Antimalarial Activity of Sesquiterpene Lactones from *Vernonia cinerea*

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Two new sesquiterpene lactones, vernolides C and D as well as six known ones were isolated from the dichloromethane fraction of an aqueous extract from *Vernonia cinerea*. Their structures were elucidated by spectroscopic methods. Among the known sesquiterpene lactones, three of them were described in this plant for the first time. *In vitro* antiplasmodial evaluation showed that the three major compounds 1, 7 and 8 were active against chloroquine resistant *Plasmodium falciparum* strain (W2) with IC_{50} 3.9, 3.7 and 3.5 μM, respectively.

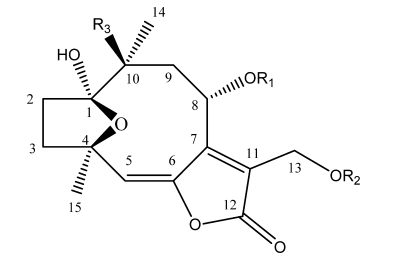
Key words antimalarial activity; *Vernonia cinerea*; vernolide C; vernolide D

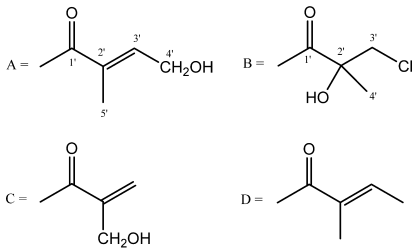
Vernonia cinerea Less (Asteraceae) is a perennial herbaceous plant mainly distribued in the tropical regions and commonly found in South-East Asia.[1,2] Many therapeutic uses were reported for this plant, especially for the treatment of malaria fever and liver diseases.[3,4]

In Cambodia, *V. cinerea* is called 'Kbal Ruy' by local residents, and is widely used as a traditional medicine for the treatment of fever and colics.[1,5] An ethnobotanical survey in different regions of Cambodia indicated that this plant was found to be used as a febrifuge against malaria.[6] It is worth noting that a far better antiplasmodial activity was found in the dichloromethane extract prepared from a decoction of the plant, compared with the dichloromethane extract directly prepared from the dried plant. The former extract was subjected to silica gel chromatography to yield two new sesquiterpene lactones, vernolides C **4** and D **8** (Fig. 1), along with six known compounds. This paper reports the identification and structural determination of the isolated compounds on the basis of spectroscopic analysis, including two-dimensional NMR and HR-ESI-MS data.

The dichloromethane extract obtained from a decoction of the whole plant was subjected to repeated column chromatography affording eight sesquiterpene lactones as described in the Experimental section. By comparison of the physical and spectroscopic data of the compounds with literature reports on the same plant,[7] **1**, **3** and **7** were identified as 8α-tigloyloxy-hirsutinolide-13-*O*-acetate, 8α-tigloyloxy-hirsutinolide and 8α-(4-hydroxymethacryloyloxy)-hirsutinolide-13-*O*-acetate, respectively. Three further known lactones: 8α-epoxymethacryloyloxy-hirsutinolide-13-*O*-acetate **2**, hirsutinolide-13-*O*-acetate **5** and piptocarphin D **6**, described here for the first time in this plant, have been identified by comparison of their spectroscopic data with those published in the literature.[8—14]

Vernolide C **4** was shown to be a chlorinated compound according to the typical cluster of ions in the mass spectrum at *m*/*z* 481 and 483; high resolution analysis of these ions as well as matching of isotopes allowed the composition to be determined as $C_{21}H_{27}ClO_9$ (*m*/*z* $[M+Na]^+$ 481.1236 *vs.* calc. 481.1241). This compound was thus the equivalent of an HCl addition product of epoxide **2**. This was confirmed by the observation in the ^{13}C-NMR spectrum of 21 separated resonances including four methyls, five methylenes, three methines and nine quaternary carbons amongst which three carbonyl functions (δ_C 172.3, 170.1, 166.7), a tetrasubstituted double bond (δ_C 148.1, 130.5), a trisubstituted double bond (δ_C 145.3) and a resonance (δ_C 108.7) suggesting one ketal group.[15] Detailed examination of the chemical shifts and long range CH correlations confirmed that the compound belonged to the hirsutinolide series with a proton onto C-10. That the hydrochlorination had taken place on the epoxide was shown by the presence of an AB quartet featuring H-3′ of the ester side chain; all of the expected CH couplings be-





	R_1	R_2	R_3
1	D	Ac	H
2	E	Ac	H
3	D	H	H
4	B	Ac	H
5	H	Ac	H
6	H	Ac	OH
7	C	Ac	H
8	A	Ac	H

Fig. 1. Sesquiterpene Lactones from *Vernonia cinerea*

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data[a)] of Compound **4** (in $CDCl_3$)

ATOM	δ ^{1}H	δ ^{13}C	HMBC (C→H)
1	—	108.7	H-3, 9, 14
2	2.17 m	36.9	H-3
	1.97 m		
3	2.13 m	38.1	H-15
4	—	80.2	H-3, 5, 15
5	5.98 s	127.1	H-3, 15
6	—	145.3	H-5, 8
7	—	148.1	H-5, 9, 13
8	6.44 d (6.9)	69.0	H-9
9	2.34 dd (16.0, 12.5)	35.7	H-8, 14
	1.87 ddd (16.0, 7.2, 1.9)		
10	1.97 m	39.5	H-8, 14
11	—	130.5	H-8, 13
12	—	166.7	H-13
13	5.03 d (13.1)	55.3	
	4.99 d (13.1)		
14	0.97 d (6.6)	17.3	H-9
15	1.59 s	28.7	H-3, 5
CO	—	170.1	H-13
CH_3	2.09 s	20.7	
1′	—	172.4	H-8, 3′, 4′
2′	—	75.2	H-3′, 4′
3′	3.85 d (11.0)	51.5	H-4′
	3.58 d (11.0)		
4′	1.50 s	23.6	H-3′

a) Chemical shifts (δ) and coupling constants (*J*) are given respectively in ppm and Hz. Assignments followed from the combination of 1D NMR data (^{1}H and ^{13}C-JMOD) and 2D NMR experiments (COSY, multiplicity-edited HSQC and HMBC).

tween the atoms of the chain were detected in the HMBC experiment. Thus the flat structure of **4** was unambiguously elucidated as 8-α-(3-chloro-2-hydroxy-2-methylpropanoyloxy)-hirsutinolide-13-*O*-acetate. The configurations of the asymmetric carbon atoms of the sesquiterpene skeleton are assumed to be similar to the one previously established for similar compounds in the same series (*viz* **2**); lack of material hampered all attempts to determine the absolute configuration in the ester side chain.

Vernolide D **8** was obtained as colourless crystals with the molecular formula $C_{22}H_{28}O_9$ as determined by the positive-ion high-resolution electrospray ionization mass spectrum (HR-ESI-MS), showing an accurate $[M+Na]^+$ ion at *m/z* 459.1626 (calc. 459.1631). The ^{1}H- and ^{13}C-NMR data of **8**, similar to those of **4**, were characteristic of an 8-*O*-substituted hirsutinolide type sesquiterpene. Five additional resonances [δ_C 167.1 (C-1′), 128.0 (C-2′), 141.7 (C-3′), 60.0 (C-4′), 12.7 (C-5′)] were indicative of 4-hydroxytigloyloxy moiety from HMBC correlations and literature data.[16)] This latter functional group was attached at the C-8 as indicated by the HMBC correlation peak between H-8 and C-1′ (Table 2). Thus the structure of **8** was established as 8-α-(4-hydroxytigloyloxy)-hirsutinolide-13-*O*-acetate.

In vitro antiplasmodial activity was performed on chloroquine resistant *Plasmodium falciparum* strain (W2).[6)] Three major sesquiterpene lactones were tested. Compounds **1**, **7** and **8** exhibited significative antiplasmodial activity with IC_{50} 3.9, 3.7 and 3.5 μM, respectively. Chloroquine was used as positive control with IC_{50} 0.52 μM. It is interesting to note that sesquiterpene lactones were isolated from decoction which is used in traditional medicine. That may confirm the potential use of this plant for the treatment of malaria.

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data[a)] of Compound **8** (in $CDCl_3$)

ATOM	δ ^{1}H	δ ^{13}C	HMBC (C→H)
1	—	108.4	H-3, 9, 14
2	2.21 m	37.2	H-3
	1.90 m		
3	2.08 m	38.6	H-15
4	—	81.0	H-3, 5, 15
5	5.89 s	126.6	H-3, 15
6	—	146.2	H-5, 8
7	—	149.9	H-5, 9, 13
8	6.27 d (6.5)	68.5	H-9
9	2.36 dd (15.9, 11.9)	35.9	H-8, 14
	1.86 dd (15.8, 7.9)		
10	1.90 m	41.2	H-8, 14
11	—	129.8	H-8, 13
12	—	166.1	H-13
13	5.08 d (13.0)	55.6	
	5.00 d (13.0)		
14	0.95 d (6.9)	17.2	H-9
15	1.49 s	28.3	H-3, 5
CO	—	170.3	H-13
CH_3	2.07 s	20.8	
1′	—	167.1	H-8, 3′, 5′
2′	—	128.0	H-3′, 4′, 5′
3′	7.03 t (6.0)	141.7	H-4′, 5′
4′	4.37 dd (15.0, 6.0)	60.0	H-3′, 5′
	4.33 dd (15.0, 6.0)		
5′	1.81 br s	12.7	H-3′, 4′

a) Chemical shifts (δ) and coupling constants (*J*) are given respectively in ppm and Hz. Assignments followed from the combination of gs-COSY, gs-HMQC and gs-HMBC experiments.

Experimental

General ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solutions on a Bruker Avance II 500 spectrometer equipped with a Dual ^{13}C/^{1}H Cryoprobe (compound **4**) and on a Bruker DRX 500 spectrometer (compound **8**). TMS was used as an internal standard in ^{1}H and ^{13}C measurements. Standard Bruker pulse sequences were used for two-dimensional experiments (gradient selected COSY, HMQC and HMBC). HR-ESI-MS were performed on a Bruker (MicroTOF) instrument. Melting point was determined on an Electrothermal IA 9300 apparatus. Optical rotations $[\alpha]_D^{20}$ were measured on a 241 MC Perkin-Elmer polarimeter with a sodium lamp and a double-jacketed cell at 20 °C. All the solvents used were of analytical grade (Carlo Erba). Silica gel 60 (Kieselgel 60, 0.040—0.063 mm, Merck) was used for column chromatography, and precoated gel plate (Kieselgel 60F254, Merck) was used for TLC.

Extraction and Separation The whole plant of *V. cinerea* collected in the Kampong Speu province of Cambodia was air-dried at room temperature during two weeks, with no direct sunlight. A voucher specimen, identified by Pr. S. K. Cheng, was deposited in the herbarium of the faculty of Pharmacy, Phnom Penh, Cambodia. The dried and powdered plant (200 g) was extracted with 3 l of boiling distilled water for 10 min and filtered. The aqueous extract was freeze-dried to give 24 g of a green powder. This powder was dissolved in 100 ml of distilled water to form a suspension and partitioned with dichloromethane (4×100 ml). The combined dichloromethane layers were filtered, dried and evaporated to give 1.1 g of dichloromethane extract which was firstly chromatographed over silica gel 60 (Kieselgel 60, 0.040—0.063 mm, Merck) eluting with CH_2Cl_2–CH_3OH [(100 : 0); (80 : 20); (60 : 40), respectively] to give three compounds **1** (70 mg), **2** (3 mg), **3** (2.5 mg) and two fractions F1 an F2. Subsequently, fraction F1 was subjected to silica gel chromatography to yield **4** (3 mg), **5** (2.8 mg) and **6** (2.6 mg). Fraction F2 was processed on a Sephadex LH-20 column eluting with CH_2Cl_2–CH_3OH (80 : 20) to afford **7** (43 mg) and **8** (17.2 mg).

Antiplasmodial Assay An *in vitro* antiplasmodial activity was performed as previously reported.[6)]

Vernolide C **4**: Brown amorphous powder; $[\alpha]_D^{20}$ +20° (c=0.26, $CHCl_3$). HR-ESI-MS *m/z* 481.1236 $[M+Na]^+$ (Calcd for $C_{21}H_{27}ClO_9Na$: 481.1241). ^{1}H- and ^{13}C-NMR data see Table 1.

Vernolide D **8**: Colourless crystals; mp 150 °C; $[\alpha]_D^{20}$ +103° (c=0.7, $CHCl_3$). HR-ESI-MS *m/z* 459.1626 $[M+Na]^+$ (Calcd for $C_{22}H_{28}O_9Na$: 459.1631). ^{1}H- and ^{13}C-NMR data see Table 2.

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