New Components Including Cyclopeptides from Barks of *Christiana africana* DC. (Tiliaceae)

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Phytochemical investigation of barks of *Christiana africana* led to the identification of cyclopeptide alkaloids, flavonoids, coumarinolignans, iridoids, sesquiterpenoids, and triterpenes. This plant was classified so far in the Tiliaceae family. This study was started while the genomic study of numerous specimens was described in order to establish new criteria for Malvales botanical classification. In the present work, twenty components were identified, belonging to the three major classes of secondary metabolites: alkaloids, phenols, and terpenes. In the first class, cyclopeptides are well-known compounds in Rhamnaceae and Sterculiaceae. Their presence in Malvaceae (in APG2 sensus) suggests a possible chemical link between the ex-Tiliaceae and the Malvaceae.

Introduction. – *Christiana africana* DC. is a tropical tree well-known in Cameroon for its use in folk medicine for hypertension treatment. It was classified by *Hutchinson* [1], *Cronquist* [2], and *Judd* and *Manchester* [3] in Malvales order, and Tiliaceae family. To establish new criteria for Malvales botanical classification, two genomic studies of numerous specimens were described in 1999 [4][5]. Eleven families were studied including the Malvaceae. According to the genomic profile, most of the Tiliaceae could join this family, while Tilioidae (ex-Tiliaceae) were composed of the genus *Tilia* and *Craigia*. Thus in the APG2 classification, the Malvaceae included ten sub-families, among them the Sterculioideae (ex-Sterculiaceae) and the Brownlowioidae. *C. africana* belongs to the latter, together with eight other genuses [6]. But until now, no chemical study had been published on *Christiana sp.*

In our course in studying the chemistry of natural products and to better understand biodiversity of the plant kingdom, it seemed valuable to investigate the chemical composition of this species in order to access new bioactive compounds, but also to see if the chemical profile presents a good phenotype expression regarding the APG2 classification.

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Results and Discussion. – This report deals with the isolation and identification of cyclopeptide alkaloids (frangulanin and melonovin), phenylpropanoid (aviculin), coumarinolignan (cleomiscosin A), iridoids (loganin, deoxyloganin, geniposide, dihydrogeniposide), chromone, sesquiterpenoids, and triterpenes from the barks of *C. africana*. Cyclopeptide alkaloids could be surprising in Tiliaceae, but not in Malvaceae. Indeed, in the same order (Malvales), the Rhamnaceae are well-known for containing these phytoconstituents. Thus, their presence would be discussed in term of interest as a first chemical link in the new classification.

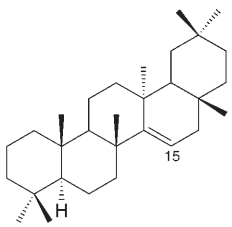
From different crude extracts, the purified compounds were identified on the basis of their spectroscopic data and comparison with the literature. Together with the known components, five new compounds were also identified: three sesquiterpenoids, a chromone, and a triterpene. Two different procedures were realized in order to obtain *a*) crude extracts of increasing polarity and *b*) a crude alkaloid extract.

1. *Structure Elucidation.* From the hexane extract, the four triterpenes and two sterols were identified as taraxerol (**1**) [7][8], lupeol, α- and β-amyrin [9], β-sitosterol, and stigmasterol [10], respectively.

From the MeOH extract, nine compounds were identified as two cyclopeptide alkaloids (frangulanin (**2**) [11][12] and melonovin (**3**) [13]), four iridoids (loganin [14], geniposide [15], dihydrogeniposide [16][17], deoxyloganin (**4**) [18]), three phenolic derivatives (scopoletol [10][19], aviculin (**5**) [20], and cleomiscosin A (**6**) [21–23]).

From the CH_2Cl_2/MeOH (1:1) extract, six of these known compounds were also identified (frangulanin (**2**) [11][12], melonovin (**3**) [13], and **4**–**6**), together with three new sesquiterpenoids (*i.e.*, **7**–**9**) and a new triterpene **10**.

Analysis of the EI-MS of **7** revealed a pseudo-molecular ion at *m*/*z* 232 (M^+) corresponding to the molecular formula $C_{15}H_{20}O_2$. ^{1}H- and ^{13}C-NMR spectra showed signals for four Me, two CH_2, and four CH groups, as well as five quaternary C-atoms (see *Table 1*). The ^{1}H,^{1}H-COSY exhibited two spin systems with, on one hand, two Me groups at δ(H) 1.70 (*d*, J = 6.8, H–C(13)) and 1.15 (*d*, J = 7.2, H–C(12)), and a CH_2 group at 3.00 (br. *hept*, J = 6.9, H–C(11)), which indicated an ^{i}Pr group. The second spin system included nine H-atoms. A CH_2 group at δ(H) 2.68 (*dd*, J = 6.8, 18.0, H–C(2))/2.26 (*dd*, J = 2.0, 18.0, H–C(2′)) was coupled to a CH group at 2.57–2.63 (*m*, H–C(1)), which showed a cross-peak with H–C(10) at 1.82–1.85 (*m*). H–C(10) was correlated with a Me group at 1.19 (*d*, J = 6.4, H–C(15)) and a CH_2 group at 2.93 (*dd*, J = 4.8, 12.0, H–C(9))/2.44 (*dd*, J = 4.0, 12.0, H–C(9′)). Finally, long-range couplings were observed between an olefinic H-atom (6.89, br. *s*, H–C(6)) and H–C(11) on one side, and a Me group at 1.88 (*d*, J = 1.6, Me(14)), on the other side. The ^{13}C-NMR spectrum showed 15 C-atoms, thereof two CO groups at δ(C) 204.3 (C(8))/208.0 (C(3)), three quaternary C-atoms at 164.0 (C(5))/155.3 (C(7))/139.5 (C(4)), four CH groups at 126.7 (C(6))/47.3 (C(1))/36.6 (C(10))/31.4 (C(11)), two CH_2 groups at 41.9 (C(2))/51.6 (C(9)), and four Me groups at 8.62 (C(14))/22.1 (C(15))/21.5 (C(13))/21.3 (C(12)). Examination of the results of the ^{1}H,^{1}H-COSY, HMQC and HMBC experiments (see *Table 1* and *Fig.*) allowed the identification of two substructures: O=C–C(Me)=C–CH=C(^{i}Pr)–C=O, and O=C–CH_2–CH–CH(Me)–CH_2–C=O. As H–C(2/2′) showed cross-peaks with C(5)/C(3)/C(1), H–C(9/9′)/H–C(6) with C(8)/C(1), and finally H–C(14) with C(5)/C(3), *i*) C(5) was connected to C(1), and *ii*) C(3) and C(8) were common to both substructures. Altogether, these data were



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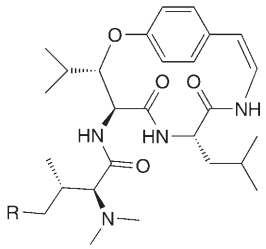
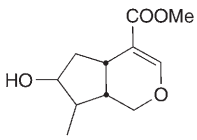


2 R = H
3 R = Me



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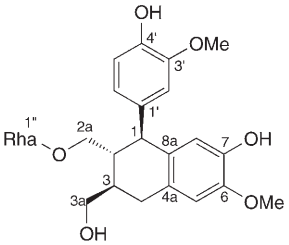

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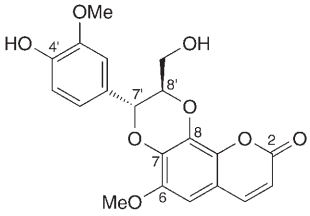

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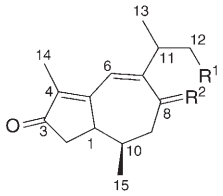
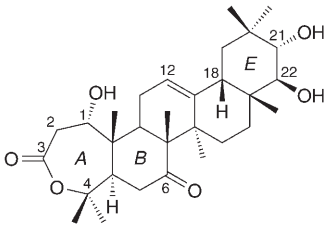
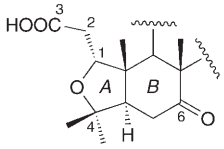


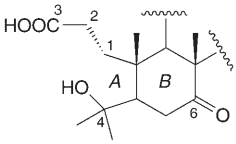
7 R^1 = H, R^2 = O
8 R^1 = H, R^2 = OH, H
9 R^1 = OH, R^2 = H, H



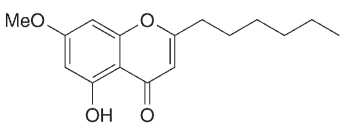
10a



10b



10c



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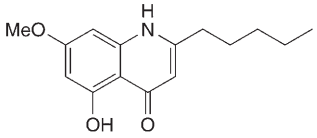


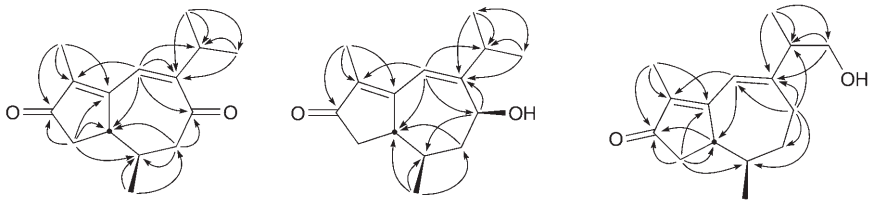
11a

used to identify **7** as 3,3a,4,5-tetrahydro-1,4-dimethyl-7-(1-methylethyl)azulene-2,6-dione or (1β,10αH)-guaia-4,6-dien-3,8-dione. The relative configuration of C(1) and C(10) was established by means of the coupling constants between H–C(2)/H–C(2′)/H–C(1)/H–C(10), together with NOESY data. The NOESY experiment exhibited NOE effects between H–C(15) and H–C(1)/H–C(9′)/H–C(2′), which showed that they are placed on the same side of the molecule. This deduction was supported by the large coupling constants J(1,2) (6.8 Hz) and J(1,10) (9.6 Hz), suggesting that H–C(1) and H–C(10) are *trans* to each other [24]. NOE cross-peaks were also observed

Table 1. *^{1}H- and ^{13}C-NMR Data of* **7**–**9**. At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

	7		**8**		**9**	
	δ(H)	δ(C)	δ(H)[a])	δ(C)[a])	δ(H)	δ(C)
1	2.57–2.63 (*m*)	47.3	2.50–2.60 (*m*)	49.0	2.58–2.72 (*m*)	47.0
2	2.68 (*dd*, *J* = 6.8, 18.0)	41.9	2.65 (*dd*, *J* = 6.0, 18.4)	41.4	2.57 (*dd*, *J* = 6.5, 18.0)	42.5
2′	2.26 (*dd*, *J* = 2.0, 18.0)		2.19 (*dd*, *J* = 2.4, 18.4)		2.12 (*dd*, *J* = 1.4, 17.8)	
3	–	208.0	–	204.5	–	206.4
4	–	139.5	–	135.7	–	135.9
5	–	164.0	–	169.6	–	168.3
6	6.89 (br. *s*)	126.7	6.38 (br. *s*)	118.4	6.40 (br. *s*)	123.0
7	–	155.3	–	161.9	–	157.8
8	–	204.3	4.50 (*dd*, *J* = 1.2, 7.6)	67.9	2.19 (*dd*, *J* = 8.5, 17.0)[b])	26.5
8′	–		–		2.45 (*dd*, *J* = 7.4, 17.5)[b])	
9	2.93 (*dd*, *J* = 4.8, 12.0)	51.6	2.10 (*ddd*, *J* = 4.0, 7.6, 14.0)	44.3	1.80–1.90 (*m*)	
9′	2.44 (*dd*, *J* = 4.0, 12.0)		1.75 (*ddd*, *J* = 1.6, 8.4, 14.0)		1.50–1.70 (*m*)	35.6
10	1.82–1.85 (*m*)	36.6	1.88–1.92 (*m*)	33.3	1.50–1.70 (*m*)	39.2
11	3.00 (br. *hept*, *J* = 6.9)	31.4	2.77 (br. *hept*, *J* = 6.8)	34.7	2.58–2.72 (*m*)	47.4
12	1.15, (*d*, *J* = 7.2)	21.3	1.16 (*d*, *J* = 6.8)	21.2	3.59–3.70 (*m*)/	66.1
					3.21 (*dd*, *J* = 6.1, 10.5)[b])	
12′	–		–		3.59–3.70 (*m*)/	
					3.29 (*dd*, *J* = 7.8, 10.7)[b])	
13	1.70 (*d*, *J* = 6.8)	21.5	1.19 (*d*, *J* = 6.8)	21.3	1.08 (*d*, *J* = 6.6)	16.0
14	1.88 (*d*, *J* = 1.6)	8.62	1.72 (*d*, *J* = 1.6)	6.7	1.77 (br. *s*)	8.6
15	1.19 (*d*, *J* = 6.4)	22.1	1.12 (*d*, *J* = 6.4)	20.9	1.04 (*d*, *J* = 6.5)	22.3

[a]) Data in $CDCl_3$ plus three drops of CD_3OD. [b]) Data in (D_5)pyridine.



7 **8** **9**

Figure. *HMBC Correlations of* **7**–**9**

between H–C(6) and H–C(13)/H–C(14), confirming that these groups were on the same side of the molecule.

The ^{1}H-NMR spectrum of **8** was compared to that of **7**. A double *doublet* was observed at δ 4.50 (J = 1.2, 7.6, 1 H, H–C(8)). In the COSY spectrum, cross-peaks of H–C(8) with two H-atoms of a CH_2 group at 2.10 and 1.75 (*ddd*, J = 4.0, 7.6, 14.0, H–C(9) and *ddd*, J = 1.6, 8.4, 14.0, H–C(9′), resp.) were observed. The signal of H–C(9) showed a long-range coupling with the signal of H–C(1) (2.50–2.60, *m*), and the latter was coupled with the signal of H–C(10) (1.88–1.92, *m*), which correlated with the signal of Me(15) (1.12, *d*, J = 6.4). The signal of H–C(1) exhibited other cross-peaks with the signals of a CH_2 group at 2.65 (*dd*, J = 6.0, 18.4, H–C(2)) and 2.19 (*dd*,

$J = 2.4$, 18.4, H–C(2′)). Beside these signals, the spectra exhibited two Me groups as *doublet*s at 1.19 and 1.16 ($J = 6.8$, Me(13) and Me(12)). They both belong to an ^{i}Pr group, as they showed a cross-peak with a methine at 2.77 (br. *hept*, $J = 6.8$, H–C(11)). HMQC and HMBC experiments allowed the assignment of all 15 C-atoms of the molecule (see *Table 1* and *Fig.*). The FAB-MS data confirmed the molecular weight with a pseudo-molecular ion peak at *m*/*z* 235 ($C_{15}H_{23}O_2$, $[M+H]^+$). Altogether, **8** was identified as the new guaiane-type sesquiterpene 6,7,8,8a-tetrahydro-6-hydroxy-5-(1-methylethyl)-3,8-dimethyl-1*H*-azulen-2-one or (1β,8β,10αH)-8-hydroxyguaia-4,6-dien-3-one. The relative configuration was deduced from the coupling constants and NOESY data. The coupling constant $J(8,9) = 7.6$ Hz suggested that H–C(8) and H–C(9) are *trans* to each other [24]. The coupling constants $J(1,2)$ of 6.0 and $J(1,2')$ of 2.4 Hz, suggested that H–C(1) and H–C(2) were eclipsed on the same side of the five-membered ring, while H–C(2′) was on the opposite side [24]. Nuclear *Overhauser* effects were noticed through cross-peaks between H–C(6) and H–C(14)/H–C(11)/H–C(12), between H–C(8) and H–C(11)/H–C(15)/H–C(1), between H–C(15) and H–C(2′)/H–C(9), confirming the relative configuration at C(8), C(10), and C(1).

Compound **9** was closely related to **7** and **8**. The positive FAB-MS exhibited a pseudomolecular ion at *m*/*z* 257 ($[M+Na]^+$) and 235 ($[M+H]^+$), corresponding to the same molecular formula as **8**. Fragmentation of this ion gave a peak at *m*/*z* 207 ($[M-CO]^+$), indicative of the loss of a CO or an ethylene subunit. The ^{1}H-NMR spectrum exhibited peaks between δ 1.00 and 6.40, with 2 Me *doublet*s ($J = 6.5$ and 6.6) at 1.04 and 1.08. Beside these signals, aliphatic H-atoms were observed between 1.61 and 2.60, and a *multiplet* at 3.62 (2 H), which were assigned through the ^{1}H,^{1}H-COSY correlations. The spectrum of the same compound in (D_5)pyridine exhibited some differences: the *multiplet* at 3.62 was changed into two double *doublet*s at 3.29 ($J = 10.7$, 7.8, H–C(12)) and 3.21 ($J = 10.5$, 6.1, H–C(12′)) (*Table 1*). Cross-peaks in the HMQC spectrum allowed to assign these H-atoms to a CH_2OH group (δ(C) 66.1, C(12)). The COSY experiment and the ^{1}H-NMR shifts showed two spin systems, identifying the following substructures: RO–CH_2–CH(Me)–C=, and =C–CH_2–CH_2–CH(Me)–CH–CH_2–C=. The ^{13}C-NMR spectra analysis led to four C=C C-atoms (168.3 (*s*), 157.8 (*s*), 135.9 (*s*), 123.0 (*d*)), three aliphatic CH (47.4, 47.0, 39.2), four CH_2 (66.1, 42.5, 35.6, 26.5) and three Me (22.3, 16.0, 8.6) groups. The HMBC correlations were used to establish the connectivities between these substructures (*Fig.*). Comparing these data to those of **7** and **8**, **9** was identified as 6,7,8,8a-tetrahydro-5-(2-hydroxy-1-methylethyl)-3,8-dimethyl-1*H*-azulen-2-one or (1β,10αH)-12-hydroxyguaia-4,6-dien-3-one, a third guaiane-type sesquiterpene. As before, the relative configuration at C(1) and C(10) was established by means of the size of the coupling constants and NOESY data. NOESY Correlations observed between H–C(6) and Me(14)/Me(13), and between H–C(1) and Me(15) suggested that H–C(6), Me(14), and Me(13) were co-planar and that H–C(1) and Me(15) were on the same side of the molecule (*Fig.*).

The ^{1}H-NMR spectrum of **10** exhibited signals between δ 5.43 and 0.90, suggesting a triterpenic structure [7]. Eight Me group *singlet*s at 1.51 (Me(24)), 1.42 (Me(23)), 1.26 (Me(26)), 1.23 (Me(27)), 1.12 (Me(25)), 1.01 (Me(28)), 1.00 (Me(29)), 0.90 (Me(30)), four CH groups at 5.43 (br. *d*, $J = 3.2$, H–C(12)), 3.87 (br. *d*, $J = 7.6$, H–C(1)), 3.35 (*d*, $J = 10$, H–C(21)) and 3.28 (*d*, $J = 10$, H–C(22)), and numerous signals between 3.32 and 1.81 ppm were indicative of a polyoxygenated triterpene. The ^{13}C-NMR signals

were in accordance with this hypothesis: 30 C-atoms were detected between δ 171.8 and 14.5 (*Table 2*), including eight Me, six CH_2, and seven CH groups, as well as nine quaternary C-atoms. The presence of a C=C bond and the oxygenated positions were confirmed as follows: 142.9 (C(13))/124.2 (C(12)), and the C-atoms at 215.1 (C(7))/171.8 (C(3))/84.5 (C(4))/79.9 (C(22))/77.5 (C(21))/69.1 (C(1)). A ^{1}H,^{1}H-COSY experiment allowed the identification of six spin systems. Thus, C(21) and C(22), and C(1) and C(2) were deduced to be vicinal C-atoms. Cross-peaks between H–C(12) (δ(H) 5.43) and the CH_2 group H–C(11) (2.00–2.06)/H–C(11′) (2.12–2.17) pointed to the substructure –C=CH–CH_2–C. A HMQC experiment allowed the assignment of H-substituted C-atoms. Combined ^{1}H,^{1}H-COSY, HSQC, and HMBC data were used to elucidate the structure of **10** as a new metabolite, a homo-3a-oxa-1,21,22-trihydroxy-olean-12-ene-3,6-dione (*Table 2*).

A NOESY experiment was helpful for the determination of the relative configuration (*Table 2*). D/CI-MS spectra exhibited a pseudo-molecular ion at *m/z* 503 assigned to $[M+H]^+$. Fission of ring *C* led to two main ions with *m/z* 253 (99.7%) and 251 (47.9%). These data were in accordance with the molecular formula $C_{30}H_{46}O_6$. From these data, two structures were proposed for the ring *A* (**10a** and **10b**). A definitive choice was made based on the chemical shifts of C(1), C(2), C(3), and C(4). According to the literature, a C=O C-atom in a lactone as in the structure of **10a** exhibited an upfield shift at δ(C) 175.6, while the tertiary carbinol C-atom (C(4)) was shifted downfield at δ(C) 85.6 [25]. In the *seco*-ring form (**10c**) the COOH group was observed at δ(C) 180.6 and the carbinol C-atom at δ(C) 75.9. The same conclusion was deduced from data of limonoids, which presented the same ring *A* as **10** [26]. The shape of ring *A* in **10b** was observed also in the limonoid class [27]. In this case, C(1) to C(4) were detected at δ(C) 81.6, 36.9, 172.1 and 78.1, respectively. From this analysis, the structure **10a** is proposed for the structure of this novel metabolite **10**.

From the basic crude extract, the major compounds were identified by means of their spectral data as the cyclodipeptides frangulanin (**2**) [11][12] and melonovin (**3**) [13]. Beside these cyclopeptides, another compound was isolated. The EI-MS of **11** exhibited an ion peak at *m/z* 261 (97, $[M-Me]^+$) and fragments at *m/z* 232 (76, $[M-44]^+$), 218 (20, $[M-58]^+$), 205 (100, $[M-71]^+$), and 177 (15, $[M-99]^+$). The base peak most likely arises from benzylic cleavage of an aliphatic C_6 chain. Positive FAB-MS analysis showed the molecular ion at *m/z* 277 ($[M+H]^+$), which was consistent with the formula $C_{16}H_{20}O_4$. The UV spectrum in MeOH showed five bands at λ 334, 321, 292 (sh), 244, and 216 nm. With $AlCl_3$, a bathochromic effect was observed on bands 1 and 2. This effect was permanent after addition of HCl (25%). This suggested the presence of a γ-hydroxyketone group. The ^{1}H-NMR spectrum of **11** was closely related to that of the 5-hydroxy-7-methoxy-2-pentyl-4-chromone described by *Jimenèz et al.* [28]. At δ(H) 6.40 (H–C(6)) and 6.34 (H–C(8)), two *doublet*s (J = 2.3 Hz) suggested two aromatic H-atoms in *meta*-position to each other. At 6.19, a *singlet* was assigned to H–C(3). A MeO group was observed at 3.83 (*s*). Additionally, the aliphatic H-atoms showed the following signals: a *triplet* at 2.48 (J = 7.7, CH_2(1′)), two *multiplet*s between 1.30 and 1.70 (CH_2(2′) to CH_2(5′)), and a *triplet* at 0.89 (J = 7.1, Me(6′)). These signals, together with the MS data, were consistent with the presence of an *n*-hexyl group. A broad *singlet* was observed at 12.43, and was assigned to HO–C(5). The ^{13}C-NMR spectrum exhibited 15 C-atoms with two Me (δ(C) 13.9 and

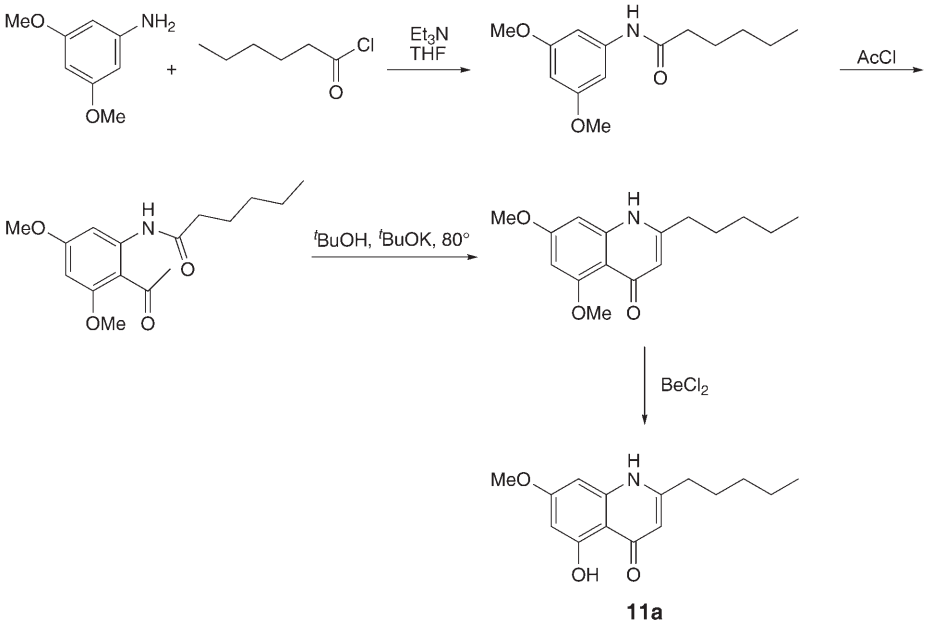
Table 2. *^{1}H- and ^{13}C-NMR Data of* **10**–**10c**. At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

	10			**10a**[a])	**10a**[b])	**10b**[c])	**10c**[a])
	δ(H)	NOESY	δ(C)	δ(C)	δ(C)	δ(C)	δ(C)
1	3.87 (br. *d*, *J* = 7.6)	2, 9, 11, 25	69.1		70.0	81.6	
2a	3.01 (*dd*, *J* = 7.6, 16.0)	25	39.8		40.1	36.9	
2b	3.32 (*d*, *J* = 15.6)						
3	–		171.8	175.6	174.3	172.1	180.6
4	–		84.5	85.6	86.3	78.1	75.9
5	2.67 (*dd*, *J* = 6.0, 13.2)	24, 9, 27	46.5				
6a	2.55 (*dd*, *J* = 6.0, 18.3)	24	42.1				
6b	2.32–2.40 (*m*)	23, 25					
7	–		215.1				
8	–		45.6				
9	2.87 (*dd*, *J* = 4.1, 12.0)	5, 27	41.7				
10	–		44.2				
11a	2.12–2.17 (*m*)	1	23.7				
11b	2.00–2.06 (*m*)	1					
12	5.43 (br. *d*, *J* = 3.2)	18	124.2				
13	–		142.9				
14	–		55.0				
15a	1.93–2.00 (*m*)	27	27.3				
15b	1.60–1.65 (*m*)	26, 28					
16a	1.65–1.72 (*m*)		21.2				
16b	1.49–1.56 (*m*)						
17	–		39.9				
18	2.06–2.12 (*m*)	21, 28, 30	48.8				
19a	1.81 (*t*, *J* = 13.6)		44.8				
19b	1.20–1.25 (*m*)						
20	–		36.1				
21	3.35 (*d*, *J* = 10.0)	18, 28, 30	77.5				
22	3.28 (*d*, *J* = 10.0)	19, 16, 29	79.9				
23b	1.42 (*s*)	2, 6	22.6				
24a	1.51 (*s*)	5,6	33.6				
25b	1.12 (*s*)	1, 6, 11	15.6				
26b	1.26 (*s*)	15	17.6				
27a	1.23 (*s*)	5, 6, 9, 15	27.4				
28b	1.01 (*s*)	15, 18, 21	25.4				
29a	1.00 (*s*)	19, 22	14.5				
30b	0.90 (*s*)	18, 21, 22	30.0				

[a]) From [25]. [b]) From [26]. [c]) From [27].

55.4), five CH_2 (22.3–33.3), and three CH (105.3, 99.5 and 99.3) groups, as well as six quaternary C-atoms (179.2, 166.3, 165.1, 157.2, 140.8, and 114.5). As observed in the ^{1}H-NMR spectrum, the ^{13}C-NMR shifts were closely related to the pertinent reference value [28]. Thus, the spectral data suggested a flavone type of structure, namely 5-hydroxy-2-hexyl-7-methoxy-4-chromone. As quinolones were identified in Sterculiaceae [29], and to insure that **11** was not an alkaloid, the quinolone **11a** was synthesized starting from 3,5-dimethoxyaniline and hexanoyl chloride, using a method described by

Scheme. *Synthesis of* **11a**



11a

Hadjeri et al. [30] (*Scheme*). Spectroscopic data of **11a** showed that it was different from our natural product **11**.

2. *Interest of the Metabolites in the APG2 Classification*. Compounds **7**–**9** were identified for the first time in the plant kingdom. Up to now, the guaiane-type sesquiterpenoids were essentially found in Asteraceae [31], and occasionally in Apiaceae, Thymeleaceae, Valerianaceae, Zingibearaceae, Rutaceae, in liverworts and in marine sponges [32–38]. Dimers were also identified in *Xylopia vielana* (Annonaceae) [39–41]. But this is so far the first report of guaianes in Malvaceae.

Seco-triterpenes have been reported from unrelated families without any botanical link: Celastraceae, Meliaceae, Apiaceae, Saxifragaceae [42–45]. Only few biological activities have been detected in these compounds.

Finally, beside the identification of new compounds, the surprising feature in this work is the identification of cyclopeptides. It is a newly reported approach to consider these compounds as potential chemotaxonomic markers. This interest arose with the recent APG2 classification. Indeed, in 2003, *C. africana* (together with the Brownlowioideae sub-family) was reclassified as belonging to the Malvaceae, instead of Tiliaceae. It is the second time that these compounds are reported in this family (in APG2 sensus): *Emile et al.* found frangulanin (**2**) in *Melochia odorata* [46]. Furthermore, the 14-membered dipeptides are reported to be present in 7 out of 27 (26%) core eudicot orders [47]. As the APG2 classification is based on genomic criteria, cyclopeptides can be looked upon as the first phenotype expression of the APG and the very first chemical link between *C. africana* and other species already known for containing this class of metabolites and belonging to Rosid plants.

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Experimental Part

General. Column chromatography (CC) was performed on silica gel (SiO_2) *60* (40–63 μm; *Merck*), on *Sephadex LH-20 gel* (*Amersham Pharmacia*, Sweden), or on polyamide (*NN-Polyamid-SC6*, < 0.07 mm; *Macherey-Nagel*). Vacuum Liquid Chromatography (VLC) was performed on SiO_2 (40–63 μm, *Merck*). Solid Phase Extraction (SPE) was performed on C_{18} reverse phase: *Lichroprep 60 RP-18* (40–63 μm, *Merck*). TLC: SiO_2 *60* F_{254s} (*Merck*). Prep. TLC: SiO_2 *60* GF_{254s} (*Merck*). UV Spectra: *Hitachi U2000* spectrophotometer; λ_{max} in nm. Optical rotations: *Perkin-Elmer 241* polarimeter, at 20°. NMR Spectra: *Bruker AC* spectrometer, at 200/400 (^1H) and 50/100 MHz (^{13}C) resp.; chemical shift δ in ppm, coupling constant J in Hz. ^1H,^1H-COSY, HSQC, HMBC, and NOESY Spectra: *Bruker DRX-500* spectrometer. EI-MS, D/CI-MS and FAB-MS: *R210C* quadripolar spectrometer. HR-ESI-MS: *Thermo-Finnigan Mat 95 XL*; in *m/z*.

Plant Material. Barks of *C. africana* were collected in 1996, in the region of Ngoumé (5°30′ N, 11°22′ E, Cameroon). A voucher specimen has been deposited with our laboratory (N° 1995R1).

Extraction and Isolation. The dried barks of *C. africana* (2.77 kg) were successively extracted with four solvents of increasing polarity, to give cyclohexane (23.5 g), CH_2Cl_2 (20.0 g), CH_2Cl_2/MeOH (68.0 g), and MeOH (422.3 g) extracts.

The CH_2Cl_2/MeOH (1:1) crude extract (25 g) was treated with NaCl. After filtration, the aq. phase was partitioned against CH_2Cl_2, AcOEt, and BuOH to give the three extracts *F1* (262 mg), *F2* (886 mg) and *F3* (2.72 g). *F1* was subjected to purification on VLC using SiO_2 as stationary phase and a gradient of CH_2Cl_2/MeOH as mobile phase. Nine fractions were obtained (*F1a–F1i*). *F1b* (15 mg) gave **7** (3.2 mg) and scopoletol [10][19] (1 mg) after separation by CC on SiO_2 with a step gradient of CH_2Cl_2/MeOH/NH_4OH (98:2:1, 130 ml), (95:5:1, 50 ml) and then MeOH (25 ml). Compound **7** and scopoletol [10][19] were eluted in the first and the second step, resp. *F1d* (72 mg) was submitted subsequently to a filtration over *Sephadex LH-20* in MeOH/H_2O (9:1) and SPE over C_{18} using a gradient of H_2O/MeOH (100:0 → 0:100), to yield **9** (3.9 mg), **4** (4 mg), and **6** (3 mg). *F1e* (58 mg) was purified by CC over SiO_2 using a mixture of CH_2Cl_2/MeOH/NH_4OH (97:3:1) to give 7 fractions. The first one (7 mg) yielded frangulanin (**2**) [11][12] (2 mg) after additional CC over SiO_2 with CH_2Cl_2/MeOH/NH_4OH (98:2:1). The fifth fraction (11 mg) yielded **10** (1.3 mg), after being purified by SPE over C_{18} using a gradient of H_2O/MeOH (90:10 → 0:100).

F2 (886 mg) was subjected to VLC over C_{18} using a gradient of H_2O/MeOH (100:0 → 0:100) to give 17 fractions (*F2a–F2o*). *F2l* (177 mg) was submitted to CC on polyamide using a gradient of H_2O/MeOH (100:0 → 60:40) to give 8 fractions. The fourth fraction yielded **5** (2 mg) after purification by CC over SiO_2 using a mixture of AcOEt/MeOH/H_2O (100:16.5:13.5).

After the removal of the tannins [48], and evaporation under vacuum, the MeOH extract (2 g) was dissolved in H_2O, and partitioned with CH_2Cl_2, to give 300 mg of an org. residue. The latter was purified by CC on SiO_2 using a gradient of cyclohexane/CH_2Cl_2 (2:8 → 0:100), and then CH_2Cl_2/MeOH/NH_4OH (10%) (100:0:0 → 95:4:1). The fraction eluted with the step gradient of 97:2:1 was further purified by prep. TLC in the same solvent to yield frangulanin (**2**) [11][12] (2 mg). Finally, the fraction eluted with CH_2Cl_2/MeOH/NH_4OH (10%) (95:4:1) led to melonovin (**3**) [13] after prep. TLC under the same conditions.

A second purification of the MeOH extract (28 g) with skin powder and partitioning with CH_2Cl_2 and BuOH led to three extracts. The aq. extract (959 mg) was successively purified by *1*) CC over *Sephadex LH-20* in MeOH, and *2*) SPE over SiO_2 with a gradient CH_2Cl_2/MeOH/H_2O. The fraction eluted with 80:20:0 (213 mg) was then purified by SPE over C_{18} using a H_2O/MeOH gradient. The first fraction (9 mg) was further purified by prep. TLC in CH_2Cl_2/MeOH (8:2) and gave loganin [14] (2.5 mg).

Finally, an alkaloid type extract was prepared from 1 kg of bark, according to a slightly modified procedure originally described by *Ghedira et al.* [49]. After maceration in NH_4OH (10% aq), the powder

was extracted with CH_2Cl_2 (16 l) for 65 h. Partitioning with H_2O and evaporation of the org. phase under reduced pressure led to 7 g of a crude extract. This extract (7 g) was subjected to a *Sephadex LH-20* CC in a mixture of cyclohexane/CH_2Cl_2 (4:6) and led to 21 fractions. *Fr. A8* (160 mg) was then subsequently purified by LPLC (using C_{18} and a gradient H_2O/MeOH as solvent), by SPE on SiO_2 (gradient cyclohexane/CH_2Cl_2/MeOH), and finally by prep. TLC on SiO_2 with CH_2Cl_2/MeOH (93:7) as the mobile phase. This yielded **8** (4 mg) and **11** (3 mg). *Fr. A21* (443 mg) was purified by HPLC (C_{18}, H_2O/MeOH 1:1) and then by *Visiprep* on SiO_2 with a gradient of cyclohexane/CH_2Cl_2/MeOH.

*(4*R*)-3,3a,4,5-Tetrahydro-1,4-dimethyl-7-(1-methylethyl)azulene-2,6-dione* (**7**). Colorless oil. $[\alpha]_D^{20} = -13.5$ ($c = 0.16$), $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 232 (M^+), 217 ([M − Me]$^+$), 190 ([M − (Me−CH=CH_2)]$^+$). HR-ESI-MS: 255.31535 ([M + Na]$^+$, $C_{15}H_{20}NaO_2^+$; calc. 255.13610).

*(6*S*,8*R*)-6,7,8,8a-Tetrahydro-6-hydroxy-3,8-dimethyl-5-(1-methylethyl)-1*H*-azulen-2-one* (**8**). Colorless oil. $[\alpha]_D^{20} = -21.3$ ($c = 0.2$), $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1*. FAB-MS (pos.): 257 ([M + Na]$^+$), 235 ([M + H]$^+$), 207 ([M − CO]$^+$). EI-MS: 234 (M^+). HR-ESI-MS: 257.15195 ([M + Na]$^+$, $C_{15}H_{22}NaO_2^+$; calc. 257.15175).

*(8*R*)-6,7,8,8a-Tetrahydro-5-(2-hydroxy-1-methylethyl)-3,8-dimethyl-1*H*-azulen-2-one* (**9**). Colorless oil. $[\alpha]_D^{20} = 93$ ($c = 0.2$), $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1*. FAB-MS: 257 ([M + Na]$^+$), 235 ([M + H]$^+$). EI-MS: 234 (M^+). HR-ESI-MS: 257.15190 ([M + Na]$^+$, $C_{15}H_{22}NaO_2^+$; calc. 257.15175).

*(1*S*,5a*R*,7a*R*,7b*R*,9a*R*,10*S*,11*S*,13a*S*,15b*R*)-1,5a,6,7a,7b,8,9,9a,10,11,12,13,13a,15,15a,15b-Hexadecahydro-1,10,11-trihydroxy-5,5,7a,7b,9a,12,12,15b-octamethylchryseno[2,1-*c*]oxepine-3,7(2*H*,5*H*)-dione* (**10**). Colorless oil. $[\alpha]_D^{20} = 9.2$ ($c = 0.065$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 2*. D/CI-MS: 503 ([M + H]$^+$), 253, 251. HR-CI-MS: 503.33780 ([M + H]$^+$, $C_{30}H_{47}O_6^+$; calc. 503.3373).

*2-Hexyl-5-hydroxy-7-methoxy-4*H*-1-benzopyran-4-one* (**11**). Yellowish lacquer. UV (MeOH): 216, 244, 292 (sh), 321, 334; (MeOH + $AlCl_3$) 238, 345; (MeOH + $AlCl_3$ + HCl) 238, 345. ^{1}H-NMR (400 MHz, $CDCl_3$): 12.43 (*s*, HO−C(5)); 6.40 (*d*, $J = 2.3$, H−C(6)); 6.34 (*d*, $J = 2.3$, H−C(8)); 6.19 (*s*, H−C(3)); 3.83 (*s*, MeO−C(7)); 2.48 (*t*, $J = 7.7$, CH_2(1′)); 1.70–1.61, 1.39–1.31 (2*m*, CH_2(2′) to CH_2(5′)); 0.89 (*t*, $J = 7.1$, Me(6′)). ^{13}C-NMR (100 MHz, $CDCl_3$): 179.2 (C(4)); 166.3 (C(7)); 165.1 (C(5)); 157.2 (C(9)); 140.8 (C(2)); 114.5 (C(10)); 105.3 (C(3)); 99.5 (C(6/8)); 99.3 (C(8/6)); 55.4 (*Me*O−C(7)); 33.3 (C(1′)); 31.1 (C(2′)); 29.7 (C(3′)); 27.7 (C(4′)); 22.3 (C(5′)); 13.9 (C(6′)). FAB-MS (pos.): 277 ([M + H]$^+$), 262. EI-MS (70 eV): 261 (97, [M − Me]$^+$), 218 (20, [M − 58]$^+$), 205 (100, [M − 71]$^+$), 177 (15, [M − 99]$^+$). HR-ESI-MS: 299.12611 ([M + Na]$^+$, $C_{16}H_{20}NaO_4^+$; calc. 299.12593).

*5-Hydroxy-7-methoxy-2-pentyl-1*H*-quinolin-4-one* (**11a**). For the preparation procedure, see *Scheme* and [30]. Colorless lacquer. ^{1}H-NMR (200 MHz, $CDCl_3$): 8.10 (br. *s*, H−N(1)); 6.29 (*d*, $J = 2.0$, H−C(6)); 6.15 (*d*, $J = 2.0$, H−C(8)); 5.98 (*s*, H−C(3)); 3.84 (*s*, *Me*O−C(7)); 2.57 (*t*, $J = 7.6$, CH_2(1′)); 1.70–1.23 (*m*, CH_2(2′) to CH_2(5′)); 0.92 (*t*, $J = 7.1$, Me(6′)). ^{13}C-NMR (50 MHz, $CDCl_3$): 182.5 (C(4)); 164.3 (C(7)); 163.5 (C(5)); 153.0 (C(9)); 141.5 (C(2)); 107.7 (C(10)); 107.0 (C(3)); 96.6 (C(6/8)); 89.9 (C(8/6)); 55.5 (*Me*O−C(7)); 34.3 (C(1′)); 31.1 (C(2′)); 29.9 (C(3′)); 27.9 (C(4′)); 22.3 (C(5′)); 13.8 (C(6′)). D/CI-MS: 262 ([M + H]$^+$).

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