Further Cytotoxic Sesquiterpene Lactones from *Elephantopus mollis* KUNTH

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Three new sesquiterpene lactones, (4βH)-5α-hydroxy-8α-(2-methylbut-2-enoyloxy)-2-oxo-1(10),11(13)-guaiadien-12,6α-olide (1), (4βH)-8α-(2-methylbut-2-enoyloxy)-2-oxo-1(5),10(14),11(13)-guaiatrien-12,6α-olide (2) and 2,5-epoxy-2β-hydroxy-4α-methoxy-8α-(2-methylbut-2-enoyloxy)-4(15),10(14),11(13)-germacratrien-12,6α-olide (3), have been isolated from roots and stems of *Elephantopus mollis* together with two known sesquiterpene lactones (4, 5). The identification of the isolates was accomplished by spectroscopic methods. Compounds (1—5) exhibited significant cytotoxic activities against mouse neuroblastoma B104 cells.

Key words neuroblastoma; sesquiterpene lactone; *Elephantopus mollis*

Elephantopus mollis KUNTH, belonging to the Asteraceae, is used in Cameroon for the treatment of cancer, fracture, abdominal pains, defective lactation and snake bite.[1)] In our continuing search for novel bioactive compounds from Cameroon medicinal plants, we have previously isolated sesquiterpene lactones with cytotoxic activities.[2)] Sesquiterpene lactones have been isolated from numerous genera of the Asteraceae family and are described as the active constituents of a variety of medicinal plants used in traditional medicine.[3—7)] Because of an interest in cytotoxic sesquiterpene lactones, the methanol extract of roots and stems of *E. mollis* was re-examined and three new cytotoxic sesquiterpene lactones, namely, (4βH)-5α-hydroxy-8α-(2-methylbut-2-enoyloxy)-2-oxo-1(**10**),11(**13**)-guaiadien-12,6α-olide (**1**), (4βH)-8α-(2-methylbut-2-enoyloxy)-2-oxo-1(5),10(14),11(13)-guaiatrien-12,6α-olide (**2**) and 2,5-epoxy-2β-hydroxy-4α-methoxy-8α-(2-methylbut-2-enoyloxy)-4(15),10(14),11(13)-germacratrien-12,6α-olide (**3**) were isolated, together with 2β-methoxy-2-deethoxyphantomolin (**4**),[8)] 2β-methoxy-2-deethoxy-8-*O*-deacylphantomolin-8-*O*-tiglinate (**5**),[8)] molephantin and molephantinin.[2)] These compounds were evaluated for their cytotoxic activities against neuroblastoma B104.

Results and Discussion

The chloroform extract, which was obtained from methanol extract of the whole bodies of *E. mollis*, was subjected to repeated column chromatography followed by preparative thin-layer chromatography (TLC) to give three new (**1**—**3**) and two known (**4**, **5**) compounds.

Compound **1** was assigned the molecular formula $C_{20}H_{24}O_6$ by HR-ESI-MS (*m*/*z* 361.1651 for $[M+H]^+$). The IR spectrum suggested the presence of a hydroxyl group (3472 cm^{-1}), an α,β-unsaturated ester carbonyl (1715 cm^{-1}) and a lactone carbonyl group (1772 cm^{-1}). The ^{1}H- and ^{13}C-NMR data (Table 1) revealed the presence of a ketone carbonyl (δ_C 204.0), an α-methylene-γ-butyrolactone moiety [δ_H 5.52, 6.01 (CH_2); δ_C 121.1 (CH_2), 138.9 (C), 169.1 (carbonyl)] and a 2-methylbut-2-enoyloxy moiety [δ_H 6.88 (CH), 1.65 and 1.83 ($2CH_3$); δ_C 165.6 (carbonyl), 128.5 (C), 138.5 (CH), 12.2 and 14.7 ($2CH_3$)]. In addition, signals of a tetrasubstituted olefin (δ_C 136.9, 151.9), a quaternary oxygenated carbon (δ_C 78.0), four methine carbons two of which were oxygenated (δ_C 85.9, 71.4), two methylene carbons (δ_C 46.7, 43.8) and two methyl groups (δ_C 15.1, 23.2) were observed. The connectivity from H-6 to H-9 was confirmed by COSY spectroscopy. Analysis of 2D NMR data (COSY, heteronuclear single quantum correlation (HSQC), HMBC) led to the complete assignment of NMR signals. HMBC correlations of H_3-15 to C-3, C-4 and C-5 and from H_3-14 to C-1, C-9, and C-10 revealed that Me-14 and Me-15 are attached to C-10 and C-4, respectively. Further HMBC correlations were observed between H_2-3 and C-1, C-2, C-4 and C-5 and suggested that compound **1** is a guaiane-type sesquiterpene. The configuration at C-4, C-5, C-6, C-7, and C-8 was determined by NOESY (correlations between H-4 and H-6, H-6 and H-8, H-7 and H-9a were observed) and by comparison of coupling constants with those of naturally occurring germacranolide and guaiane-type sesquiterpenes.[10—12)] Further confirmation of the structure was made by comparison of data with those of (4βH)-5α-hydroxy-8α-(2-methylpropenoyloxy)-1(10),11(13)-guaiadien-12,6α-olide.[3)] The relative configuration of the 2-methylbut-2-enoyloxy residue was deduced from coupling constants (Table 1) and by NOE data obtained from NOESY spectrum (Fig. 1).

Compound **2** was shown to have the molecular formula $C_{20}H_{22}O_5$ by HR-ESI-MS (*m*/*z* 343.1549 $[M+H]^+$). The IR spectrum indicated the presence of an α,β-unsaturated ester carbonyl (1708 cm^{-1}) and a lactone carbonyl group (1769 cm^{-1}). The ^{1}H- and ^{13}C-NMR data (Table 1) were similar to those of **1** with a ketone carbonyl, an α-methylene-γ-butyrolactone and a 2-methylbut-2-enoyloxy moiety. However, the quaternary oxygenated carbon observed in compound **1** is replaced by an exomethylene group [δ_H 5.24, 5.75; δ_C 122.2 (CH_2), 133.5 (C)]. Since an olefin carbon [δ_C 174.6 (C)] was shifted downfield, it was thought to be located at the β position of a conjugated system with a carbonyl group. This was confirmed by the HMBC correlations between H-4 and C-1, C-2, C-3, C-5 and C-6. Structure of **2** was established by comparing NMR data with those of

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Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1**—**3** in $CDCl_3$ (*J* Values (Hz) in Parentheses)

Position	**1**		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	136.9		138.5		51.8	2.46 (dd, 14, 1)
						2.52 (dd, 14, 1)
2	204.0		206.4		107.1	
3	46.7	2.11 (dd, 17, 13)	46.0	2.15 (dd, 19, 1)	45.4	2.00 (dd, 14, 1)
		2.27 (dd, 17, 9)		2.81 (dd, 19, 7)		2.64 (dd, 14, 1)
4	37.0	2.37 (m)	35.3	3.24 (qdd, 7, 7, 1)	83.8	
5	78.0		174.6		85.9	4.38 (d, 4)
6	85.9	4.25 (d, 10)	78.6	5.53 (d, 10)	79.4	4.16 (dd, 6, 4)
7	47.1	3.90 (m)	50.3	3.50 (m)	39.9	3.77 (m)
8	71.4	5.01 (ddd, 10, 10, 2)	75.1	5.38 (ddd, 9, 4, 4)	75.4	5.06 (ddd, 10, 3, 2)
9	43.8	2.28 (dd, 13, 2)	41.6	2.61 (dd, 15, 4)	34.3	2.63 (dd, 16, 2)
		3.05 (dd, 13, 10)		2.86 (dd, 15, 7)		2.72 (dd, 16, 3)
10	151.9		133.5		140.3	
11	138.9		138.3		136.4	
12	169.1		169.8		169.4	
13	121.1	5.52 (d, 3)	122.6	5.58 (d, 3)	124.6	5.71 (dd, 3, 1)
		6.01 (d, 3)		6.14 (d, 3)		6.21 (dd, 3, 1)
14	23.2	2.18 (s)	122.2	5.24 (d, 2)	121.7	5.20 (br s)
				5.75 (d, 2)		5.37 (br s)
15	15.1	1.15 (d, 7)	21.8	1.35 (d, 7)	26.0	1.35 (s)
1′	165.6		165.3		166.0	
2′	128.5		128.5		128.4	
3′	138.5	6.88 (dq, 7, 2)	138.6	6.95 (dq, 7, 2)	138.6	6.96 (dq, 7, 2)
4′	12.2	1.83 (dq, 2, 2)	12.4	1.87 (dq, 2, 2)	12.2	1.90 (dq, 2, 2)
5′	14.7	1.65 (dq, 7, 2)	14.8	1.62 (dq, 7, 2)	14.6	1.61 (dq, 7, 2)
OMe					51.8	3.12 (s)

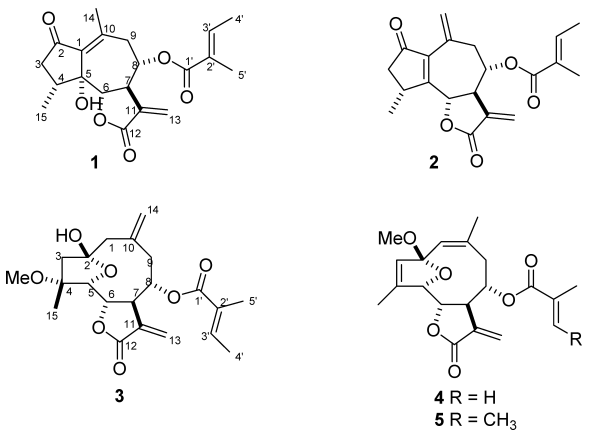

Fig. 1. Chemical Structures of Compounds **1**—**5**

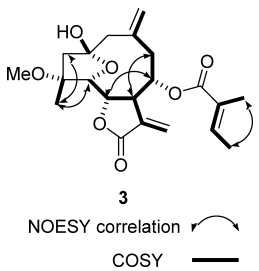

Fig. 2. Key COSY and Observed NOESY Correlations for Compound **3**

(4βH)-8α-(2-methylpropenoyloxy)-2-oxo-1(5),10(14),11(13)-guaiatrien-12,6α-olide isolated from the same plant.[2)]

The molecular formula of compound **3** was determined to be $C_{21}H_{28}O_7$ by the HR-ESI-MS (*m*/*z* 393.1917 $[M+H]^+$), ^{13}C-NMR and ^{13}C DEPT NMR analyses. The IR spectrum showed a strong broad hydroxyl band (3544 cm^{-1}), an α,β-unsaturated ester carbonyl (1718 cm^{-1}) and a lactone carbonyl group (1781 cm^{-1}). Comparison of NMR spectra with those of **1** and **2** suggested the absence of unsaturated carbonyl in **3**, but the presence of an acetal carbon (δ_C 107.1). In the HMBC spectrum, cross peaks were observed between C-2, H-1, H-3 and H-5 respectively, indicating that acetal carbon was situated at C-2. Compound **3** was assumed to be a germacranolide-type sesquiterpene with ether linkage between C-2 and C-5. The ^{1}H- and ^{13}C-NMR indicated the presence of an α-methylene-γ-butyrolactone moiety [δ_H 5.71, 6.21 (CH_2); δ_C 124.6 (CH_2), 136.4 (C), 169.4 (carbonyl)], a 2-methylbut-2-enoyloxy moiety [δ_H 6.96 (CH), 1.61 and 1.90 ($2CH_3$); δ_C 166.0 (carbonyl), 128.4 (C), 138.6 (CH), 12.2 and 14.6 ($2CH_3$)], an exomethylene [δ_H 5.20, 5.37 (CH_2); 121.7 (CH_2), 140.3 (C)] and a methoxyl group (δ_H 3.12; δ_C 51.8). The disposition of the methoxyl group was determined by the analysis of the NOESY spectra. Analysis of the ^{1}H- and ^{13}C-NMR data, HMBC, HSQC and NOESY spectrum provided evidence that **3** possesses the same skeleton and stereochemistry as 2,5-epoxy-2β-hy-

Table 2. Cytotoxic Activity of Compounds **1**—**5** against Neuroblastoma B104

Compound	IC_{50} (μM)
1	1.93
2	2.13
3	1.58
4	2.57
5	3.85

droxy-8α-(2-methylbut-2-enoyloxy)-4(15),10(14),11(13)-germacratrien-12,6α-olide recently isolated from *E. mollis*.[2)]

The antiproliferative effects of these isolated compounds were evaluated *in vitro*, against neuroblastoma B104 by using the sulforhodamine B assay.[9)] The five sesquiterpene lactones **1**—**5** exhibited significant cytotoxic activities (Table 2).

Experimental

General IR spectra were obtained with a Nicolet AVATAR 320 FT-IR spectrophotometer. ^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, NOESY, HSQC and HMBC experiments were performed on a Bruker Avance DPX instrument (300 MHz for ^{1}H and 75 MHz for ^{13}C). All chemical shifts were given in ppm, with tetramethylsilane as an internal standard. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Bio-TeK spectrophotometer. Silica gel 60 F254 (Merk) was used for TLC. Mass spectra were recorded on a Bruker Daltonics microTOF mass spectrometer.

Plant Material The roots and stems of *E. mollis* were collected from Ebolowa in the southern province of Cameroon and identified by Dr. Tchiengue Barthelemy of the National Herbarium of Cameroon where a voucher specimen (N° 48561) is deposited.

Extraction and Isolation Air-dried whole plant (300 g) of *E. mollis* was extracted with methanol (2 l×3) at room temperature. The solvent was evaporated in vacuum to yield a dark residue. The residue was suspended in H_2O and extracted with $CHCl_3$ and 25 g of extract were obtained. Part of the crude extract (15 g) was dissolved in MeOH and mixed with silica gel, dried at room temperature, and subjected to vacuum liquid chromatography (VLC) on silica gel (40—63 μm, 9×73 cm) using a step-gradient of $CHCl_3$–AcOEt. Ten fractions (A: 10 : 0; B: 9 : 1; C: 8 : 2; D: 7 : 3; E: 6 : 4; F: 5 : 5; G: 4 : 6; H: 3 : 7; I: 2 : 8; J: 0 : 10; each 2×250 ml) was obtained on the basis of TLC analysis. All the fractions were subjected to a cytotoxicity test on neuroblastoma B104, and only D, E and F showed appreciable activities (IC_{50} 7.85, 5.32, 13.63 μg/ml, respectively). Fraction D (820 mg) was crystallized from MeOH to give molephantin (315 mg) and molephantinin (148 mg). Fraction E (132 mg) was chromatographed over Sephadex LH-20 with MeOH as eluent to give three main subfractions (E1, E2, E3). Fraction E1 (37 mg) was subjected to preparative TLC. Compounds **1** (6 mg, *n*-heptane/AcOEt, 4 : 6, *Rf*=0.43) and **2** (13 mg, *n*-heptane/AcOEt, 4 : 6, *Rf*=0.67) were obtained. Subfraction E2 (118 mg) was rechromatographed on a silica gel column (40—63 μm, 5×25 cm) using *n*-heptane–AcOEt (6 : 4) to give **3** (7 mg). Subfraction E3 was passed through silica gel with *n*-heptane/AcOEt (60 : 40) and purified by preparative TLC to afford **4** (37 mg, *n*-heptane/AcOEt, 1 : 1, *Rf*=0.38). Further repeated silica gel chromatography of fraction F with *n*-heptane/AcOEt (55 : 45) yielded **5** (28 mg).

Compound **1**: Colourless oil; $[\alpha]_D^{25}$ +39.4° (c=0.1, CH_2Cl_2); UV (MeOH): λ_{max} (log ε)=218 (4.14) nm; IR ($CHCl_3$): ν_{max}=3472, 1772, 1715, 1668, and 1642 cm^{-1}; ^{1}H- and ^{13}C-NMR (Table 1); HR-ESI-MS: *m*/*z* 361.1651 $[M+H]^+$ (Calcd for $C_{20}H_{25}O_6$: 361.1646).

Compound **2**: Colourless oil, $[\alpha]_D^{25}$ +37.3° (c=0.1, CH_2Cl_2); UV (MeOH): λ_{max} (log ε)=215 (4.37) nm; IR ($CHCl_3$): ν_{max}=2917, 1769 and 1708 cm^{-1}; ^{1}H- and ^{13}C-NMR (Table 1); HR-ESI-MS: *m*/*z* 343.1556 $[M+H]^+$ (Calcd for $C_{20}H_{23}O_5$: 343.1549).

Compound **3**: Colourless oil; $[\alpha]_D^{25}$ +87.6° (c=0.1, CH_2Cl_2); UV (MeOH): λ_{max} (log ε)=215 (4.12) nm; IR ($CHCl_3$): ν_{max}=3544, 1781, 1718, 1677 and 1629 cm^{-1}; ^{1}H- and ^{13}C-NMR (Table 1); HR-ESI-MS: *m*/*z* 393.1917 $[M+H]^+$ (Calcd for $C_{21}H_{29}O_7$: 393.1922).

***In Vitro* Cytotoxicity** The sulforhodamine B (SRB) assay against mouse neuroblastoma B104 was carried out according to the procedures by Skehan *et al.*[9)]

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