.11, dd (8.7, 2.4)	131.4	7.11, m	131.4	7.11, m	131.4	7.11, m	131.4	7.11, dd (8.5, 1.5)	131.3
14	-	131.6	-	131.8	-	131.6	-	131.6	-	131.3
15	7.11, dd (8.7, 2.4)	131.0	7.11, m	130.3	7.11, m	130.5	7.11, m	130.3	7.12, dd (8.5, 1.5)	130.2
16	7.27, dd (8.7, 2.4)	120.9	7.27, d (8.5)	120.8	7.27, d (8.1)	120.9	7.27, d (8.9)	120.8	7.20, m	120.3
17	1.65, m	28.2	1.67, m	28.2	1.65, m	28.1	1.64, m	28.2	1.54, m	28.6
	2.21, dd (11.5, 4.5)		2.21, dd (12.3, 4.9)		2.21, dd (12.1, 5.8)		2.22, dd (12.5, 6.1)		2.01, dd (11.3, 4.8)	
18	1.75, m	23.3	1.75, m	23.3	1.75, m	23.3	1.75, m	23.3	1.67, m	23.5
	1.95, m		1.97, m		1.95, m		1.97, m		1.82, m	
19	3.55, brt (9.8)	46.7	3.55, brt (10.8)	46.7	3.55, brt (9.8)	46.7	3.58, brt (9.3)	46.8	3.42, brt (9.2)	47.0
	3.85, m		3.90, ddd (10.8, 7.1, 3.2)		3.85, m		3.85, ddd (10.1, 9.3, 7.4)		3.71, m	
20	2.13, m	28.7	2.12, dq (6.8, 1.7)	28.7	2.13, m	28.7	2.11, m	28.7	2.10, m	28.6
21	1.32, d (6.7)	19.5	1.32, d (6.8)	19.4	1.32, d (6.8)	19.5	1.32, d (6.8)	19.4	1.27, d (6.8)	19.3
22	1.05, d (6.7)	13.9	1.07, d (6.8)	14.0	1.06, d (6.8)	13.9	1.07, d (6.8)	14.0	1.02, d (6.8)	14.0
24	-	165.2	-	164.3	-	165.3	-	164.4	-	165.0
25	3.57, d (5.4)	66.7	3.59, d (5.3)	72.7	3.62, d (5.0)	66.2	3.62, d (4.8)	72.2	4.10, dd (10.4, 4.5)	68.1
R₁	<i>N</i> -CH ₃		<i>N</i> -CH ₃		<i>N</i> -CH ₃		<i>N</i> -CH ₃		<i>N</i> -CH ₃	
	2.68, s	31.8	2.92, s	40.3	2.66, s	31.8	2.91, s	39.7	2.97, s	41.1
R₂			<i>N</i> -CH ₃				<i>N</i> -CH ₃		<i>N</i> -CH ₃	
			2.92, s	41.9			2.91, s	42.2	2.97, s	41.1
R₃	Val		Val		iLeu		iLeu		Phe	
1'	2.13, m	30.2	2.45, m	27.2	1.88, m	36.8	2.16, m	33.9	3.12, dd (13.8, 10.6)	34.3
									3.40, m	
2'	0.95, d (6.7)	16.7	0.93, d (6.7)	15.1	1.02, m	25.1	0.77, m	26.3		134.1
					1.47, m		1.42, m			
3'	0.96, d (6.7)	17.5	0.97, d (6.7)	18.7	0.92, t (6.8)	10.3	0.93, t (6.9)	10.4	7.20, m	129.0
4'					0.96, d (6.8)	13.1	0.98, d (6.9)	11.5	7.30, m	128.5
5'									7.21, m	127.5
6'									7.30, m	128.5
7'									7.20, m	129.0

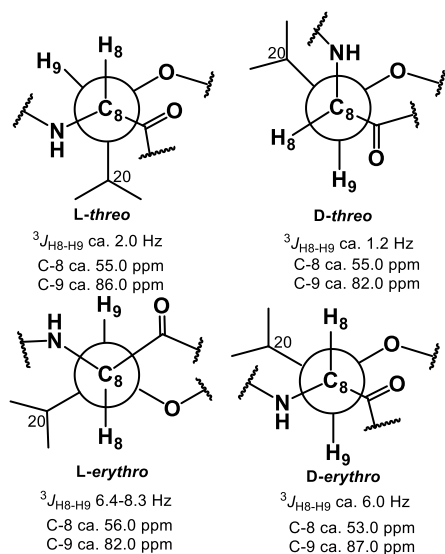
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188 The stereochemistry of the cyclopeptide alkaloids **1** - **6**, was proposed from the ¹H NMR
189 coupling constants, ¹³C NMR data, and NOESY analysis and by determining the absolute
190 configuration of the amino acids by chiral HPLC after acid hydrolysis. With this purpose,
191 compounds **1** - **6** were hydrolyzed and their amino acids analyzed through the chiral HPLC. In
192 cycloheisterin A-E and **6**, proline has the L configuration and *N*-methyl-valine, *N,N*-dimethyl-
193 valine, *N*-methyl-isoleucine, *N,N*-dimethyl-isoleucine, and *N,N*-dimethyl phenylalanine in
194 cycloheisterin A-E, respectively and *N,N*-dimethyl-leucine in **6** were in the L form. The ¹³C-
195 NMR chemical shift values of the α -amino acid of the macrocycle (proline in all five alkaloids)
196 and the terminal units (*N*-methyl-valine in **1**, *N,N*-dimethyl-valine in **2**, *N*-methyl-isoleucine in
197 **3**, *N,N*-dimethyl-isoleucine in **4**, *N,N*-dimethyl-phenylalanine in **5**, and *N,N*-dimethyl-leucine
198 in **6**) match well with those previously reported for similar compounds and was in agreement
199 with the fact that the majority of plant cyclopeptides are composed of L-amino acids(El-Seedi
200 et al. 2005, Kang et al. 2015, Maldaner et al. 2011, Medina et al. 2016, Suksamrarn et al. 2005,
201 Tuenter et al. 2017).

202 The configuration of the β -hydroxyleucine was established based on the available NMR data.
203 In the case of the *erythro* form, $J_{\alpha,\beta}$ ca. 8.0 Hz, whereas for *threo* compounds $J_{\alpha,\beta}$ ca. 2.0 Hz
204 (Fig. 3) (Dias et al. 2007, Gournelis et al. 1997, Mostardeiro et al. 2013, Tuenter et al. 2016).
205 The coupling constant of the doublet corresponding to H-9 ($J_{\alpha,\beta}$) of compounds **1** – **5** ca. 8.3
206 Hz, clearly indicative of an *erythro* configuration. ¹³C NMR spectroscopy is used for the
207 elucidation of the absolute configuration of the β -hydroxy amino acids. For both *L-threo* and *D-*
208 *threo* series, the signal of the α carbon appears at ca. δ_C 55.0, whereas for the β carbon, its signal
209 appears at ca. δ_C 82.0 for the *D-threo* and ca. δ_C 86.0 for the *L-threo* (Fig. 3) (Mostardeiro et al.
210 2013). For the *L-erythro* series, the signal of the α carbon (C-8) appears at ca. δ_C 55.0, whereas
211 for the *D-erythro* it appears at ca. δ_C 53.0. Important information is also observed for the β
212 carbon (C-9): in the *L-erythro* series, the signal appears at ca. δ_C 81.5, whereas for the *D-erythro*
213 configuration it appears at ca. δ_C 87.0 (Abu-Zarga et al. 1995, Caro et al. 2012, Dongo et al.
214 1989, Gournelis et al. 1997, Medina et al. 2016, Mostardeiro et al. 2013, Tuenter et al. 2016).
215 These data show that the chemical shift of the β carbon is most indicative for the L and D forms
216 of a β -hydroxy amino acids ($\Delta\delta$ 4 - 5 ppm) than α carbon ($\Delta\delta$ 0 - 3 ppm). The chemical shift of
217 C-9 in compounds **1** – **5** was around δ_{C-9} 83.3, clearly suggestive for the *L-erythro* form, whereas
218 the chemical shift of C-8 was around δ_C 53.0. Furthermore, the *J* value of the ¹H NMR signal
219 attributed to the methyl group at position C-22 was 6.7 Hz, indicative for a
220 pseudoaxial/equatorial coupling, typical for *L-erythro-β*-hydroxyleucine (Abu-Zarga et al.

221 1995, Gournelis et al. 1997, Tuenter et al. 2016). In addition, the cross-peak observed in the
 222 NOESY spectra of **1** - **5** between H-9 and H-20, H-9/H-21 and H-8/H-22 and the lack of the
 223 NOESY interaction between H-9 and H-8, suggests the *L-erythro* configuration for the β -
 224 hydroxyleucine moiety (Fig. 2). Furthermore, the NOESY effect observed between H-25 and
 225 H-1' indicated that these protons are co-facially oriented.



227 Fig. 3. Representatives and approximates NMR data for *threo* and *erythro* β -hydroxyleucine in cyclopeptide
 228 alkaloids.

229

230 3. Conclusion

231 In summary, six compounds were isolated from the crude alkaloid extract of *H. parvifolia*
 232 leaves, among them five previously undescribed cyclopeptide alkaloids from the 4(14) type, 4
 233 amino acid constituents outside and the 14-atoms of the macrocycle (inside). Their structures
 234 were established by different spectroscopic methods including 1D- and 2D-NMR experiments
 235 as well as HR-ESI-MS analysis. Compound **6** (anorldianine) that has a unique substructure
 236 containing proline, was previously isolated from *Heisteria nitida* (El-Seedi et al. 1999).
 237 Compounds **1-5** were derivatives of anorldianine and differed in only the terminal amino acid
 238 which was *N*-methyl-valine in **1**, *N,N*-dimethyl-valine in **2**, *N*-methyl-isoleucine in **3**, *N,N*-
 239 dimethyl-isoleucine in **4**, and *N,N*-dimethyl-phenylalanine in **5**. Cyclopeptide alkaloids have
 240 only been reported from a few families of the plant kingdom, in fact, they seem to be quite rare
 241 and present in small quantities. This kind of cyclopeptide alkaloids was isolated only in
 242 *Canthium anorldianum* (Rubiaceae) and *Heisteria nitida* (Olacaceae). Further phytochemical
 243 investigation on *Heisteria* species are needed to verify wether anorldianine derivative
 244 cyclopeptide alkaloids could be considered as a taxonomic markers for the genus *Heisteria*. The

245 cytotoxic activity of compounds **1-6** against the chronic myeloid leukemia (K562) cell line was
246 evaluated. Only compounds **2**, **4** and **6** exhibited an antiproliferative activity at the
247 concentration 100 μ M with cell growth inhibition of 46%, 44%, and 43%, respectively, whereas
248 compounds **1**, **3**, and **5** showed cell growth inhibition of 13%, 19%, and 36%, respectively at
249 the same concentration.

250 **4. Experimental**

251 *4.1. General experimental procedures*

252 Optical rotations were measured on a Perkin Elmer model 341 polarimeter (589 nm, 20 °C). IR
253 spectra were obtained on a Nicolet Avatar 320 FT-IR spectrometer with KBr disks. NMR
254 spectra were acquired in CD₃OD on Bruker Avance DRX III 500 instruments (¹H at 500 MHz
255 and ¹³C at 125 MHz). Standard pulse sequences and parameters were used to obtain 1D- (¹H
256 and ¹³C) and 2D- (COSY, ROESY, HSQC and HMBC) NMR spectra. HR-ESI-MS experiments
257 were performed using a Micromass Q-TOF high-resolution mass spectrometer (Manchester,
258 UK). Mass spectra were recorded in the positive-ion mode in the range *m/z* 100–2000, with a
259 mass resolution of 20000 and an acceleration voltage of 0.7 kV. Flash chromatography was
260 conducted on a Grace Reveleris system equipped with dual UV and ELSD detection using
261 Grace® cartridges (Silica gel or RP-18). A prepacked RP-C₁₈ column (Phenomenex 250 x 15
262 mm, Luna 5 μ) was used for semi-preparative HPLC. The eluting mobile phase consisted of
263 H₂O with TFA (0.0025%) and CH₃CN with a flow rate of 5 mL/min and the chromatogram was
264 monitored at 210, 250, 270, and 300 nm. TLC was performed on precoated silica gel 60 F₂₅₄
265 Merck and compounds were visualized by spraying the dried plates with Dragendorff's reagent.

266 *4.2. Plant material*

267 The leaves of *Heisteria parvifolia* Sm. were collected in Agboville forest in August 2016. They
268 are identified by Pr. Akke Assi in the national center florestic of Félix Houphouët-Boigny
269 University of Côte d'Ivoire (Ake assi 11049).

270 *4.3. Extraction and isolation*

271 The dried powdered leaves of *H. parvifolia* (1 kg) were wetted with 50% aq. NH₄OH (500 mL),
272 macerated overnight and then percolated with 15 L of EtOAc. The organic solvent was
273 concentrated under reduced pressure. The crude extract (26 g) was suspended in 2 L of EtOAc
274 and extracted with an aqueous 2% H₂SO₄ solution (3 x 2 L). The acid phase was made alkaline
275 with aqueous NH₃ and extracted with 3 x 2 L of CHCl₃. The CHCl₃ solution was washed with
276 H₂O (2 L), dried (Na₂SO₄) and evaporated *in vacuo* to give 500 mg of crude alkaloid extract
277 (yield 0.05%). The crude alkaloid extract was subjected to silica gel flash chromatography

278 eluted with increasingly polar CHCl₃/MeOH (100:00-95:05) for 25 min, to yield 26 fractions
279 (F1-26). Fractions F6, F8, F10, F12, F14 and F17 were subjected separately to semipreparative
280 HPLC RP-18 chromatography, by eluting with an isocratic gradient (28% CH₃CN). Compound
281 **4** (*t_R* 13.2 min, 31 mg) was obtained from fractions F6 and F8, compound **5** (*t_R* 14.9 min, 4 mg)
282 from fraction F10, compound **6** (*t_R* 10.6 min, 6 mg) from fraction F12, compounds **2** (*t_R* 14.6
283 min, 6 mg) and **3** (*t_R* 17.3 min, 4 mg) from fraction F14, and compound **1** (*t_R* 11.3 min, 5 mg)
284 from fraction F17.

285 4.3.1. Cycloheisterin A (**1**)

286 White amorphous powder; $[\alpha]_D^{20} = -148$ (*c* 0.5; MeOH); UV (MeOH) λ_{\max} (abs.) 222 (1.66),
287 274 (0.33); IR ν_{\max} 3395, 2972, 1682, 1508, 1205, 1133, 984, 720; ¹H and ¹³C NMR, see Table
288 1; HR-ESI-MS (positive ion mode) *m/z* 457.2807 [M + H]⁺ (calcd for C₂₅H₃₇N₄O₄, 457.2815).

289 4.3.2. Cycloheisterin B (**2**)

290 White amorphous powder; $[\alpha]_D^{20} = -187$ (*c* 0.52; MeOH); UV (MeOH) λ_{\max} (abs.) 222 (0.10),
291 282 (0.01); IR ν_{\max} 3439, 2969, 1681, 1508, 1204, 1136, 700; ¹H and ¹³C NMR, see Table 1;
292 HR-ESI-MS (positive ion mode) *m/z* 471.2979 [M + H]⁺ (calcd for C₂₆H₃₉N₄O₄, 471.2971).

293 4.3.3. Cycloheisterin C (**3**)

294 White amorphous powder; $[\alpha]_D^{20} = -135$ (*c* 0.31; MeOH); UV (MeOH) λ_{\max} (abs.) 224 (1.38),
295 276 (0.37); IR ν_{\max} 3388, 2965, 1686, 1506, 1206, 1133, 985, 719; ¹H and ¹³C NMR, see Table
296 1; HR-ESI-MS (positive ion mode) *m/z* 493.2785 [M + Na]⁺ (calcd for C₂₆H₃₈N₄O₄Na,
297 493.2791).

298 4.3.4. Cycloheisterin D (**4**)

299 White amorphous powder; $[\alpha]_D^{20} = -179$ (*c* 0.23; MeOH); UV (MeOH) λ_{\max} (abs.) 222 (3.21),
300 280 (0.3); IR ν_{\max} 3395, 2972, 1682, 1508, 1205, 1133, 720; ¹H and ¹³C NMR, see Table 1; HR-
301 ESI-MS (positive ion mode) *m/z* 485.3138 [M + H]⁺ (calcd for C₂₇H₄₁N₄O₄, 485.3128).

302 4.3.5. Cycloheisterin E (**5**)

303 White amorphous powder; $[\alpha]_D^{20} = -91$ (*c* 0.41; MeOH); UV (MeOH) λ_{\max} (abs.) 222 (0.91),
304 274 (0.5); IR ν_{\max} 3439, 2969, 1681, 1508, 1204, 1136, 700; ¹H and ¹³C NMR, see Table 1; HR-
305 ESI-MS (positive ion mode) *m/z* 541.2799 [M + Na]⁺ (calcd for C₃₀H₃₈N₄O₄Na, 541.2791).

306 4.4. General procedure for determination of amino acid configurations.

307 The absolute configurations of amino acids were determined by chiral HPLC after acid
308 hydrolysis according to literature ([Mostardeiro et al. 2013](#), [Siva et al. 1996](#), [Wang et al. 2017](#)).
309 Briefly, each solution of **1-5** (0.5 mg) in 6 N HCl (0.4 mL) was heated at 110 °C for 24 h and
310 then concentrated to dryness. The residue was dissolved in H₂O (200 μL) to obtain the test

311 solution, 10 μ L of which was injected into chiral HPLC system with a Chiralpak IC column
312 (250 mm \times 4.6 mm I.D., 5 μ m) maintained at 35 $^{\circ}$ C and detected at 254 nm. : Isopropanol/*n*-
313 hexane (90:10, v/v) containing 0.1% TFA was used as the mobile phase at a flow rate of 0.8
314 mL/min.

315 **5. Cytotoxicity bioassay by MTS**

316 K562 cells (chronic myeloid leukemia) were trypsinized, harvested, and spread onto 96-well
317 flat-bottom plates at a density of 1000 cells per well, and then incubated for 24 h in RPMI 1640
318 Medium supplemented with 10% fetal bovine serum and antibiotics. After culture, the cells
319 were treated with compounds **1-6** for 72 h. The cell cultures were then analyzed using 3-(4,5-
320 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner
321 salt (MTS) according to the manufacturer's instructions (Promega Corporation, Charbonnières,
322 France). Camptothecin was used as positive control. MTS is bio-reduced by cells into a colored
323 formazan product. Absorbance was analyzed at a wavelength of 540 nm with a Multiskan Ex
324 microplate absorbance reader (Thermo Scientific, Paris, France). Percentage of cell growth was
325 calculated as 100% \times (absorbance of the treated cells) / (absorbance of the negative control
326 cells). Control cells were treated with complete culture medium containing 0.2% DMSO. The
327 values represent averages of three independent experiments.

328 **Supporting Information**

329 HR-ESI-MS and 1D- and 2D-NMR spectra of compounds **1-5**.

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336 **References**

- 337 Abbiw, D., 1990. Useful Plants of Ghana: West African Uses of Wild and Cultivated Plants.
338 London: Intermediate Technology Publications, Royal Botanic Gardens, Kew.
- 339 Abu-Zarga, M., Sabri, M., Al-Aboudi, A., 1995. New cyclopeptide alkaloids from *Ziziphus*
340 *lotus*. *J. Nat. Prod.* 58, 504-511.
- 341 Burkill, H.M., 1997. The useful plants of West Tropical Africa. Edition 2, Vol. 4, families M-
342 R, 284-285. Kew Royal Botanic Gardens.

343 Cairo-Valera, G., De Budowski, J., Delle-Monache, F., Marini-Bettolo, G.B., 1977. A new
344 psychoactive drug : *Heisteria olivae* (Olacaceae). Atti Accad. Naz. Lincei, Cl. Sci. Fis. Mat.
345 Nat. Rend. 62, 363-364.

346 Caro, M.S., de Oliveira, L.H., Ilha, V., Burrow, R.A., Dalcol, I.I., Morel, A.F., 2012. Absolute
347 configuration of franganine. J. Nat. Prod. 75, 1220-1222.

348 Dias, G.C., Gressler, V., Hoenzel, S.C., Silva, U.F., Dalcol, I.I., Morel, A.F., 2007. Constituents
349 of the roots of *Melochia chamaedrys*. Phytochemistry 68, 668-672.

350 Dirsch, V., Wiemann, W., Wagner, H., 1992. Anti-inflammatory activity of triterpene quinone-
351 methides and proanthocyanidins from the stem bark of *Heisteria pallida* Engl. Pharm.
352 Pharmacol. Lett., 2, 184-186.

353 Dirsch, V., Neszmélyi, A., Wagner, H., 1993. A trimeric propelargonidin from stem bark of
354 *Heisteria pallida*. Phytochemistry 34, 291-293.

355 Dongo, E., Ayafor, J.F., Sondengam, B.L.; Connolly, J.D., 1989. A new peptide alkaloid from
356 *Canthium anorldianum*. J. Nat. Prod. 52, 840-843.

357 El-Seedi, H.R., Gohil, S., Perera, P., Torssell, K.B.G., Bohlin, L., 1999. Cyclopeptide alkaloids
358 from *Heisteria nitida*. Phytochemistry 52, 1739-1744.

359 El-Seedi, H.R., Larson, S., Backlund, A., 2005. Chemosystematic value of cyclopeptide
360 alkaloids from *Heisteria nitida* (Olacaceae). Biochem. Syst. Ecol. 33, 831-839.

361 El-Seedi, H.R., Zahra, M.H., Göransson, U., Verpoorte, R., 2007. Cyclopeptide alkaloids.
362 Phytochem. Rev. 6, 143-165.

363 Gournelis, D.C., Laskaris, G.G., Verpoorte, R., 1997. Cyclopeptides alkaloids. Nat. Prod. Rep.
364 14, 75-82.

365 Joullie, M.M., Richard, D.J., 2004. Cyclopeptide alkaloids: chemistry and biology. Chem.
366 Comm. 18, 2011-2015.

367 Kang, K.B., Ming, G., Kim, G.J., Ha, T.K., Choi, H, Oh, W.K., Sung, S.H., 2015. Jubanines F-
368 J, cyclopeptide alkaloids from the roots of *Ziziphus jujuba*. Phytochemistry 119, 90-95.

369 Kraus, C.M., Neszmélyi, A., Holly, S., Wiedemann, B., Nenninger, A., Torssell, K.B., Bohlin,
370 L., Wagner, H., 1998. New acetylenes isolated from the bark of *Heisteria acuminata*. J. Nat.
371 Prod. 61, 422-427.

372 Kvist, L.P., Holm-Nielsen, L.B., 1987. Ethnobotanical aspects of lowland Ecuador. Opera
373 Botanica, 92, 83-107.

374 Louppe, D., Oteng-Amoako, A.A., Brink, M., in Timbers, Plant Resources of Tropical Africa
375 (Series), 7(1), pp: 385-386. Ed. Wageningen: PROTA Foundation: Backhuys, 2008.

376 Malaisse, F., N'Gasse, G., Lognay, G., 2004. *Heisteria parvifolia* (Olacaceae), an
377 Underestimated shrub or small tree with Oil producing seeds. *Syst. Geogr. Plants.* 74, 7-25.
378 Maldaner, G., Marangon, P., Ilha, V., Caro, M.S.P., Burrow, R.A., Dalcol, I.I., Morel, A.F.,
379 2011. Cyclopeptide alkaloids from *Scutia buxifolia* Reiss. *Phytochemistry* 72, 804-809.
380 Medina, R.P., Schuquel, I.T.A., Pomini, A.M., Silva, C.C., Oliveira, C.M.A., Kato, L.,
381 Nakamura, C.V., Santin, S.M.O., 2016. Ixorine, a New Cyclopeptide Alkaloid from the
382 Branches of *Ixora brevifolia*. *J. Braz. Chem. Soc.* 27, 753-758.
383 Morel, A.F., Maldaner, G., Ilha, V., Missau, F., Silva, U.F., Dalcol, I.I., 2005. Cyclopeptide
384 alkaloids from *Scutia buxifolia* Reiss and their antimicrobial activity. *Phytochemistry* 66,
385 2571-2576.
386 Morel, A.F., Maldaner, G., Ilha, V., 2009. Cyclopeptide Alkaloids from higher plants.
387 Alkaloids. *Chem. Biol.* 67, 79-141.
388 Mostardeiro, M.A., Ilha, V., dahmer, J., Caro, M.S.B., Dalcol, I.I., Da Silva, U.F., Morel, A.F.,
389 2013. Cyclopeptide alkaloids: Stereochemistry and synthesis of the precursors of discarines
390 C and D and myrianthine A. *J. Nat. Prod.* 76, 1343-1350.
391 Russo, E.B., 1992. Headache treatments by native peoples of the Ecuadorian Amazon: a
392 preliminary cross-disciplinary assessment. *J. Ethnopharmacol.* 36, 193-206.
393 Schwing, K., Reyheller, C., Schaly, A., Kubik, S., Gerhards, M., 2011. Structural analysis of
394 an isolated cyclic tetrapeptide and its monohydrate by combined IR/UV spectroscopy.
395 *Chemphyschem.* 121981-1988.
396 Silva, U.F., Cardoso, C.D., Zanatta, N., Icheln, D., Gehrcke, B., Morel, A.F., 1996.
397 Determination of the stereochemistry of the *N,N*-dimethyl amino acid and the α -amino acid
398 residue of peptide alkaloids by chiral gas chromatography. *Phytochem. Anal.* 7, 20-23.
399 Sugawara, F., Ishimoto, M., Le Van, N., Koshino, H., Uzawa, J., Yoshida, S., Kitamura, K.,
400 1996. Insecticidal peptide from mung bean: a resistant factor against infestation with azuki
401 bean weevil. *J. Agric. Food Chem.* 44, 3360-3364.
402 Suh, D.Y., Kim, Y.C., Kang, Y.H., Han, B.H., 1997. Metabolic cleavage of frangufoline in
403 rodents: *In Vitro* and *in Vivo* Study. *J. Nat. Prod.* 60, 265-269.
404 Suksamrarn, S., Suwannapoch, N., Aunchai, N., Kuno, M., Ratananukul, P., Haritakun, R.,
405 Jansakul, C., Ruchirawat, S., 2005. Ziziphine N, O, P and Q, new antiplasmodial
406 cyclopeptide alkaloids from *Ziziphus oenoplia* var. *brunoniana*. *Tetrahedron* 61, 1175-1180.
407 Tan, N.-H., Zhou, J., 2006. Plant cyclopeptides. *Chem. Rev.* 106, 840-895.
408 Tuenter, E., Exarchou, V., Baldé, A., Cos, P., Maes, L., Apers, S., Pieters, L., 2016.
409 Cyclopeptide alkaloids from *Hymenocardia acida*. *J. Nat. Prod.* 79, 1746-51.

- 410 Tuenter, E., Foubert, K., Staerk, D., Apers, S., Pieters, L., 2017. Isolation and structure
411 elucidation of cyclopeptide alkaloids from *Ziziphus nummularia* and *Ziziphus spina-christi*
412 by HPLC-DAD-MS and HPLC-PDA-(HRMS)-SPE-NMR. *Phytochemistry* 138, 163-169.
- 413 Wang, X., Wu, H., Luo, R., Xia, D., Jiang Z., Han, H., 2017. Separation and detection of free D- and
414 L-amino acids in tea by off-line two-dimensional liquid chromatography. *Anal. Methods* 9, 6131-
415 6138.