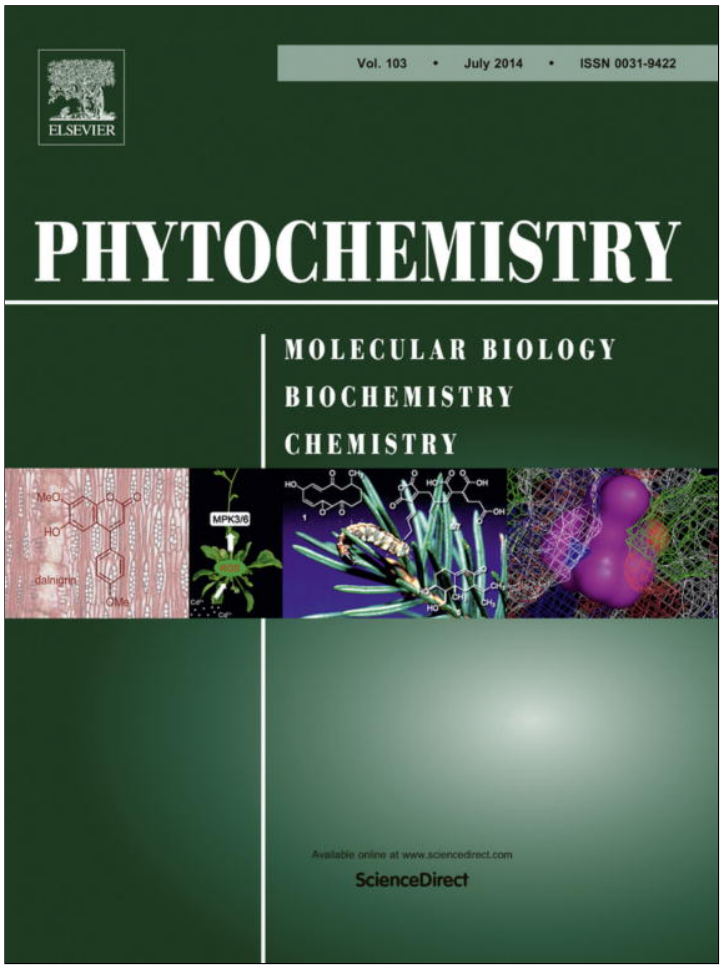

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Bisbenzylisoquinoline and hasubanane alkaloids from *Stephania abyssinica* (Dillon & A. Rich) (Menispermeceae)

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ABSTRACT

Two bisbenzylisoquinoline and one hasubanane alkaloids: (−)-pseudocurine (**1**), (−)-pseudoisocurine (**2**) and (−)-10-oxoaknadinine (**3**), were isolated from leaf extract of *Stephania abyssinica*, a plant used in traditional medicine in South Nyanza region of Kenya. They were characterized using 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, NOESY, HMQC and HMBC) NMR techniques. (−)-Pseudocurine (**1**) and (−)-pseudoisocurine (**2**) exhibited strong to moderate anti-plasmodial activity while (−)-10-oxoaknadinine (**3**) showed moderate to mild activity.

Introduction

Each year, over 500 million malaria cases and 2.7 million deaths resulting from the parasite infections are reported worldwide (Greenwood et al., 2005; WHO, 2002). The resurgence of the disease is attributed partly to development of drug resistance by the most common and virulent malaria parasite (*Plasmodium falciparum*). Consequently, there is need for the discovery and development of new drugs preferably with different modes of action. The plant kingdom is an important source of anti-parasitic drugs or lead compounds in drug discovery and development. Two of the anti-malarial drugs: quinine and artemisinin, that in use today were obtained directly from plants and more potent derivatives and analogues developed (Philipson et al., 1993). Many rural communities in Africa, Asia and South America depend on the plant kingdom for traditional anti-malarial therapy. *Stephania abyssinica* has been used for traditional anti-malarial therapy by the communities living in the South Nyanza, Kenya. Previous investigations revealed that extracts of *S. abyssinica* exhibited strong anti-plasmodial activity (Muregi et al., 2003). We report the isolation and characterization of two new anti-plasmodial bisbenzlyisoquinoline alkaloids (BBIQ): (−)-pseudocurine (**1**) and (−)-pseudoisocurine (**2**); together with a mildly active hasubanane alkaloid, 10-oxoaknadinine (**3**) from *S. abyssinica*.

Results and discussion

The NMR spectral data for the isolated compounds are summarized in Table 1–3.

The presence of two (2) N-methyl groups in **1** was suggested by the peaks at δ_H 2.31 (s) and 2.68 (s) in ^{1}H NMR and δ_C 40.1 and 40.2

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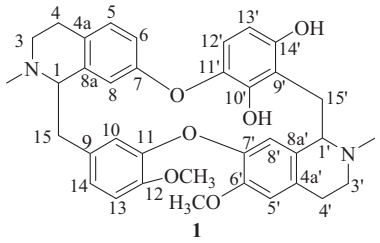

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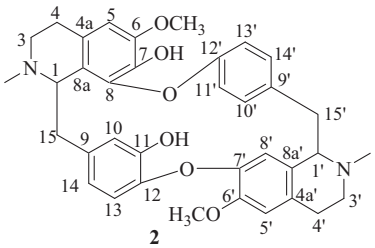

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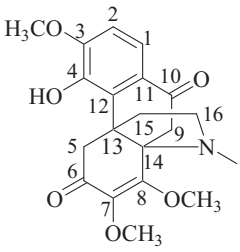

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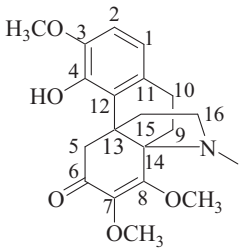

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in ^{13}C NMR spectra (Table 1). Similarly, the presence of two methylene groups attached to nitrogen atoms was indicated by the pairs of peaks at: δ_H 2.92 (m), 3.42 (ddd); and 3.07 (m), 3.50 (m) in the ^{1}H NMR and δ_C 43.6 and 45.0 in ^{13}C NMR spectra. The two –NCH$_2$ groups were found to be next to two pairs of benzylic protons at: δ_H 2.57 (dd), 3.03 (m); and 2.99 (m), 3.08 (m), respectively, from

Table 1
^{1}H (500 MHz, CD_3OD) and ^{3}C NMR data for (−)-pseudocurine (**1**).

Position	δ_C	DEPT	δ_H, m, *J* (Hz)	COSY	HMBC
1	60.2	CH	3.78 dd (13.8, 9.4)	H-15	C-15, C-8a, C-4a
3	43.6	CH_2	2.92 m	H-4, H-3 eq	C-1, C-4
			3.42 ddd (13.0, 5.2, 4.6)	H-4, H-3ax	
4	21.4	CH_2	2.57 dd (12.0, 4.6)	H-3, H-4 eq	C-3, C-5, C-4a, C-8a
			3.03 m	H-3, H-4ax	
4a	123.9	q			
5	108.0	CH	6.67 d (8.2)	H-6	C-4, C-7, C-8a
6	131.7	CH	6.43 dd (8.2, 1.7)	H-5, H-8	C-8, C-4a
7	156.0	q			
8	114.7	CH	6.67 d (1.7)	H-6	C-6, C-1, C-4a
8a	123.0	q			
9	132.6	q			
10	121.1	CH	6.45 d (1.8)	H-14	C-15, C-12, C-14
11	142.0	q			
12	146.7	q			
13	115.1	CH	6.78 d (8.3)	H-14	C-11, C-9
14	125.3	CH	7.05 dd (8.3, 1.8)	H-13, H-10	C-15, C-10, C-12
15	39.2	CH_2	2.71 dd (13.8, 9.4)	H-1, H-15 eq	C-9, C-1, C-14, C-10
			2.88 dd (13.8, 9.4)	H-1, H-15ax	
1′	64.7	CH	3.82 dd (10.0, 2.1)	H-15′	C-8a′, C-15′
3′	45.0	CH_2	3.07 m	H-4′, H-3′eq	C-4, C-4a′
			3.50 m	H-4′, H-3’ax	
4′	23.7	CH_2	2.99 m	H-3′, H-4′eq	C-8a′, C-4a′, C-3′
			3.08 m	H-3′, H-4’ax	
4′a	126.1	q			
5′	112.6	CH	6.90 s		C-8a′, C-7′, C-4′
6′	149.3	q			
7′	144.3	q			
8′	117.6	CH	5.75 s		C-6′, C-4a′, C-1′
8a′	126.6	q			
9′	130.9	q			
10′	138.8	q			
11′	148.0	q			
12′	129.2	CH	7.20 d (7.5)	H-13′	C-10′, C-14′
13′	113.5	CH	6.67 d (7.5)	H-12′	C-11′, C-9′
14′	137.7	q			
15′	37.9	CH_2	2.73 dd (10.0, 2.1)	H-1′, H-15′eq	C-8a′, C-1′, C-9′, C-14′
			3.24 dd (10.0, 2.1)	H-1′, H-15’ax	
2-NCH_3	40.1	CH_3	2.31 s		C-1, C-3
2′-NCH_3	40.2	CH_3	2.68 s		C-1′, C-3′
6′-OCH_3	55.29	CH_3	3.89 s		C-6′
12-OCH_3	55.26	CH_3	3.90 s		C-12

Table 2
^{1}H (500 MHz, CD_3OD) and ^{13}C NMR data for (−)-pseudoisocurine (**2**).

Position	δ_C	DEPT	δ_H, m, *J* (Hz)	COSY	HMBC
1	60.0	CH	4.50 d (9.8)	H-15	C-15, C-8a, C-4a
3	45.5	CH_2	3.13 m	H-4, H-3 eq	C-1, C-4
			3.70 m	H-4, H-3ax	
4	22.3	CH_2	3.14 m	H-3, H-4ax	C-5, C-4a
			2.91 m	H-3, H-4 eq	
4a	122.1	q			
5	108.0	CH	6.77 s		C-4, C-7, C-8a
6	148.7	q			
7	138.7	q			
8	137.4	q			
8a	121.4	q			
9	132.6	q			
10	121.4	CH	6.48 d (1.7)	H-14	C-14, C-12, C-15
11	142.6	q			
12	147.6	q			
13	116.7	CH	6.81 d (8.3)	H-14	C-11, C-9
14	126.3	CH	6.91 dd (8.3, 1.7)	H-13, H-10	C-15, C-10, C-12
15	38.5	CH_2	2.88 dd (16.0, 9.8)	H-1, H-15 eq	C-9, C-1, C-14, C-10
			3.00 dd (16.0, 9.8)	H-1, H-15ax	
1′	64.2	CH	4.15 d (7.0)	H-15′	C-8a′
3′	45.3	CH_2	3.77 m	H-4′, H-3′ax	C-4a′
			3.28 m	H-4′, H-3′eq	
4′	22.9	CH_2	2.95 m	H-3′, H-4′eq	C-8a′, C-4a′, C-3′
			3.14 m	H-3′, H-4′ax	
4′a	125.3	q			
5′	112.4	CH	6.95 s		C-8a′, C-7′, C-4′
6′	149.4	q			
7′	144.9	q			
8′	116.1	CH	5.65 s		C-6′, C-4a′, C-1′, C-12
8a′	123.3	q			
9′	129.6	q			
10′	132.5	CH	6.43 bd (8.4)	H-11′	C-15′, C-12′, C-14′
11′	114.7	CH	6.89 dd (8.4, 2.2)	H-10′, H-13′	C-13′, C-9′
12′	156.0	q			
13′	113.1	CH	6.74 dd (8.4, 2.2)	H-14′, H-10′	C-9′, C-11′
14′	130.1	CH	7.25 bd (8.4)	H-13′	C-10′, C-12′
15′	37.0	CH_2	2.95 dd (14.7, 7.0)	H-1′, H-15′eq	C-8a′, C-1′, C-9′, C-14′
			3.32 dd (14.7, 7.0)	H-1′, H-15′ax	
2-NCH_3	40.3	CH_3	2.52 s		C-1, C-3
2′-NCH_3	40.1	CH_3	2.81 s		C-1′, C-3′
6-OCH_3	55.4	CH_3	3.92 s		C-6
6′-OCH_3	55.3	CH_3	3.91 s		C-6′

COSY spectra. The presence of two (2) methine groups attached to nitrogen atoms was evident from the peaks at δ_H 3.78 and 3.82 in the ^{1}H NMR and δ_C 60.2 and 64.7 in the ^{13}C NMR spectra. Similarly, the two –NCH– groups were found to be next to another set of two pairs of benzylic protons at: δ_H 2.71 (dd), 2.88 (dd); and 2.73 (dd), 3.24 (dd), respectively. Furthermore, the presence of two methoxyl groups attached to aromatic nuclei was confirmed by the peaks at: δ_H 3.89 (s) and 3.90 (s) in the ^{1}H NMR and δ_C 55.29 and 55.26 in the ^{13}C NMR spectra, respectively. The rest of the NMR signals were in the aromatic region: δ_H 5.75–7.05 and δ_C 108–156. The above spectral information strongly suggested the presence of a bisbenzylisoquinoline (BBIQ) scaffold in the compound.

The electron-impact mass spectrum (EIMS) of **1** showed a molecular ion peak at *m*/*z* 594 $[M]^+$ and a prominent peak at *m*/*z* at 298 indicative of a head to tail linked bisbenzylisoquinoline (BBIQ) (Baldas et al., 1972). HR-EIMS revealed the molecular ion peak at 594.2741 $C_{36}H_{38}N_2O_6$ requires 594.2730. Position of attachment of the two diaryl ether bridges between C-7, C11′; and C-11 and C7′ together with the location of hydroxyl groups at C-10′ and C-14′ were established through interpretation of the HMBC spectrum of the compound. Complete ^{1}H and ^{13}C NMR assignment and structure characterization of **1** was achieved by 1D (^{1}H, ^{13}C and DEPT), 2D NMR spectral (COSY, NOESY, HMQC, HMBC) (Table 1) (Fig 1) and optical data analysis. Compound **1** was thus identified as (−)-pseudocurine.

The ^{1}H and ^{13}C NMR spectral data of **2** (Table 2) had features similar to those highlighted in **1**: two N-methyls (δ_H 2.52 (s), 2.81 (s) and δ_C 40.3, 40.1), two methylene groups (δ_H 3.13 (m), 3.70 (m); 3.28 (m), 3.77 (m) and δ_C 44.5, 45.3) attached to nitrogen atoms, two methine groups (δ_H 4.5 (d), 4.15 (d) and δ_C 60.0, 64.2) attached to nitrogen atoms and two methoxyl groups (δ_H 3.91 (s), 3.92 (s) and δ_C 55.3, 55.4) were identified with the rest of the signals in the aromatic region. The ^{13}C and ^{1}H NMR data strongly suggested a BBIQ skeleton but the chemical shifts suggested a slightly different arrangement of the constituent atoms, in **2** especially the substitution pattern on the two isoquinoline scaffolds. The EIMS of **2** was also basically similar to that of **1** with the highest ion peak at *m*/*z* 595 $[M+1]^+$ and a prominent peak at *m*/*z* 298 consistent with a BBIQ scaffold. HR-EIMS revealed the molecular ion peak at 594.2735 $C_{36}H_{38}N_2O_6$ requires 594.2730. The complete spectral assignment and structure determination of **2** was achieved by 1D (^{1}H, ^{13}C and DEPT), 2D NMR spectral (COSY, NOESY, HMQC, HMBC) (Table 2) (Fig 2) and optical data analysis. NOESY was observed on 6-OCH_3 (δ_H 3.92) when H-13′ (δ_H 6.74) was irradiated and vice versa suggesting that the two groups are close in place. Similarly, NOESY was observed on 6′-OCH_3 (δ_H 3.91) when H-13 (δ_H 6.81) was irradiated and vice versa suggesting that the two groups are close in space. The diaryl ether bridges were finally located between C-8, C-12′ and C-12, C-7′ in **2** and the two methoxyl groups at C-6 and C-6′. Detailed comparison of 1D and 2D NMR

Table 3
^{1}H (400 MHz, CD_3OD) and ^{13}C NMR data for (−)-10-oxoaknadinine (**3**) and aknadinine (**4**).

Position	**3**					**4**	
	∂_C	DEPT	∂_H	COSY	HMBC	∂_C	∂_H
1	120.0	CH	6.51 d (8.3)	H-2	C-3, C-12, C-10	119.13	6.56 d (8.2)
2	110.6	CH	6.71 d (8.3)	H-1	C-11, C-4	108.64	6.66 d (8.2)
3	147.3					145.01	
4	144.4					143.76	
5	44.0	CH_2	2.70 d (16.0)	H-5 eq	C-13, C-6	43.28	2.64 d (16.0)
			3.40 d (16.0)	H-5ax			3.50 d (16.0)
6	194.0					194.82	
7	139.9					138.12	
8	166.0					165.16	
9	23.7	CH_2	2.00 d (16.2)	H-9 eq	C-14, C-10	23.05	1.90 ddd (13.4, 11.3, 4.6)
			2.19 d (16.2)	H-9ax			2.19 ddd (13.4, 4.9, 4.9)
10	196.0					25.22	2.56 ddd (16.2, 4.9, 4.6
							2.79 ddd (16.2, 11.3, 4.9)
11	129.9					128.77	
12	128.0					128.41	
13	47.7					47.13	
14	69.9					67.81	
15	35.2	CH_2	2.33 m	H-16, H-15 eq	C-13, C-16	33.96	2.11 ddd (14.0, 9.5, 4.0)
			2.50 m	H-16, H-15ax			2.47 ddd (14.0, 10.1, 6.4)
16	52.9	CH_2	2.58 m	H-15, H-16 eq	N-CH_3, C-15	51.34	2.67 ddd (9.7, 9.5, 6.4)
			2.72 m	H-15, H-16ax			2.83 ddd (10.1, 9.7, 4.0)
3-OMe	56.6		3.81 s		C-3	56.22	3.83
7-OMe	61.2		3.55 s		C-7	60.73	3.65
8-OMe	61.4		4.08 s		C-8	60.55	4.07
N-Me	36.9		2.53 s		C-14, C-16	36.36	2.53

Data for aknadinine (**4**) is adopted from Kashiwaba et al., 1996 and were run in $CDCl_3$ at 600 MHz.

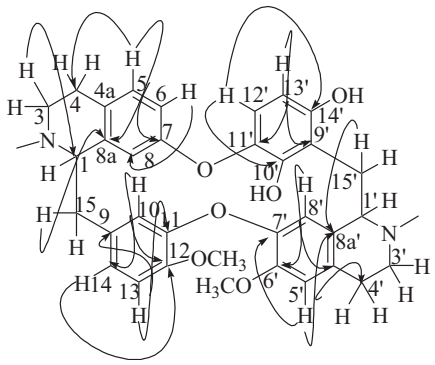

Fig. 1. Diagnostic HMBCs for **1**.

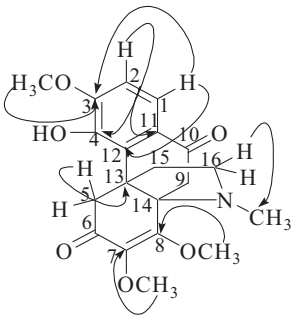

Fig. 3. Diagnostic HMBCs for **3**.

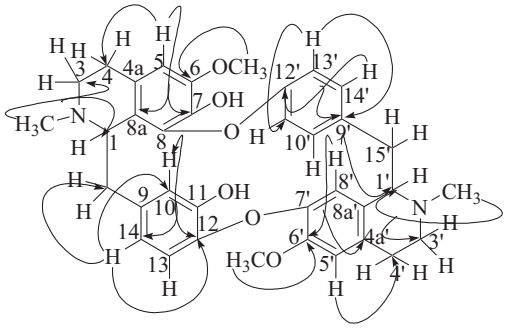

Fig. 2. Diagnostic HMBCs for **2**.

data of the two compounds revealed the following key differences: (i) one of the methoxyl groups was attached to C-6 in **2** instead of C-12 in **1**; and (ii) the hydroxyl groups were found at C-11 and C-7 in **2**, instead of C-10′ and C-14′ in **1**. Compound **2** was therefore identified as (−)-pseudoisocurine.

Compound **3** gave a positive reaction for alkaloids with Dragendoff's reagent. The presence of one N-methyl and three O-methyl groups were obvious from ^{1}H and ^{13}C NMR spectral data from the peaks observed at δ_H 2.53, 3.81, 3.55 and 4.08 and δ_C 36.9, 56.1, 61.2 and 61.4 (Table 3). Similarly, the presence of one –NCH_3 group was also deduced from the peaks at δ_H 2.72 (m), 2.58 (m) and δ_C 52.9 neighbouring the two diastereotopic protons at δ_H 2.50 m, 2.33 (m) and δ_C 35.2. Two carbonyl groups were confirmed at δ_C 194.0 and 196.0. The presence of two pairs of isolated methylene groups was also noted from the peaks at δ_H 2.0 d, 2.19 d, δ_C 23.7 and δ_H 2.70 (d), 3.40 (d), δ_C 44.0. Two neighbouring aromatic protons (AB system) were also observed at δ_H 6.51 (H-1, d, *J* 8.3 Hz) and 6.71 (H-2, d, *J* 8.3 Hz); and δ_C 120.0 and 110.6. A peak in the UV spectrum of **3** at λ_{max} 280 nm indicated the presence of α, β-unsaturated carbonyl moiety. The above strongly suggested the presence of a hasubanane scaffold. The complete assignment and structure determination was achieved on the basis of 1D (^{1}H, ^{13}C and DEPT) and 2D (HMQC, HMBC and COSY) (Table 3) (Fig 3) NMR spectral and optical data analysis. MS showed a higher peak at *m*/*z* 373 and base peak at *m*/*z* 315. HR-EIMS revealed the molecular ion peak at 373.1532 $C_{20}H_{23}NO_6$ requires 373.1525. The data obtained were comparable to that of aknadinine (**4**) previously iso-

lated from *Stephania cepharantha hayata* (Kashiwaba et al., 1996). The key difference was the presence of carbonyl group at C-10 (δ_C 196) for (−)-10-oxoaknadinine while in aknadinine there is a methylene group [H_{ax} (δ_H 2.56), H_{eq} (δ 2.79) and δ_C 25.2]. Compound **3** was therefore identified as (-)-10-oxoaknadinine and is being reported for the first time.

In vitro anti-plasmodial activity for the isolated compounds, chloroquine (CQ) and artemisinin is summarized in Table 4. (−)-Pseudocurine (**1**) showed strong anti-plasmodial activity against both strains of *P. falciparum* D6 (CQ-susceptible) (IC_{50} = 290 ng/ml), and W2 (CQ resistant) (IC_{50} = 310 ng/ml). (−)-Pseudoisocurine (**2**) exhibited moderate activity against D6 (IC_{50} = 750 ng/ml) and mild activity against W2 (IC_{50} = 1650 ng/ml). (−)-10-Oxoaknadinine (**3**) showed mild activity against W2 (IC_{50} = 3450 ng/ml) and was inactive against D6 (IC_{50} = 10250 ng/ml). Interestingly, a number of bisbenzylisoquinoline alkaloids have been reported to display good anti-plasmodial activity (Angerhofer et al., 1999; Mambu et al., 2000). Cissamptin and isoliensinine previously isolated from *Cissampelos mucronata* showed a good anti-plasmodial activity of IC_{50} values 2596 ng/ml and 257 ng/ml, respectively against *P. falciparum* (Tshibangu et al., 2003).

Conclusion

The bisbenzylisoquinoline and hasubanane alkaloids from *Stephania abyssinica* showed a good anti-plasmodial activity and justify the use of this plant to treat malaria by local communities in southern Nyanza, Kenya. This makes these compounds good candidates for further scientific research on anti-plasmodial drug discovery and development.

Experimental

General procedures

Solvents for extraction, chromatography and re-crystallization were of analytical grade or double distilled. Column chromatography was performed using silica gel (60–200 mesh) while Sephadex LH-20 was used in gel filtration chromatography. Thin layer chromatography (TLC) was done on aluminium pre-coated silica gel G/UV_{254}. Preparative thin layer chromatography (PTLC) was done on glass coated with silica gel. Melting points were determined on Gallenkamp apparatus and are uncorrected. UV spectra were recorded from Cecil 2041 spectrophotometer in methanol or chloroform. IR spectra were recorded with Shimadzu FT-IR 8101 spectrometer using potassium bromide pellets or chloroform as solvent. Resonance frequencies were given in cm^{-1}. ^1H and ^{13}C NMR spectra of the compounds were recorded from Bruker DRX-500/400 NMR spectrometer with a gradient unit at 500/400 and 125/100 MHz, respectively, at a controlled temperature of 292.9 K. COSY, NOESY, DEPT, HMQC and HMBC spectra were recorded on Bruker DRX-500. DMSO-d_6, $CDCl_3$ and CD_3OD were used as solvents. Optical rotation was measured using Polax-2L polarimeter at 25 °C. Mass spectra were recorded on VG-12–250 (EIMS) spectrometer. The *m/z* is reported in a.m.u with corresponding intensities in parentheses.

Plant material and extraction

The plant material was collected from Kisii Highlands, Kenya in August 2006, authenticated by Mr. Simon Mathenge, and voucher specimen (RO/02/2006) deposited at the University of Nairobi, Herbarium in the Department of Botany. The plant samples were air-dried under shade and pulverized using a laboratory mill. Cold solvent extraction method was employed. Briefly, 1.7 kg of the plant chaff was soaked in solvents of increasing polarity: hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH) for 48 h each.

Table 4
In vitro anti-plasmodial activity (IC_{50}) of compounds isolated from *Stephania abyssinica* and standard drugs.

Compound	*P. falciparum* (D6) IC_{50} ± SD (μg/ml)	*P. falciparum* (W2) IC_{50} ± SD (μg/ml)
(−)-Pseudocurine	0.29 ± 0.00	0.31 ± 0.01
(−)-Pseudoisocurine	0.75 ± 0.11	1.65 ± 0.03
(−)-10-oxoaknadinine	10.25 ± 1.84	3.45 ± 2.22
Chloroquine	0.00116 ± 0.00000	0.00169 ± 0.00014
Artemisinin	0.00834 ± 0.14	0.00005687 ± 00000127

Isolation and identification

In the first procedure, DCM extract was hydrolyzed with acid and the basic alkaloid fraction isolated and stored at −20 °C until when required for bioassay or chromatographic separation. Fractionation of the product (266 mg) was carried out by gel filtration chromatography on Sephadex LH-20 eluting with a mixture of DCM–MeOH (1:1). The sub-fractions were combined based on their TLC profiles and purified by preparative thin layer chromatography (PTLC) (2% MeOH–DCM in ammonia) to give (−)-pseudocurine (**1**) (43.6 mg) and (−)-pseudoisocurine (**2**) (21.8 mg).

*(−)-Pseudocurine (**1**):* white amorphous solid; m.p. 168–170 °C; $[\alpha]_D^{25}$: −262° (2.1, CH_3OH); UV: λ_{max} ($CHCl_3$) 235 (2.43), 240 (2.48), 271 (2.51), 278 (2.44) and 287 (2.53) nm; IR: υ_{max} ($CHCl_3$) 3352.1, 2943.2, 2831.3, 1450.4, 1114.8 and 1029.9 cm^{-1}; NMR: Table 1; EIMS: *m/z* 594.2741 M^+ (100) ($C_{36}H_{38}N_2O_6$ requires 594.2730), 552 (15), 386 (23), 298 (100), 282 (67), 267 (55), 253 (28), 174 (20), 147 (22) and 107 (10).

*(−)-Pseudoisocurine (**2**):* white amorphous solid; m.p. 158–160 °C $[\alpha]_D^{25}$: −158° (3.1, CH_3OH); UV: λ_{max} ($CHCl_3$) 216 (2.17), 224 (2.31), 228 (2.50) and 280 (0.90) nm; IR: υ_{max} ($CHCl_3$) 3352.1, 2943.2, 2831.3, 1450.4, 1114.8 and 1029.9 cm^{-1}; NMR: Table 2; EIMS: *m/z* 595.2735 $[M+1]^+$ (100) ($C_{36}H_{39}N_2O_6$ requires 594.2730, 552 (23), 521 (15), 315 (12), 298 (100), 282 (28), 267 (20) and 177 (5).

In the second procedure, repeated column chromatography of the DCM extract (2 g) with hexane–EtOAc (100:0–0:100) and MeOH–EtOAc (15:85) mixtures gave hasubanane-containing fraction. This was subjected to gel filtration on Sephadex LH 20 with a mixture of MeOH–DCM (1:1) and purified by PTLC to give (-)-10-oxoaknadinine (**3**) (23.8 mg).

*(−)-10-Oxoaknadinine (**3**)*: light yellow amorphous solid; $[\alpha]_D^{25}$: −291° (1.5, CH_3OH); UV: λ_{max}($CHCl_3$) 231 (2.62), 2.37 (2.72), 250 (2.84) and 272 (2.67) and 280 (2.58) nm. NMR: Table 3. EIMS: *m/z* 373.1532 $C_{20}H_{23}NO_6$ requires 373.1525, 373 (58), 358 (19), 342 (21), 330 (16), 316 (40), 315 (100), 314 (80), 389 (10), 284 (11).

In vitro anti-plasmodial assays

The *in vitro* anti-plasmodial tests were carried out based on the inhibition of [^{3}H]-hypoxanthine uptake by *Plasmodium falciparum* D6 strain (CQ-susceptible) and W2 (CQ-resistant) cultured in human blood serum according to Desjardin et al. (1979).

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