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Antiplasmodial and Cytotoxic Triterpenoids from the Bark of the Cameroonian Medicinal Plant *Entandrophragma congoënse*

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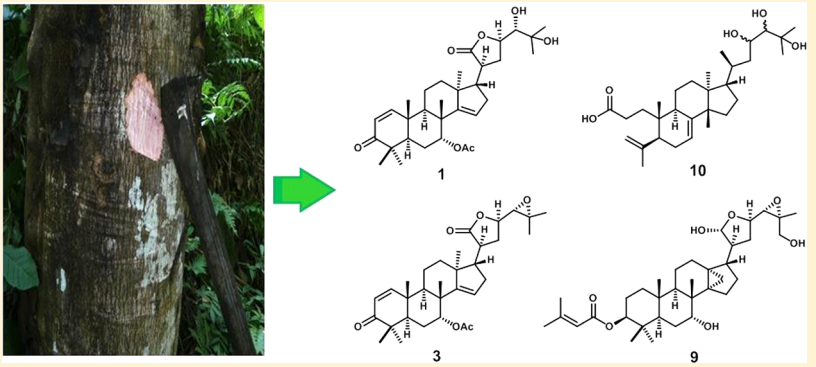
Supporting Information



ABSTRACT: Eight new triterpenoids, prototiamins A–G (**1**–**6**, **9**) and *seco*-tiaminic acid A (**10**), were isolated along with four known compounds from the bark of *Entandrophragma congoënse*. Their structures were elucidated by means of HRMS and different NMR techniques and chemical transformations. Assignments of relative and absolute configurations for the new compounds were achieved using NOESY experiments and by chemical modification including the advanced Mosher's method. Additionally, the structure and relative configuration of compound **3** were confirmed by single-crystal X-ray diffraction analysis. Compounds **1**, **3**, and **5** displayed significant in vitro antiplasmodial activity against the erythrocytic stages of chloroquine-sensitive *Plasmodium falciparum* strain NF54. Prototiamin C (**3**) was the most potent of the compounds isolated, with an IC_{50} value of 0.44 μM. All compounds tested showed low cytotoxicity for the L6 rat skeletal myoblast cell line.

Malaria is a parasitic disease endemic to tropical areas. It can be caused by any of the four *Plasmodium* species, viz., *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, that are transmitted by the female *Anopheles* mosquito.[1,2] Each year, about 300–500 million malaria episodes occur, of which 2–3 million cases lead to death, including a significant number of child mortalities, mainly in African countries. Malaria remains a major public health problem.[3,4] In the past decade, the increased incidence of malaria has been attributed to the development of resistance of the malarial parasite (*Plasmodium falciparum*) to chloroquine[5] and to the development of resistance of the vector mosquitoes to insecticides,[6] causing an urgent need to find new antimalarial agents. One possible source for affordable treatment remains the use of traditional herbal remedies. In Cameroon, the local population may use plants from the family Meliaceae for such purposes. This family comprises more than 50 genera including the genus *Entandrophragma*, which is represented by four species in Cameroon.[4]

Entandrophragma congoënse A. Chev. (Meliaceae), locally known as "Black Tiama" is very rare in Cameroon.[7] To the best of our knowledge, only one phytochemical study has so far been undertaken on this plant, which reported the presence of the limonoid gedunin.[8] Chemical investigations carried out on other species of the genus *Entandrophragma* have led to the isolation of cyclic and acyclic triterpenes,[9−11] limonoids,[12−14] and protolimonoids[15] as the most widely encountered classes of compounds. Some of these substances possess antiplasmodial activity.[4] In continuation of an ongoing search for bioactive metabolites from Cameroonian medicinal plants,[11,16,17] a phytochemical investigation was carried out on the bark of *E. congoënse*. In the present study, reported are the isolation and structure elucidation of eight new triterpenoids, prototiamins A–G (**1**–**6**, **9**) and *seco*-tiaminic acid A (**10**). The trivial names

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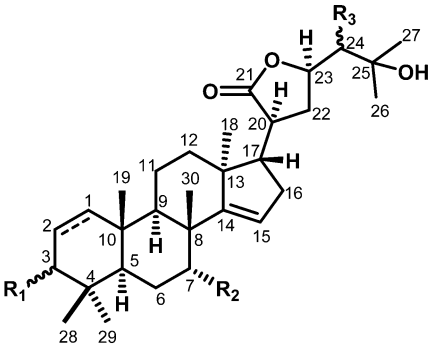
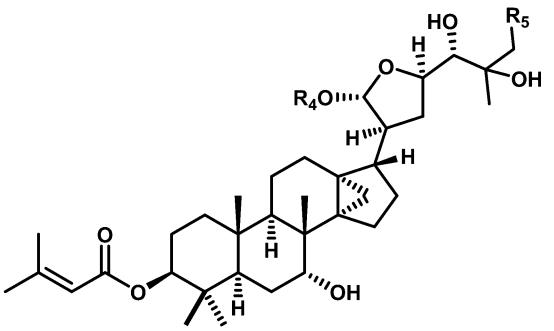
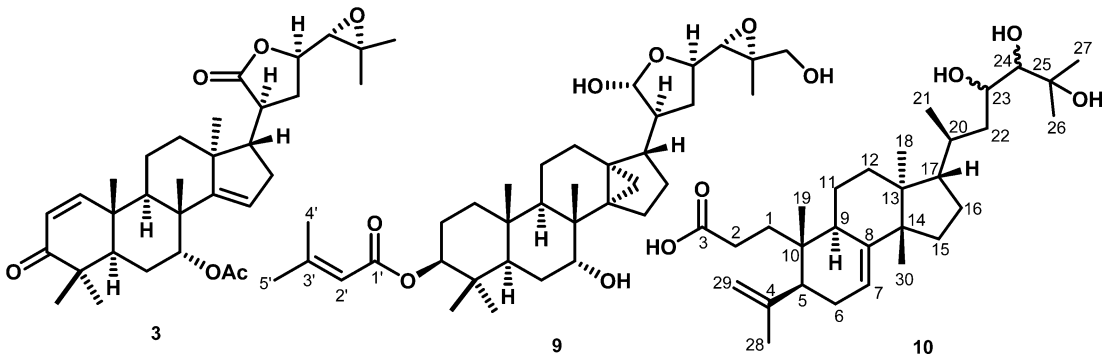
Chart 1



1	$\Delta^{1(2)}$	R_1 = O	R_2 = OAc	R_3 = α-OH
1c	$\Delta^{1(2)}$	R_1 = O	R_2 = OH	R_3 = α-OH
2	$\Delta^{1(2)}$	R_1 = O	R_2 = OH	R_3 = β-OH
4	1,2-dihydro	R_1 = α-OH	R_2 = OAc	R_3 = α-OH
5	1,2-dihydro	R_1 = α-OAc	R_2 = OAc	R_3 = α-OH



6 R_4 = R_5 = H
7 R_4 = Me R_5 = H
8 R_4 = Me R_5 = OH



3 **9** **10**

of the new secondary metabolites were given based on the local name of their plant of origin.

RESULTS AND DISCUSSION

The dichloromethane–methanol (1:1) extract of the bark of *E. congoënse* was subjected to repeated silica gel column chromatography and semipreparative HPLC to afford eight new triterpenoids, prototiamins A–F (**1**–**6**) and G (**9**) and *seco*-tiaminic acid A (**10**), and four known compounds, the apotirucallane triterpenoids **7** and **8**,[18] methyl angolensate,[19] and lupeone.[20] The structure elucidation of the new triterpenoids was carried out by means of HRMS and NMR spectroscopy and comparison with previously reported data.

Prototiamin A (**1**) was isolated as a white powder. Its molecular formula was assigned as $C_{32}H_{46}O_7$ on the basis of the HRESIMS (*m*/*z* 543.3314 $[M + H]^+$, calcd for $C_{32}H_{47}O_7$, 543.3316) and NMR data, requiring 10 double-bond equivalents. The ^{1}H NMR spectrum (Table 1) exhibited signals for seven tertiary methyls [δ 1.38, 1.32, 1.21, 1.20, 1.09 (Me × 2), and 1.07], an olefinic proton (δ 5.31), an acetyl group (δ 1.96), and two olefinic protons indicated by a *cis*-double bond [δ 7.17 (d, J = 9.9 Hz) and 5.86 (d, J = 9.9 Hz)]. The ^{13}C NMR spectrum (Table 2) revealed the presence of 32 carbon atoms, which were assigned by DEPT and HSQC experiments to one ketone carbonyl (δ 204.9), two ester carbonyls (δ 177.9 and 170.3), four sp^2 carbons (δ 158.9, 158.5, 125.8, and 119.0), eight methyls (δ 27.8, 27.4, 27.0, 26.9, 21.6, 21.5, 21.0, and 19.4), six methylenes, and seven methines. All these data coupled with a literature survey indicated that **1** is an analogue of senegalene C, an apotirucallane-type triterpenoid isolated from *Khaya senegalensis*.[21] Careful comparison of their NMR data revealed that the two compounds share the same tetracyclic moiety with important differences in the side chain. Besides the characteristic fingerprint signals for the tetracyclic rings, analysis of the ^{13}C NMR spectrum revealed that the signal of a hemiacetal carbon at δ 96.8 in senegalene C[21] was replaced by a lactone carbonyl group at δ 177.9 in **1**, which was confirmed by the 2D NMR data and the 2 mass units difference between the two compounds. In the HMBC experiment, cross-peaks were observed from proton signals at δ 1.96, 2.37 (H_2-22) and 2.75 (H-20) to the carbonyl signal at δ 177.9 (C-21) (Figure 1a), which supported the presence of a lactone ring inside the chain. The second ester carbonyl was attached to C-7, based on the cross-peak observed between the oxymethine proton signal at δ 5.23 (H-7) and the acetyl carbon signal at δ 170.3. All these findings were supported by the ^{1}H–^{1}H COSY and HMBC spectra (Figure 1a). In the ^{1}H–^{1}H COSY spectrum, the oxymethine proton signal at δ 5.23 (H-7) exhibited correlations with the methine proton signal at δ 2.21 (H-5) and the methylene proton signals at δ 2.25 and 2.41 (H_2-6). Furthermore, the olefinic proton signals at δ 7.17 (H-1) and 5.86 (H-2) showed correlations in the HMBC spectrum to the ketone carbonyl signal at δ 204.9 (C-3). In turn, the signal of the oxymethine group at δ 4.63 (H-23) displayed HMBC correlations with the carbon signals at δ 30.4 (C-22), 76.6 (C-24), and 72.9 (C-25), and the ^{1}H NMR signal at δ 3.30 (H-24) also showed correlations with the resonances at δ 72.9 (C-25), 26.9 (C-26), and 27.0 (C-27) (Figure 1a). Interpretation of NOESY data (Figure 1b) obtained for **1** revealed the same relative configuration of the tetracyclic moiety as in senegalene

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Table 1. ^{1}H NMR Data for Prototiamins A–E (1–5)[a]

	1[b]	2[c]	3[b]	4[b]	5[b]
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	7.17 (1H) d (9.9)	7.14 (1H) d (1H) (10.3)	7.18 (1H) d (10.2)	2.15 (2H) m	1.28 (1H) m 1.46 (1H) m
2	5.86 (1H) d (9.9)	5.83 (1H) d (10.2)	5.87 (1H) d (10.2)	1.17 (2H) m	1.28 (1H) m 1.46 (1H) m
3				3.42 (1H) t (2.3)	4.68 (1H) t (2.3)
5	2.21 (1H) m	2.39 (1H) m	2.20 (1H) d (2.3)	1.84 (1H) d (1.5)	1.83 (1H) m
6	2.25 (1H) m 2.41 (1H) m	1.85 (2H) m	1.94 (2H) m	1.63 (2H) m	1.72 (1H) m 1.78 (1H) m
7	5.23 (1H) brs	3.98 (1H) t (2.6)	5.26 (1H) t (2.7)	5.18 (1H) t (2.7)	5.20 (1H) t (2.8)
9	2.24 (1H) m	2.21 (1H) m	2.23 (1H) m	2.04 (1H) m	2.07 (1H) m
11	2.26 (2H) m	1.52 (1H) m 2.22 (1H) m	1.82 (1H) m 1.92 (1H) m	2.15 (2H) m	1.28 (1H) m 1.46 (1H) m
12	2.25 (2H) m	1.73 (1H) m 1.98 (1H) m	1.58 (1H) m 2.29 (1H) m	1.39 (2H) m	2.12 (1H) m 2.19 (1H) m
15	5.31 (1H) brs	5.52 (1H) d (3.2)	5.33 (1H) dd (2.3, 2.3)	5.25 (1H) brs	5.29 (1H) brs
16	2.20 (2H) m	2.18 (2H) m	2.13 (1H) dd (2.3, 10.9) 2.29 (1H) dd (2.3, 10.9)	2.13 (2H) m	2.17 (2H) m
17	2.24 (1H) m	2.30 (1H) m	2.24 (1H) m	2.20 (1H) m	2.26 (1H) m
18	1.20 (3H) s	1.05 (3H) s	1.07 (3H) s	1.04 (3H) s	1.10 (3H) s
19	1.07 (3H) s	1.17 (3H) s	1.20 (3H) s	0.92 (3H) s	0.95 (3H) s
20	2.75 (1H) m	2.76 (1H) m	2.77 (1H) m	2.75 (1H) m	2.78 (1H) m
22	1.96 (1H) m 2.37 (1H) m	2.23 (1H) m 2.40 (1H) m	1.98 (1H) m 2.37 (1H) m	2.23 (1H) m 2.37 (1H) m	2.23 (1H) m 2.43 (1H) m
23	4.63 (1H) ddd (2.0, 6.0, 10.0)	4.63 (1H) ddd (2.0, 6.5, 9.9)	4.20 (1H) ddd (1.3, 4.5, 7.4)	4.61 (1H) ddd (1.9, 6.1, 9.9)	4.63 (1H) ddd (1.9, 6.2, 10.2)
24	3.30 (1H) brs	3.28 (1H) d (6.5)	2.83 (1H) d (7.4)	3.29 (1H) d (1.9)	3.30 (1H) brd (1.9)
26	1.32 (3H) s	1.30 (3H) s	1.36 (3H) s	1.35 (3H) s	1.32 (3H) s
27	1.38 (3H) s	1.35 (3H) s	1.38 (3H) s	1.30 (3H) s	1.38 (3H) s
28	1.09 (3H) s	1.09 (3H) s	1.09 (3H) s	0.84 (3H) s	0.91 (3H) s
29	1.09 (3H) s	1.16 (3H) s	1.09 (3H) s	0.86 (3H) s	0.78 (3H) s
30	1.21 (3H) s	1.14 (3H) s	1.21 (3H) s	1.11 (3H) s	1.14 (3H) s
H_3COCO-3					2.09 (3H) s
H_3COCO-7	1.96 (3H) s		1.96 (3H) s	1.96 (3H) s	1.99 (3H) s

[a]The chemical shifts are in δ values (ppm) from TMS. [b]Recorded in $CDCl_3$ at 500 MHz. [c]Recorded in $CDCl_3$ at 600 MHz.

C.[21] Thus, cross-peaks observed between Me-19/Me-28, Me-19/Me-30, and Me-30/H-7 indicated these to be cofacial, and they were assigned as β-oriented, whereas the NOESY correlations of H-5/H-9, H-9/Me-18, and H-20/H-23 supported their α-orientation. The absolute configuration of the chiral center at C-24 was determined by application of the advanced Mosher's method (Figure 2).[22,23] Accordingly, the absolute configuration at C-24 was determined as *S* with the equatorial arrangement of H-24. The hydrogen bond formation between OH-25 and the O atom in the tetrahydrofuran ring confers a limited conformational mobility to the side chain (Figure 3a).[24,25] The value of the coupling constant ($J \approx 0$ Hz), in conjunction with the NOESY correlation observed between H-23 and H-24, was then indicative of the axial–equatorial orientation of the protons involved. On the basis of all the evidence obtained, the structure of compound **1** was deduced as 7α-acetoxy-24*S*,25-dihydroxy-21,23-epoxyapotirucalla-1,14-diene-3,21-dione.

Compound **2** was isolated as a white powder. Its molecular formula was established on the basis of its HRESIMS (m/z 501.3223 $[M + H]^+$, calcd for $C_{30}H_{45}O_6$, 501.3216) and was found to be the same as that of compound **1c** obtained by hydrolysis of **1** using aqueous base (5% NaOH). This suggested that compounds **2** and **1c** are isomers. The NMR data of compound **2** showed resonances nearly identical to those of prototiamin A (**1**), except that signals for the acetyl group were missing, and the coupling constant between protons H-23 and H-24 was different ($J \approx 0$ Hz in **1** and 6.5 Hz in **2**). The relative configuration was found to be identical to that of compound **1**, based on the NOESY experiment (Figure 1b). Furthermore, the signal at δ 5.23 (oxymethine group, H-7) in **1** shifted to δ 3.50, confirming the lack of an acetyl group in compound **2**, while the large coupling constant between H-23/H-24 suggested an axial–axial arrangement of the protons involved due to the H-bond formation between OH-25 and the O atom in the tetrahydrofuran ring (Figure 3b).[24,25] Accordingly, the *R* configuration at C-24 was assigned for **2**, suggesting that this compound is the C-24 epimer of **1c**. The structure of compound **2** (prototiamin B) was determined as 7α,24*R*,25-trihydroxy-21,23-epoxyapotirucalla-1,14-diene-3,21-dione.

Compound **3**, prototiamin C, was obtained as white crystals. Its molecular formula was found to be $C_{32}H_{44}O_6$ based on the HRESIMS data (m/z 525.3219 $[M + H]^+$, calcd for $C_{32}H_{45}O_6$, 525.3211), representing 18 amu less than that of **1** and 24 amu more than that of **2**. A comparison of its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) with those of compounds **1** and **2** revealed all three structures to be closely related. Compound **3** is a homologue of prototiamins A (**1**) and B (**2**) described

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Table 2. ^{13}C NMR Data for Compounds 1−5[a]

	1[b]	2[c]	3[b]	4[b]	5[b]
position	δ_C mult.	δ_C mult.	δ_C mult.	δ_C mult.	δ_C mult.
1	158.5 CH	158.4 CH	158.8 CH	32.3 CH_2	33.7 CH_2
2	125.8 CH	125.6 CH	125.4 CH	18.8 CH_2	33.7 CH_2
3	204.9 C	205.3 C	204.5 C	75.9 CH	78.4 CH
4	44.5 C	44.4 C	44.1 C	36.9 C	36.5 C
5	46.6 CH	44.8 CH	46.2 CH	41.9 CH	43.5 CH
6	29.8 CH_2	24.5 CH_2	30.6 CH_2	22.8 CH_2	23.4 CH_2
7	75.0 CH	71.9 CH	74.6 CH	75.6 CH	75.9 CH
8	43.2 C	45.1 C	42.8 C	42.0 C	42.7 C
9	38.9 CH	37.0 CH	38.5 CH	43.3 CH	43.7 CH
10	40.3 C	40.4 C	39.9 C	37.4 C	37.8 C
11	32.7 CH_2	32.3 CH_2	23.8 CH_2	32.3 CH_2	33.7 CH_2
12	39.9 CH_2	16.5 CH_2	32.7 CH_2	32.5 CH_2	33.2 CH_2
13	47.0 C	47.0 C	46.7 C	46.3 C	47.1 C
14	158.9 C	161.0 C	158.1 C	159.2 C	159.6 C
15	119.0 CH	119.8 CH	118.3 CH	117.8 CH	118.4 CH
16	32.6 CH_2	32.7 CH_2	32.9 CH_2	31.9 CH_2	32.8 CH_2
17	54.5 CH	54.4 CH	54.2 CH	53.7 CH	54.5 CH
18	19.4 CH_3	20.5 CH_3	20.6 CH_3	20.3 CH_3	20.7 CH_3
19	21.0 CH_3	19.1 CH_3	16.5 CH_3	15.3 CH_3	15.7 CH_3
20	44.5 CH	39.8 CH	39.9 CH	39.6 CH	40.1 CH
21	177.9 C	177.7 C	177.2 C	178.0 C	178.0 C
22	30.4 CH_2	30.0 CH_2	30.6 CH_2	29.6 CH_2	30.3 CH_2
23	77.8 CH	77.7 CH	78.0 CH	77.1 CH	78.4 CH
24	76.6 CH	76.3 CH	64.3 CH	76.3 CH	76.5 CH
25	72.9 C	72.7 C	57.2 C	72.2 C	72.9 C
26	26.9 CH_3	26.7 CH_3	19.4 CH_3	26.4 CH_3	27.0 CH_3
27	27.0 CH_3	26.8 CH_3	24.7 CH_3	26.5 CH_3	27.0 CH_3
28	21.6 CH_3	21.7 CH_3	21.2 CH_3	21.8 CH_3	21.9 CH_3
29	27.4 CH_3	27.3 CH_3	27.0 CH_3	27.9 CH_3	28.0 CH_3
30	27.8 CH_3	27.9 CH_3	27.5 CH_3	27.6 CH_3	28.0 CH_3
H_3COCO-3					170.9 C
					21.6 CH_3
H_3COCO-7	170.3 C		169.9 C	170.4 C	170.4 C
	21.5 CH_3		21.1 CH_3	21.3 CH_3	21.5 CH_3

[a]The chemical shifts are in δ values (ppm) from TMS. ^{13}C multiplicities were determined by HSQC experiment. [b]Recorded in $CDCl_3$ at 125 MHz. [c]Recorded in $CDCl_3$ at 150 MHz.

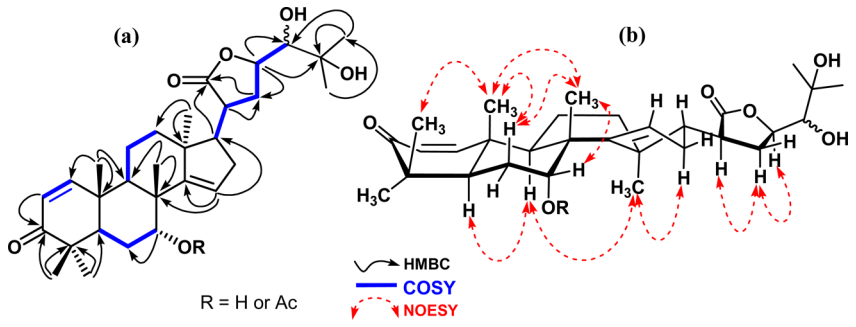

Figure 1. Important HMBC and COSY correlations (a) and key NOESY correlations (b) for **1**, **1c**, and **2**.

above. Differences were observed in the chemical shift of the two oxygenated carbon signals in the side chain of **1** and **2** at ca. δ 76 (C-24) and 72 (C-25), which were shifted upfield to δ 64.3 (C-24) and 57.2 (C-25) in **3**, suggesting the formation of an epoxide ring. Further evidence was obtained from the HMBC spectrum, in which cross-peaks were observed between the proton signals of two terminal methyl groups at δ 1.36 (Me-26) and 1.38 (Me-27) and the carbon signals at δ 64.3 (C-24) and 57.2 (C-25). The relative configuration of **3** was found to be identical to those of prototiamin A (**1**) on the basis of NOESY correlations of Me-28/Me-19, H-5/H-9, H-9/Me-18, H-20/H-23, and H-7/Me-30. In addition, the chemical shifts of C-24 (δ 64.3) and C-25 (δ 57.2) and the coupling constant ($J_{23,24}$ = 7.4 Hz) between H-23 and H-24 supported the *S* configuration of C-24 for compound **3**.[3,26] In order to confirm the proposed configuration and substitution patterns in **3**, the

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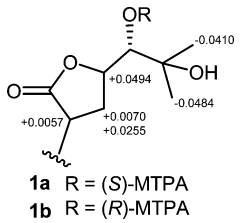

Figure 2. $\Delta\delta(\delta_R - \delta_S)$ values (in ppm) for the MTPA esters of **1**.

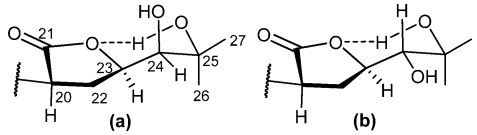

Figure 3. (a) C-24*S* induces an axial−equatorial coupling of H-23/H-24. (b) C-24*R* induces an axial−axial coupling of H-23/H-24.

sample was recrystallized from dichloromethane−methanol (19:1) and subjected to single-crystal X-ray diffraction analysis. The data obtained confirmed the structure of **3** as well as the relative configuration (Figure 4). Thus, the structure of prototiamin C (**3**) was assigned as 7α-acetoxy-21,23-epoxyapotirucall-24,25-epoxide-1,14-diene-3,21-dione.

A fourth new compound, prototiamin D (**4**), was obtained as an optically active red oil ($[\alpha]^{20}_D$ −71), with the same molecular formula as chisiamol G[26] by HRESIMS (*m*/*z* 547.3642 $[M + H]^+$, calcd for $C_{32}H_{51}O_7$, 547.3629), suggesting that they have closely related structures. Comparison of the NMR data of compounds **1**, **2**, and **4** showed all three compounds to have the same skeleton, with a key difference being the absence of the C-1/C-2 double bond in **4**. Careful analysis of ^{1}H and ^{13}C NMR spectra of **4** confirmed that its structure is close to that of chisiamol G with the same substituent pattern including an acetyl group (δ 5.18 and 1.96).[26] In the HMBC spectrum (Figure 5a), proton signals of the *gem*-dimethyl groups at δ 0.84 (Me-28) and 0.86 (Me-29) showed cross-peaks with the oxymethine group at δ 75.9 (C-3), and the oxymethine signal at δ 5.18 exhibited cross-peaks with the ^{13}C NMR signals at δ 159.2 (C-14) and 170.4 ($\underline{C}O\underline{C}H_3$). These data indicated the acetyl group to be located at C-7 instead of C-3 as in chisiamol G. In the ^{1}H NMR spectrum, the resonance at δ 3.42 (t, J = 2.3 Hz) was attributed to H-3. The small coupling constant was indicative of the α-orientation of the hydroxy group (OH-3). The relative configurations of **4** were deduced from the NOESY spectrum, which showed important cross-peaks due to correlations of Me-28/Me-19, H-5/H-9, H-9/Me-18, Me-30/H-7, and H-20/H-23 (Figure 5b). Moreover, the *S* configuration at C-24 was assigned on the basis of the small coupling constant of H-24 (J = 1.9 Hz). Thus, the structure of prototiamin D (**4**) was proposed as 7α-acetoxy-21,3α,24*S*,25-trihydroxy-23-epoxyapotirucall-14-en-21-one.

Prototiamin E (**5**) was obtained as an amorphous, white powder. A pseudomolecular ion peak $[M + H]^+$ at *m*/*z* 589.3737 (calcd for $C_{34}H_{53}O_8$, 589.3735) in the HRESIMS was used to assign its molecular formula as $C_{34}H_{52}O_8$. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **5** were almost superimposable on those of **4**, except for the presence of signals at δ 170.9, 21.5 and δ 2.09 corresponding to an additional acetyl group, which suggested that **5** is a homologue of **4** with an additional acetyl group. In the ^{1}H NMR spectrum of **5**, the signal attributed to H-3 shifted at δ 4.68 (t, J = 2.3 Hz), indicating the acetyl group to reside at position 3. Further confirmation was observed in the HMBC experiment in which cross-peaks were observed between proton signals of the *gem*-dimethyl groups at δ 0.91 (Me-28) and 0.78 (Me-29) and the oxymethine group at δ 78.4 (C-3) and between the proton signal at δ 4.68 (H-3) and the acetyl carbon signal at δ 170.9. The α-orientation of the OH-3 was deduced on the basis of the small coupling constant $J_{H\text{-}3/H\text{-}2}$ = 2.3 Hz. The relative configuration of compound **5** was determined to be the same as that of **4** based on a NOESY experiment (Figure 5b). Furthermore, the *S* configuration was also assigned to C-24 on the basis of the small coupling constant observed for H-24 (J = 1.9 Hz). Thus, the structure of prototiamin E (**5**) was determined unambiguously as 3α,7α-diacetoxy-24*S*,25-dihydroxy-21,23-epoxyapotirucall-14-en-21-one.

Prototiamin F (**6**) was isolated as an amorphous, white powder. Its molecular formula was established as $C_{35}H_{56}O_7$ from the pseudomolecular ion peak $[M + H]^+$ at *m*/*z* 589.4100 (calcd for $C_{35}H_{57}O_7$, 589.4099), obtained by HRESIMS. Its ^{1}H NMR spectrum (Table 3) displayed signals for eight tertiary methyl groups (δ 2.18, 1.90, 1.30, 1.28, 1.04, 0.91, 0.88, and 0.87), a hemiacetal group (δ 5.36), an olefinic proton (δ 5.69), and a cyclopropyl methylene group at δ 0.68 and 0.48 (brd, J = 4.4 Hz). Its ^{13}C NMR spectrum revealed 35 carbon signals, which were sorted into eight methyls, nine methylenes, 10 methines, and eight quaternary carbons, including one ester

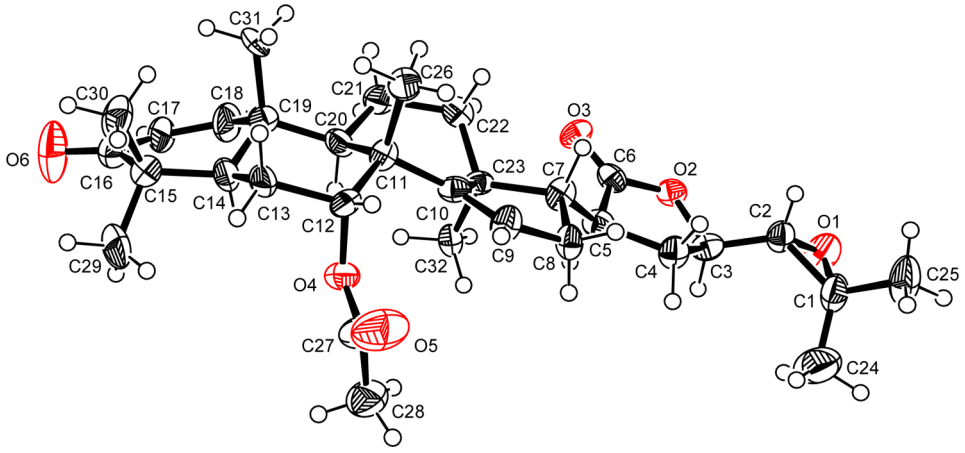

Figure 4. Molecular structure and relative configuration of prototiamin C (**3**). Displacement ellipsoids are drawn at the 50% probability level.

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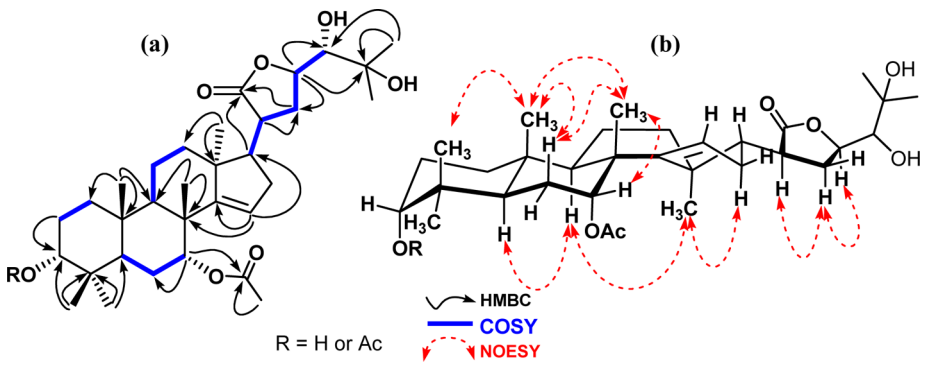

Figure 5. Important HMBC and COSY correlations (a) and key NOESY correlations (b) for **4** and **5**.

carbonyl carbon at δ 166.5 (Table 3). These data suggested **6** to be a glabretal-type triterpenoid.[18] An additional five resonances in the ^{1}H and ^{13}C NMR spectra were consistent with a senecioyl moiety [δ_H 5.69 (s, 1H), 2.18 (CH_3, s), and 1.90 (CH_3, s) and δ_C 166.5, 155.7, 116.6, 27.3, and 20.2]. The location of this ester group at C-3 was inferred from the HMBC spectrum from the cross-peak between the carbonyl signal at δ 166.5 (C-1′) and the proton signal at δ 4.56 (H-3) (Figure 6a). The structure of compound **6** was found to be close to that of the known apotirucallane triterpenoid **7**.[18] Comparison of their NMR data indicated the only difference to be the absence of the *O*-methyl group resonances (δ 3.35, 55.7) in **6**. Interpretation of the NOESY data (Figure 6b) obtained for **6** revealed the same relative configuration as that of **7**.[18] The *S* configuration of C-24 was established on the basis of the coupling constant of H-24 ($J \approx 0$ Hz).[24,25] Thus, structure **6** was elucidated as shown and assigned the trivial name prototiamin F.

Prototiamin G (**9**) was isolated as a red oil. Its molecular formula was determined to be $C_{35}H_{54}O_7$ by the pseudomolecular ion peak $[M + H]^+$ at m/z 587.3955 (calcd for $C_{35}H_{55}O_7$, 587.3942) in the HRESIMS. The ^{1}H and ^{13}C NMR spectra (Table 3) of **9** were similar to those of **6**, suggesting that compound **9** is also a glabretal-type triterpene.[18] Characteristic signals were observed for cyclopropylmethylene protons [δ 0.48 and 0.72 (1H, d, J = 4.5 Hz)] and a senecioyl moiety [5.79 (1H, s), 1.92 (CH_3, s), and 2.20 (CH_3, s)]. Further comparison of their ^{13}C NMR data revealed the absence of a signal due to the tertiary methyl at δ 1.28 (Me-27) and signals corresponding to two oxygenated carbons at δ 75.0 (C-24) and 73.4 (C-25) in the ^{13}C NMR spectrum of **6** and the presence of signals of an oxymethylene group at δ 65.0 (C-27) and epoxy group at δ 63.5 (C-24) and 61.0 (C-25) in the ^{13}C NMR spectrum of **9**. The epoxy function was confirmed by resonances of the corresponding proton at δ 3.18 (d, J = 7.7 Hz, H-24) in its ^{1}H NMR spectrum. In the HMBC spectrum, cross-peaks due to the signals for H-27 (δ 3.57, 3.64) with the carbon signals at δ 63.5 (C-24), 61.0 (C-25), and 15.9 (C-26) were observed, and consequently it was inferred that the oxymethylene group is attached at C-25. Careful analysis of the NOESY spectrum showed cross-peaks similar to those observed for compound **6**, indicating the similarity in the configuration of the two compounds **6** and **9**. As established above for compound **3**, the chemical shifts of C-24 (δ 63.5) and C-25 (δ 61.0) and the coupling constant ($J_{23,24}$ = 7.7 Hz) between H-23 and H-24 supported the α-orientation of the epoxide group in **9**, and consequently the *R* configuration was established for C-24.[3,26] The assignments (^{1}H and ^{13}C NMR data) of **9** were established using a comprehensive analysis of the 2D NMR spectra including COSY, HSQC, HMBC, and NOESY experiments and by comparison of its ^{1}H and ^{13}C NMR data with those reported for a glabretal-type triterpene.[18] On the basis of all evidence obtained, the structure of compound **9** (prototiamin G) was determined as shown.

seco-Tiaminic acid A (**10**) was obtained as colorless needles, and its molecular formula was determined to be $C_{30}H_{50}O_5$ by HRESIMS, which showed a pseudomolecular ion peak $[M + H]^+$ at m/z 491.3734 (calcd for 491.3736, $C_{30}H_{51}O_5$). The HRESIMS exhibited characteristic fragments for a 3,4-*seco*-triterpenoid with a prominent peak at m/z 418.3318 $[M + H - C_2H_4COOH]^+$.[27] The ^{1}H NMR spectrum (Table 3) indicated six resonances of methyl groups at δ 1.25, 1.23, 1.05, 0.91, and 0.86 (singlet each) and at δ 0.96 (d, J = 6.5 Hz). Resonances for three characteristic methylene groups were also observed at δ 1.63, 1.65, and 4.86 (brs). The ^{13}C NMR spectrum (Table 3) in conjunction with the HSQC experiment exhibited 30 carbon signals, which were sorted into seven methyls, nine methylenes, seven methines, and seven quaternary carbons, including one carboxylic acid group at δ 177.0, one sp^2 methylene group at δ 147.8 and 113.3, and three oxygenated carbons at δ 75.6, 73.7, and 68.9. In the HMBC spectrum (Figure 7a), signals of the methylene group at δ 1.63 and 1.65 displayed cross-peaks with the carbonyl group at δ 177.0. These data suggested that compound **10** is a 3,4-*seco*-tirucallane triterpenoid.[27] Further analysis of the HMBC spectrum showed cross-peaks between the broad singlet at δ 4.86 (sp^2 methylene protons) and carbon signals at δ 49.4 (C-5), 21.9 (C-28), and 147.8 (C-4), confirming the presence of an isopropenyl group with a double bond located between C-4/C-29. Furthermore, another double bond could be located between C-7/C-8, based on the long-range correlations observed in the HMBC spectrum between the olefinic proton at δ 5.27 and the carbon signals at δ 49.4 (C-5), 30.3 (C-6), and 147.8 (C-8). Additional cross-peaks were observed in the HMBC spectrum between the proton signals at δ 4.05 (H-23) and 3.16 (H-24) and between the tertiary oxygenated carbon signal at δ 73.7 (C-25) and the methylene signal at δ 41.1 (C-22). This indicated the presence of three hydroxy groups in the side chain (Figure 7a). In the NOESY spectrum, typical cross-peaks were observed indicating that the relative configuration of the tetracyclic core in **10** was identical to that of 3,4-secotirucalla-23-oxo-4(28),7,24-trien-21-al-3-oic acid isolated from *Entandrophragma angolense*.[27] Briefly, cross-peaks between H-5/H-9 and H-9/Me-18 revealed that H-5, H-9, and Me-18 are α-oriented (Figure 7b). However, it was not possible to determine the configurations at C-23 and C-24. On the basis of the spectroscopic evidence obtained, the

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Table 3. ^{1}H and ^{13}C NMR Data for Compounds 6, 9, and 10[a]

	6[b]		9[b]		10[c]	
position	δ_C mult.	δ_H (J in Hz)	δ_C mult.	δ_H (J in Hz)	δ_C mult.	δ_H (J in Hz)
1	38.3 CH_2	1.06 (1H) m	38.0 CH_2	1.03 (1H) m	32.1 CH_2	1.63 (2H) m
		1.63 (1H) m		1.60 (1H) m		
2	24.1 CH_2	1.60 (1H) m	24.1 CH_2	1.58 (1H) m	28.0 CH_2	2.22 (1H) m
		1.74 (1H) m		1.72 (1H) m		2.39 (1H) m
3	79.7 CH	4.56 (1H) dd (5.3, 11.5)	78.0 CH	4.70 (1H) m	177.0 C	
4	37.1 C		36.8 C		147.8 C	
5	46.0 CH	1.61 (1H) m	46.0 CH	1.56 (1H) m	49.4 CH	2.48 (1H) dd (5.7, 12.2)
6	24.1 CH_2	1.60 (1H) m	24.1 CH_2	1.58 (1H) m	30.3 CH_2	2.03 (1H) m
		1.74 (1H) m		1.72 (1H) m		2.24 (1H) m
7	74.3 CH	3.79 (1H) m	74.4 CH	3.77 (1H) m	118.1 CH	5.27 (1H) brd (3.4)
8	38.8 C		38.7 C		147.8 C	
9	44.1 CH	1.24 (1H) m	44.1 CH	1.23 (1H) m	41.0 CH	2.55 (1H) m
10	36.9 C		37.1 C		36.8 C	
11	25.6 CH_2	1.74 (1H) m	25.6 CH_2	1.75 (1H) m	18.6 CH_2	1.57 (2H) m
		2.10 (1H) m		2.10 (1H) m		
12	26.2 CH_2	1.55 (1H) m	25.4 CH_2	1.84 (1H) m	34.1 CH_2	1.49 (1H) m
		1.94 (1H) m		1.95 (1H) m		1.74 (1H) m
13	28.8 C		28.9 C		43.4 C	
14	36.9 C		36.8 C		51.5 C	
15	25.6 CH_2	1.74 (1H) m	25.6 CH_2	1.75 (1H) m	34.2 CH_2	1.74 (1H) m
		2.10 (1H) m		2.10 (1H) m		1.89 (1H) m
16	26.2 CH_2	1.55 (1H) m	25.4 CH_2	1.84 (1H) m	28.3 CH_2	1.38 (1H) m
		1.94 (1H) m		1.95 (1H) m		2.03 (1H) m
17	44.9 CH	2.19 (1H) m	44.7 CH	2.19 (1H) m	54.2 CH	1.57 (1H) m
18	13.7 CH_2	0.48 (1H) d (4.4)	14.1 CH_2	0.48 (1H) d (4.5)	21.7 CH_3	0.91 (3H) s
		0.68 (1H) d (4.4)		0.72 (1H) d (4.5)		
19	15.9 CH_3	0.91 (3H) s	16.3 CH_3	0.92 (3H) s	15.5 CH_3	0.86 (3H) s
20	48.7 CH	1.86 (1H) m	49.2 CH	1.86 (1H) m	33.8 CH	1.48 (1H) m
21	97.6 CH	5.36 (1H) brs	98.2 CH	5.43 (1H) d (3.9)	18.5 CH_3	0.96 (3H) d (6.5)
22	29.4 CH	1.85 (1H) m	28.9 CH	1.74 (1H) m	41.1 CH_2	1.17 (1H) m
		1.98 (1H) m		2.02 (1H) m		1.90 (1H) m
23	78.7 CH	4.49 (1H) t (7.3)	79.7 CH	4.55 (1H) ddd (1.1, 3.5, 7.7)	68.9 CH	4.05 (1H) dd (4.9, 8.8)
24	75.0 CH	3.17 (1H) brs	63.5 CH	3.18 (1H) d (7.7)	75.6 CH	3.16 (1H) brs
25	73.4 C		61.0 C		73.7 C	
26	26.7 CH_3	1.30 (3H) s	15.9 CH_3	1.33 (3H) s	25.4 CH_3	1.23 (3H) s
27	26.7 CH_3	1.28 (3H) s	65.0 CH_2	3.57 (1H) m	26.0 CH_3	1.25 (3H) s
				3.64 (1H) m		
28	16.8 CH_3	0.87 (3H) s	28.4 CH_3	0.91 (3H) s	21.9 CH_3	1.80 (3H) s
29	27.7 CH_3	0.88 (3H) s	21.0 CH_3	0.87 (3H) s	113.3 CH_2	4.86 (2H) brs
30	19.5 CH_3	1.04 (3H) s	19.5 CH_3	1.07 (3H) s	27.0 CH_3	1.05 (3H) s
1′	166.5 C		166.5 C			
2′	116.6 CH	5.69 (1H) s	116.6 CH	5.79 (1H) s		
3′	155.7 C		155.8 C			
4′	27.3 CH_3	1.90 (3H) s	27.6 CH_3	1.92 (3H) s		
5′	20.2 CH_3	2.18 (3H) s	20.2 CH_3	2.20 (3H) s		

[a]The chemical shifts are in δ values (ppm) from TMS. ^{13}C multiplicities were determined by HSQC experiment. [b]Recorded in $CDCl_3$ at 500 MHz (^{1}H) and 125 MHz (^{13}C). [c]Recorded in CD_3OD at 500 MHz (^{1}H) and 100 MHz (^{13}C).

structure of compound **10** (*seco*-tiaminic acid A) was assigned as shown.

The isolated compounds (**1**–**6**, **8**–**10**) were evaluated against a chloroquine-sensitive strain of the malaria parasite (*Plasmodium falciparum* NF54). The cytotoxicity of the compounds was also evaluated using a rat skeletal myoblast (L6) cell line, as described earlier.[28] Compounds **1**, **3**, and **5** displayed strong antiplasmodial activity (0.44 to 0.87 μM). Compound **1** displayed strong selectivity for the *P. falciparum* NF54 strain (SI 104), while **3** and **5** had selective indices of 12 (see Table 4). Compounds **2**, **4**, **6**, and **8** were active against *P. falciparum*, with IC_{50} values ranging from 1.3 to 2.0 μM, and were less selective, while compound **10** was found to be inactive.

The present investigation of the bark of *E. congoënse* has yielded eight new compounds (**1**–**6**, **9**, **10**), which showed some diversity in the production of bioactive secondary metabolites. This may be exemplified in the functional group variation of the basic carbon skeleton ranging from changes in the degree of oxidation, alkylation, or acylation to chain and ring-closed forms. The new compounds **1**, **3**, and **5** showed

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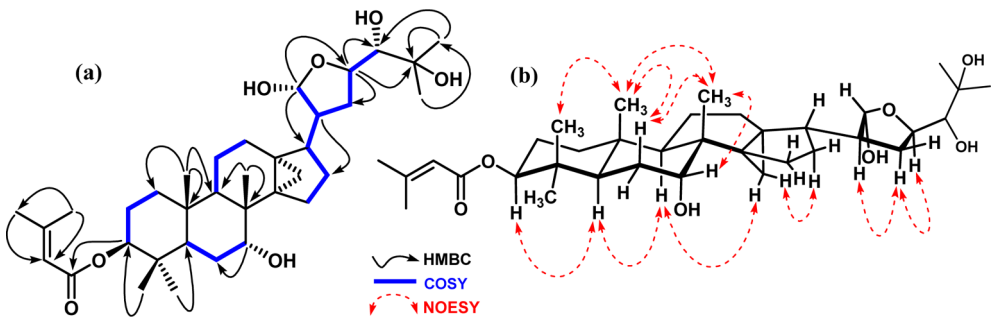

Figure 6. Important HMBC and COSY correlations (a) and key NOESY correlations (b) for **6**.

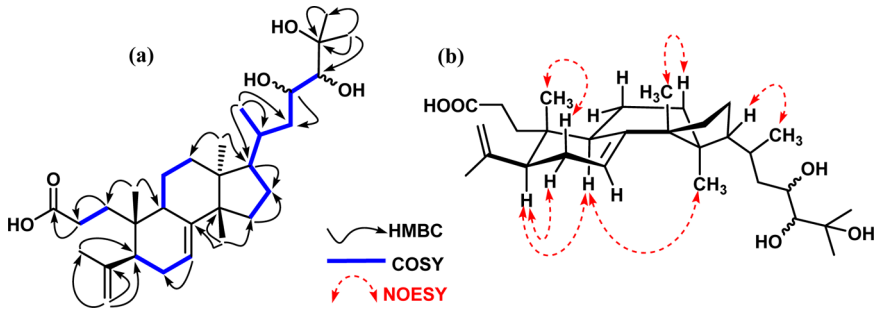

Figure 7. Important HMBC and COSY correlations (a) and key NOESY correlations (b) for **10**.

Table 4. Antiplasmodial and Cytotoxic Activity Data of Compounds 1−6 and 8−10 Compared to Standard References (Chloroquine and Podophyllotoxin)[a]

	antiplasmodial activity	cytotoxic activity	
compound	*Plasmodium falciparum* NF54	rat skeletal myoblasts L6	selectivity index
1	0.67	70.6	104.7
2	1.3	32.3	25.2
3	0.44	5.3	12.1
4	2.0	42.4	21.0
5	0.87	11.0	12.7
6	1.4	6.5	4.4
8	1.3	11.9	9.0
9	1.3	8.6	6.8
10	19.3	56.1	2.9
chloroquine	0.006		
podophyllotoxin		0.012	

[a]IC_{50} values (in μM) are the means of two independent assays.

significant antiplasmodial activity in vitro, which supports the use of this plant in traditional medicine in Cameroon.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Gallenkamp melting point apparatus (Loughborough, U.K.) and are uncorrected. Optical rotations were measured on a PerkinElmer polarimeter, model 241. The UV spectra were recorded on a PerkinElmer Lambda 35 UV/vis spectrometer. To monitor analytical HPLC elution, a photodiode array detector (λ = 205 nm) was used in the wavelength range 200−800 nm. Xcalibur software (Thermo Fisher Scientific, Bremen, Germany) was used for data acquisition and for manually browsing the acquired data. IR measurements were obtained on a PerkinElmer (model 1600) FTIR spectrometer. The NMR spectra were recorded in $CDCl_3$ and CD_3OD using Bruker Avance DRX 400 or 500 MHz and Varian Unity Inova 600 MHz NMR spectrometers. Chemical shifts (δ) are stated in parts per million (ppm) from the internal standard, tetramethylsilane (TMS). The $^3J_{C,H}$ couplings were measured by means of pulsed field gradient HMBC spectra recorded by varying the *J*-refocusing time between t = 0.04 and 0.14 s. The high-resolution mass spectra were obtained with an LTQ Orbitrap spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a HESI-II source. Flash column chromatography was performed using silica gel 60 (Merck, 0.040−0.063 mm). Preparative reversed-phase HPLC was carried out with a Gilson system consisting of a 322 pump with a UV detector 152 (λ = 205 nm) using a Nucleodur gravity column from Macherey-Nagel (Düren, Germany) (250 × 16 mm, 5 μm particle size). Separation was achieved by using an H_2O (A)−MeOH (B) gradient program as follows (flow rate 6 mL·min^{-1}): 50% A isocratic for 2 min, followed by variation from 50% B to 100% B for 4 min, after 100% B isocratic for 9 min. Afterward, the system was returned to its initial condition (50% A) within 2 min and was equilibrated for 3 min. Single-crystal X-ray diffraction analysis was performed on an Oxford Diffraction Xcalibur S diffractometer at 173(2) K using graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å).

Plant Material. The bark of *Entandrophragma congoënse* was collected in June 2012 at Nkomokui in the Centre region of Cameroon (near Yaoundé). The plant material was identified by Mr. Victor Nana, an experienced botanist at the National Herbarium of Yaoundé, where a voucher specimen (No. 43234 HNC) was deposited.

Extraction and Isolation. The dried and powdered stem bark of *E. congoënse* (5.1 kg) was macerated two times with a mixture of dichloromethane−methanol (1:1) for 48 and 8 h, respectively. Evaporation under reduced pressure afforded a crude extract (386 g). A part of the crude extract (350 g) was subjected to silica gel flash column chromatography using a stepwise gradient of *n*-hexane/EtOAc followed by a gradient of EtOAc/MeOH, to afford six fractions: A (65 g, pure *n*-hexane), B (112 g, *n*-hexane/EtOAc 50%), C (23 g, pure EtOAc), D (21 g, EtOAc/MeOH 30%), E (32 g, EtOAc/MeOH 50%), and F (15 g, pure MeOH). Fraction A was found to contain mainly fatty acids. Part of fraction B (110 g) was submitted to open silica gel column chromatography eluting with a gradient of *n*-hexane/EtOAc (0 to 75%) to afford compounds lupeone (86 mg), methyl angolensate (102 mg), and **3** (8 mg), respectively. Fractions C and D were combined and subjected to a silica gel column chromatographic

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separation using a stepwise gradient of CH_2Cl_2–MeOH to give seven subfractions, coded JM1–7. Subfractions JM3 (11.8 g) and JM4 (10.2 g) were further purified by silica gel column chromatography using a cyclohexane–ethyl acetate (15 to 95%) gradient to afford two series of fractions, F1–48 from JM3 and H1–63 from JM4, which were combined based on their TLC profiles.

Fractions F23–36 (10.5 g, cyclohexane–ethyl acetate 40%) were rechromatographed on a silica gel column using a mixture of cyclohexane–ethyl acetate with increasing polarity (from 10% to 50%) to give 40 fractions of ca. 150 mL, which were combined on the basis of their TLC and LC-MS profile to five series (I–V). From series II (2.0 g, cyclohexane/ethyl acetate 30%), compound **1** (48 mg) was obtained, and series III (2.7 g, cyclohexane/ethyl acetate 35%) gave compound **5** (16 mg). Series IV (3.0 g, cyclohexane/ethyl acetate 45%) was submitted for further purification using reversed-phase semipreparative HPLC (see General Experimental Procedures), to give two compounds, **6** (21 mg, t_R 7.64 min) and **2** (14 mg, t_R 5.02 min).

Fractions H12–27 (8.0 g, cyclohexane–ethyl acetate 30%) were further subjected to reversed-phase semipreparative HPLC, as described above, to afford compounds **4** (10.5 mg, t_R 4.63 min), **7** (6.0 mg, t_R 8.67 min), and **8** (11.6 mg, t_R 9.51 min). Fractions H41–54 (cyclohexane–ethyl acetate 60%) were also purified using semipreparative HPLC to give two compounds, **10** (20.0 mg, t_R 8.65 min) and **9** (12.0 mg, t_R 9.67 min).

*Prototiamin A (**1**):* white powder (CH_2Cl_2); mp 150–151 °C; $[\alpha]^{20}_D$ −53 (*c* 0.1, $CHCl_3$); UV (MeOH) (log ε) λ_{max} 246 (2.60), 227 (0.44) nm; IR (KBr) ν_{max} 3649, 2979, 2322, 1768, 1666, 1377, 1245, 1030, 668 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HRESIMS *m/z* 543.3314 $[M + H]^+$ (calcd for $C_{32}H_{47}O_7$, 543.3316).

*Prototiamin B (**2**):* white powder (MeOH); mp 176–177 °C; $[\alpha]^{20}_D$ −60 (*c* 0.03, $CHCl_3$); UV (MeOH) (log ε) λ_{max} 243 (1.99), 225 (0.46) nm; IR (KBr) ν_{max} 3460, 2733, 2712, 2671, 1738, 1244, 754 cm^{-1}; ^1H ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Tables 1 and 2; HRESIMS *m/z* 501.3223 $[M + H]^+$ (calcd for $C_{30}H_{45}O_6$, 501.3211).

*Prototiamin C (**3**):* white crystals (CH_2Cl_2); mp 161–162 °C; $[\alpha]^{20}_D$ +11 (*c* 0.1, $CHCl_3$); UV (MeOH) (log ε) λ_{max} 246 (2.47) nm; IR (KBr) ν_{max} 3456, 2948, 1718, 1387, 1163, 756 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HRESIMS *m/z* 525.3219 $[M + H]^+$ (calcd for $C_{32}H_{45}O_6$, 525.3211).

*Prototiamin D (**4**):* red oil; $[\alpha]^{20}_D$ −71 (*c* 0.1, $CHCl_3$); UV (MeOH) (log ε) λ_{max} 243 (2.13) nm; IR (KBr) ν_{max} 3466, 2943, 1765, 1713, 1658, 1379, 1255, 1029, 764 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HRESIMS *m/z* 547.3642 $[M + H]^+$ (calcd for $C_{32}H_{51}O_7$, 547.3629).

*Prototiamin E (**5**):* white powder (CH_2Cl_2); mp 203–204 °C; $[\alpha]^{20}_D$ −69 (*c* 0.1, $CHCl_3$); UV (MeOH) (log ε) λ_{max} 241 (0.92) nm; IR (KBr) ν_{max} 3629, 2945, 1768, 1725, 1374, 1249, 1026, 781 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; HRESIMS *m/z* 589.3737 $[M + H]^+$ (calcd for $C_{34}H_{53}O_8$, 589.3735).

*Prototiamin F (**6**):* white powder (MeOH); mp 135–136 °C; $[\alpha]^{20}_D$ +15.3 (*c* 0.15, $CHCl_3$); UV (MeOH) (log ε) λ_{max} 246 (2.67), 216 (0.89), 212 (0.78), 201 (0.55) nm; IR (KBr) ν_{max} 3411, 2945, 1710, 1338, 1229, 1150, 1078, 997, 755 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 3; HRESIMS *m/z* 589.4100 $[M + H]^+$ (calcd for $C_{35}H_{57}O_7$, 589.4099).

*Prototiamin G (**9**):* red oil; $[\alpha]^{20}_D$ +7 (*c* 0.1, $CHCl_3$); UV (MeOH) (log ε) λ_{max} 243 (2.35), 222 (0.72), 210 (0.66), 205 (0.72) nm; IR (KBr) ν_{max} 3457, 2946, 1710, 1385, 1149, 754 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 3; HRESIMS *m/z* 587.3955 $[M + H]^+$ (calcd for $C_{35}H_{55}O_7$, 587.3942).

*seco-Tiaminic acid A (**10**):* colorless needles (MeOH); mp 145–146 °C; $[\alpha]^{20}_D$ −3 (*c* 0.1, $CHCl_3$); UV (MeOH) (log ε) λ_{max} 232 (3.27) nm; IR (KBr) ν_{max} 3649, 2949, 1707, 1378, 755 cm^{-1}; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 3; HRESIMS *m/z* 491.3734 $[M + H]^+$ (calcd for $C_{30}H_{51}O_5$, 491.3736).

Hydrolysis of Prototiamin A (1). To a solution of prototiamin A (**1**, 10 mg, 0.018 mmol) was added 5 mL of a solution of NaOH (5%), and the mixture was stirred at room temperature under reflux conditions for 6 h. The reaction medium was monitored constantly by TLC, which revealed at a given time the disappearance of the starting material. Then, the reaction medium was quenched with 2 mL of 2% H_2SO_4 and extracted five times (3 mL of ethyl acetate each), and the organic solution obtained was dried with anhydrous Na_2SO_4. The solvent was then evaporated under reduced pressure, and the residue subjected to preparative HPLC using the same solvent system and conditions as described above (see General Experimental Procedures) to afford compound **1c** (6.1 mg, t_R 5.14 min) as a white powder: UV (MeOH) (log ε) λ_{max} 242 (1.97), 226 (0.49) nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.17 (1H, d, *J* = 10.3 Hz, H-1), 5.86 (1H, d, *J* = 10.3 Hz, H-2), 2.21 (1H, m, H-5), 2.26 (1H, m, H-6a), 2.41 (1H, m, H-6b), 3.50 (1H, brs, H-7), 2.24 (1H, m, H-9), 2.26 (2H, m, H_2-11), 2.25 (2H, m, H_2-12), 5.31 (1H, brs, H-15), 2.17 (2H, m, H_2-16), 2.24 (1H, m, H-17), 1.20 (3H, s, CH_3-18), 1.06 (3H, s, CH_3-19), 2.76 (1H, m, H-20), 2.00 (1H, m, H-22a), 2.39 (1H, m, H-22b), 4.64 (1H, ddd, *J* = 2.0, 6.3, 10.3 Hz, H-23), 3.30 (1H, brs, H-24), 1.32 (3H, s, CH_3-26), 1.37 (3H, s, CH_3-27), 1.09 (3H, s, CH_3-28), 1.09 (3H, s, CH_3-29), 1.21 (3H, s, CH_3-30); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.5 (CH, C-1), 125.8 (CH, C-2), 205.5 (C, C-3), 45.0 (C-4), 45.3 (CH, C-5), 24.7 (CH_2, C-6), 72.0 (CH, C-7), 44.6 (C, C-8), 37.2 (CH, C-9), 40.6 (C, C-10), 32.9 (CH_2, C-11), 16.6 (CH_2, C-12), 47.2 (C, C-13), 158.5 (C, C-14), 119.9 (CH, C-15), 32.5 (CH_2, C-16), 54.6 (CH, C-17), 19.2 (CH_3, C-18), 20.7 (CH_3, C-19), 44.6 (CH, C-20), 177.9 (C, C-21), 30.3 (CH_2, C-22), 77.8 (CH, C-23), 76.5 (CH, C-24), 72.9 (C, C-25), 26.9 (CH_3, C-26), 26.9 (CH_3, C-27), 21.8 (CH_3, C-28), 27.4 (CH_3, C-29), 28.1 (CH_3, C-30); HRESIMS *m/z* 501.3222 $[M + H]^+$ (calcd for $C_{30}H_{45}O_6$, 501.3216).

Esterification of Prototiamin A (1) with (*R*)- and (*S*)-MTPA Chloride. Two portions of prototiamin A (**1**) (11.4 mg, 0.021 mmol each) were treated with (*R*)- and (*S*)-MTPA chloride in 4-(dimethylamino)pyridine (DMAP) and triethylamine, stirring at room temperature. The two reactions were conducted in parallel, and the progress was monitored by LC-HRMS. After complete consumption of **1**, the solvent was evaporated and the reaction mixture extracted with ethyl acetate, and then the extract obtained was submitted to preparative HPLC (gradient 50–100% CH_3OH in 20 min) to afford the (*S*)-MTPA (**1a**, t_R 13.32 min) and (*R*)-MTPA (**1b**, t_R 12.01 min) esters of prototiamin A (**1**).

X-ray Diffraction Analysis. The crystals of compound **3** were covered with an inert oil (perfluoropoly alkyl ether). Additionally, the crystal structures were solved with direct methods (SHELXS-97)[29] and refined against F^2 with the full-matrix least-squares method (SHELXL-97).[30,31] A multiscan absorption correction using the implemented CrysAlis RED program was employed. The non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed in geometrically calculated positions, and each was assigned a fixed isotropic displacement parameter based on a riding model.

Crystallographic data of prototiamin C (**3**) (for key parameters see Table S1 in the Supporting Information) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 1053913). A copy of these data can be obtained free of charge via the Internet at www.ccdc.cam.ac.uk/conts/retrieving.html or on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. [tel: (+44) 1223-336-408; fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk].

Antiplasmodial Activity. In vitro activity against the erythrocytic stages of chloroquine-sensitive *Plasmodium falciparum* strain NF54 was assayed using a ^3H-hypoxanthine incorporation assay,[32−34] the chloroquine- and pyrimethamine-resistant NF54 strain that originated from Thailand,[33] and the standard drug chloroquine (Sigma-Aldrich). Compounds were dissolved in DMSO at 10 μg/mL and added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), $NaHCO_3$ (2.1 g/L), neomycin (100 U/mL), Albumax, and washed human red cells A^+ at 2.5% hematocrit (0.3% parasitemia). Serial drug dilutions of 11

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3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4% CO_2, 3% O_2, and 93% N_2. After 48 h, 50 μL of [^{3}H] hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fiber filter were then washed with distilled water. The dried filters were inserted into a plastic foil with 10 mL of scintillation fluid and counted in a Betaplate liquid scintillation counter (Wallac). IC_{50} values were calculated from sigmoidal inhibition curves by linear regression.

In Vitro Cytotoxicity with L6 Cells. Assays were performed in 96-well microtiter plates, with each well containing 100 μL of RPMI 1640 medium, supplemented with 1% L-glutamine (200 mM) and 10% fetal bovine serum, and 4000 L6 cells (a primary cell line derived from rat skeletal myoblasts).[35] Serial drug dilutions of 11 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. After 70 h of incubation, the plates were inspected under an inverted microscope to ensure growth of the controls and sterile conditions. Then, 10 μL of Alamar Blue was added to each well, and the plates were incubated for another 2 h. Next, the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. The IC_{50} values were calculated by linear regression from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Corporation). The IC_{50} values in μmol/mL are the means of two independent assays; the individual values varied less than ±50%.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **1**–**6**, **9**, and **10** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This paper is dedicated to the memory of Prof. Dr. Karsten Krohn, who passed away on October 10, 2013.

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