Two New Antibacterial Sesquiterpenoids from *Centipeda minima*

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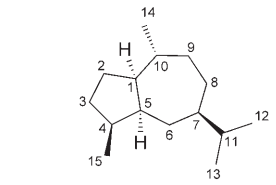
Two new guaiane-type sesquiterpene lactones, compounds **1** and **2**, along with three known guaianolide- or pseudoguaianolides, were isolated from *Centipeda minima* (whole plant). Their structures were identified by spectroscopic and mass-spectrometric analyses. The configuration at C(5) of the guaiane framework of **1** was rationalized by quantum-mechanical calculations (*Table 2*). All compounds were found to be active against eight different microbial pathogens (*Table 3*), with *MIC* values in the range of 6.25–100 μg/ml.

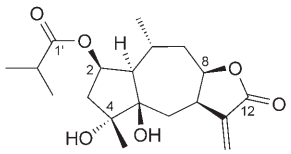
Introduction. – The widespread use of chemically modified antibiotics produced by fermentation in human medicine and agriculture has caused serious problems in terms of bacterial resistance [1]. Therefore, plant-derived antimicrobial agents with high potency and low mammalian toxicity have gained special interest in the recent decades [2–4]. *Centipeda minima* (L.) is a species of the Compositae family, spreading over South Asia and Oceania [5], and used against cold, nasal allergy, diarrhea, malaria, and asthma in China [6]. Flavonoids and sesquiterpenes are considered to be active components of this plant [7–10].

During our search for new antimicrobial agents from traditional Chinese medicines, we embarked on an investigation on *C. minima*. Herein, we report the isolation, structural identification, and antibacterial properties of the sesquiterpenoids **1**–**5** from the whole plant of *C. minima*, compounds **1** and **2** being new natural products.

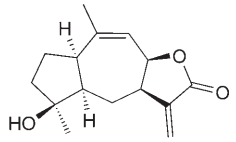
Results and Discussion. – 1. *Structure Elucidation*. Compound **1** was obtained as a colorless oil. HR-ESI-MS Analysis of **1** showed the quasi-molecular ion peak at *m*/*z* 375.1780 ($[M + Na]^+$; calc. 375.1784), corresponding to the formula $C_{19}H_{28}O_6$, requiring six degrees of unsaturation. The similarities of the NMR spectra of **1** (*Table 1*) and the known isolate 2β-(isobutyryloxy)florilenalin (**3**) suggested that **1** was also a sesquiterpene lactone with a guaianolide skeleton, esterified with isobutyric acid. The main difference between the ^{13}C-NMR spectra of **1** and **3** was that the CH group at δ(C) 51.6 and the CH_2 group at δ(C) 115.6 in **3** were replaced by an oxygenated



10βH-Guaiane



1



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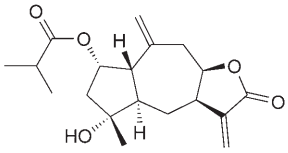

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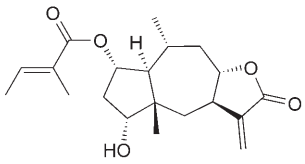

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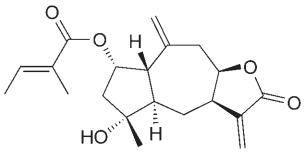

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quaternary C-atom and a Me group (δ(C) 21.9) in **1**, respectively. The following HMBC correlations were observed: H–C(3)/C(4), H–C(3)/C(15), H–C(15)/C(3), H–C(15)/C(4), H–C(15)/C(5), H–C(1)/C(5), H–C(6)/C(4), H–C(6)/C(5), H–C(6)/C(7), H–C(6)/C(8), and H–C(6)/C(11). This indicated that the two OH groups were at C(4) and C(5), respectively. The HMBC correlation between H–C(2) and C(1′) established the linkage of the ester group with the backbone. Based on the ROESY interactions of H–C(14)/H–C(1), H–C(14)/H–C(2), H–C(8)/H–C(1), H–C(8)/H–C(7), H–C(15)/H_β–C(3), and H_α–C(3)/H–C(2), we concluded that H–C(1), H–C(2), H–C(7), H–C(8), and Me(14) were all α-configured, whereas Me(15) was in β-orientation. The observed NOE for H–C(7) upon irradiation of H–C(8) indicated that these two H-atoms were on the same side of the ring.

A literature survey showed that the 5-OH group in related sesquiterpenes had been assigned either the relative configuration α or β. Since there was not enough spectroscopic evidence to clearly discriminate between these two possibilities, we decided to perform a quantum-mechanics calculation using the Gaussian 03 software package [11]. The optimized structures of the two theoretical 5-epimers **1a** (5α-OH) and **1b** (5β-OH) are shown in *Table 2* (H-atoms being hidden for clarity), together with their calculated ^{13}C-NMR chemical shifts, as derived at the B3LYP/6-311+G(2d,p) level, using the GIAO method, the most-stable conformation being obtained at the B3LYP/6-31G* level [12]. The computed magnetic shieldings were corrected and converted into the corresponding chemical shifts, as reported before [13]. Since the *maximum* error in δ(C) was as large as 10.0 ppm for **1a** (*Entry 2*), but only 6.9 ppm for **1b** (*Entry 11*), the configuration of the 5-OH group in **1** was tentatively assumed to be β. Taken together, the structure of compound **1** was, thus, assigned as 4,5β-dihydroxy-2β-(isobutyryloxy)-10βH-guai-11(13)-en-12,8β-olide[1]).

Compound **2** was obtained as a colorless oil. Its molecular formula was determined as $C_{15}H_{20}O_3$ by HR-ESI-MS (*m*/*z* 271.1321 ([*M*+Na]$^+$; calc. 271.1310)). The ^{13}C-NMR spectrum of **2** (*Table 1*) displayed two Me and three CH_2 groups, one exocyclic

[1]) For systematic names, see *Exper. Part*.

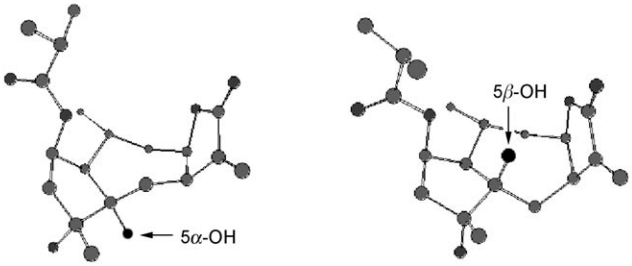
Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.76–1.79 (*m*)	59.8	2.07–2.15 (*m*)	50.9
2	4.86–4.90 (*m*)	78.4	a: 1.90–1.97 (*m*) b: 1.51–1.59 (*m*)	26.7
3	α: 2.20 (*dd*, *J*=14.0, 8.5) β: 1.93–1.97 (*m*)	44.3	1.82–1.86 (*m*)	38.8
4	–	82.0[a])	–	79.6
5	–	83.4[a])	1.76–1.82 (*m*)	51.2
6	a: 1.97–2.00 (*m*) b: 1.86–1.90 (*m*)	31.6	α: 2.28 (*dd*, *J*=13.2, 7.5) β: 1.35–1.44 (*m*)	28.3
7	3.47–3.51 (*m*)	37.9	3.40–3.46 (*m*)	44.0
8	4.75–4.79 (*m*)	77.9	5.33–5.36 (*m*)	78.8
9	a: 2.02–2.06 (*m*) b: 1.79–1.83 (*m*)	35.4	5.35–5.38 (*m*)	121.2
10	2.01–2.04 (*m*)	30.7	–	139.7
11	–	140.3	–	140.4
12	–	170.3	–	170.2
13	a: 6.26 (*d*, *J*=2.1) b: 5.60 (*d*, *J*=2.1)	121.8	a: 6.23 (*d*, *J*=2.3) b: 5.59 (*d*, *J*=2.3)	120.9
14	1.04 (*d*, *J*=6.5)	21.9	1.75 (*s*)	24.1
15	1.35 (*s*)	22.5	1.27 (*s*)	24.3
1′	–	176.7	–	–
2′	2.51 (*q*, *J*=7.0)	34.1	–	–
3′	1.15 (*d*, *J*=7.0)	18.8	–	–
4′	1.15 (*d*, *J*=7.0)	18.8	–	–

methylidene, four methines (one olefinic), a regular and an olefinic quaternary C-atom, as well as a C=O group, suggesting a sesquiterpene lactone with a guaianolide backbone.

^{1}H,^{1}H-COSY, HMQC, and HMBC experiments established that **2** had the same planar structures as the previously reported compound 4-hydroxy-1βH-guaia-9,11(13)-dien-12,8α-olide [14]. The key difference was that H–C(7) at δ(H) 3.42 and H–C(8) at δ(H) 5.34 in the ^{1}H-NMR spectrum of **2** were distinctly shifted downfield compared to the corresponding signals of the known compound (δ(H) 2.54 and 4.72, resp.) due to different configurations at C(7) and C(8) [15]. Clear NOEs between H–C(7) and H_α–C(6), H–C(5), and H–C(8) were observed for **2** upon irradiation of H–C(7), which indicated that H–C(7) and H–C(8) were both α-configured and at the same side of the seven-membered ring. In addition, NOEs were observed between H–C(15) and both H_α–C(6) and H–C(1), when irradiating H–C(15); also, ROESY cross-peaks were found between H–C(1) and both H–C(7) and H–C(8). These data, thus, suggested that H–C(1), H–C(5), H–C(7), H–C(8), and Me(15) were all α-configured. Thus, the structure of compound **2** was deduced as 4-hydroxyguaia-9,11(13)-dien-12,8β-olide. Compound **2** is a new stereoisomer of the known compound 4-hydroxy-1βH-guaia-9,11(13)-dien-12,8α-olide [14].

Table 2. *Observed* vs. *Calculated* ^{13}*C-NMR Chemical Shifts of the Possible Epimers* **1a** (5α-OH; left) *and* **1b** (5β-OH, right).



Position	$\delta(C)_{obs}$	$\delta(C)_{calc}$		$\Delta\delta(C)$[a])	
		1a	**1b**	**1a**	**1b**
1	59.8	59.3	57.3	0.5	2.5
2	78.4	68.4	75.1	10.0	3.3
3	44.3	45.6	49.9	−1.3	−5.6
4	82.0	83.2	84.2	−1.2	−2.2
5	83.4	82.0	85.6	1.4	−2.2
6	31.6	36.4	37.5	−4.8	−5.9
7	37.9	43.2	42.8	−5.3	−4.9
8	77.9	79.8	76.5	−1.9	1.4
9	35.4	33.8	37.0	1.6	−1.6
10	30.7	24.9	24.3	5.8	6.4
11	140.3	146.9	147.2	−6.6	−6.9
12	170.3	167.7	167.3	2.6	3.0
13	121.8	118.7	116.3	3.1	5.5
14	21.9	22.0	19.3	−0.1	2.6
15	22.5	21.0	20.4	1.5	2.1
1′	176.7	176.7	175.1	0.0	1.6
2′	34.1	38.2	36.0	−4.1	−1.9
3′	18.8	19.1	16.0	−0.3	2.8
4′	18.8	19.8	19.0	−1.0	−0.2

[a]) Relative chemical-shift difference calculated as $\delta(C)_{obs}-\delta(C)_{calc}$.

The three known sesquiterpenes were identified as 2β-(isobutyryloxy)florilenalin (**3**) [16], pulchellin-2α-*O*-tiglate (**4**) [17], and florilenalin-2α-*O*-tiglate (**5**) [16] by comparison of their spectroscopic data with literature values. Compound **4** was isolated for the first time from *C. minima*. Compounds **3** and **5** have been previously found in *C. minima*, but not investigated for their antibacterial effects.

2. *Bioassay.* Antibacterial tests revealed that compounds **1**–**5** all exhibited antibacterial effects against the bacteria investigated (*Table 3*). Compound **4** was more active against *Gram*-negative bacteria, and **3** was more active against *Gram*-positive bacteria. All tested bacteria were poorly sensitive towards **5**, with *MIC* values >100 μg/ml. With an *MIC* value of 6.25 μg/ml, compound **4** was found to be most effective against *Salmonella typhimurium*, *Salmonella paratyphi* A and B, and *Shigella*

flexneri. The *MIC* value of **3** against *Staphylococcus aureus*, 12.5 μg/ml, is comparable with that of the standard drug cefradine.

Table 3. *Antimicrobial Activities of Compounds* **1**–**5**. Values refer to minimum-inhibitory concentrations (*MIC*), in μg/ml.

Pathogen	**1**	**2**	**3**	**4**	**5**	Cef[a])	Gen[b])
Staphylococcus aureus	>50	50	12.5	>100	>100	15	7.5
Escherichia coli	>50	50	>100	>50	>100	7.5	7.5
Salmonella typhimurium	>50	25	>100	6.25	>100	7.5	7.5
Shigella flexneri	>50	25	25	6.25	>100	3.25	3.25
Staphylococcus epidermidis	>50	50	12.5	12.5	>100	3.25	7.5
Bacillus subtilis	>50	>50	50	>50	>100	3.25	3.25
Salmonella paratyphi A	>50	25	>100	6.25	>100	3.25	3.25
Salmonella paratyphi B	>50	25	>100	6.25	>100	3.25	3.25

[a]) Cefradine (pos. control). [b]) Gentamycin (pos. control).

Compounds **1**–**5** are guaianolide- or pseudoguaianolide-type sesquiterpenoids that are structurally related to the antimicrobial drug '6-*O*-methylacrylylplenolin', which has been isolated before from *C. minima* [10].

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Experimental Part

General. Column chromatography (CC) was performed on silica gel (200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Factory*, China), on C_{18} reverse-phase silica gel (40–63 μm, *Daiso Co.*, Japan), on *Sephadex LH-20* gel (*Amersham Pharmacia*, Sweden), or on *Diaion HP20* and *MCI CHP-20P* gel (75–150 μm, *Mitsubishikasei*, Japan). TLC was performed on silica gel GF_{254} (10–40 μm; *Qingdao*). All solvents were distilled before use. UV Spectra: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} in nm. Optical rotations: *Jasco-20C* digital polarimeter. IR Spectra: *Bruker Tensor-27* FT-IR spectrophotometer, with KBr discs; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker AM-400* spectrometer; chemical shifts δ in ppm rel. to Me_4Si, coupling constants J in Hz. ^1H,^1H-COSY, HSQC, HMQC, and HMBC Spectra: *Bruker DRX-500* spectrometer. FAB-MS (neg. or pos.): *VG AutoSpec-3000* mass spectrometer. ESI- and HR-ESI-MS: *API QSTAR Pulsar-1* mass spectrometer; in *m*/*z*.

Plant Material. Whole plants of *C. minima* were purchased from the *Yunnan Corporation of Materia Medica* (*YCMM*), Yunnan Province, P. R. China, in June 2005, and identified by Mr. *H. Y. Sun* at *YCCM*. A voucher specimen (CHYX0159) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

Extraction and Isolation. The dried and powdered plant material (10.0 kg) was extracted with 95% aq. EtOH under reflux (3×). The extracts were combined and concentrated, and the residue was suspended in H_2O, and then successively partitioned with petroleum ether (PE), AcOEt, and BuOH, resp. The AcOEt-soluble extract (170 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 8:1): *Fr. 1*–*Fr. 4*. *Fr. 2* (30 g) was decolored on *Diaion HP20* (95% EtOH), and the eluents were subjected to CC (SiO_2; $CHCl_3$/MeOH 1:0→5:1): *Fr. 2.1*–*Fr. 2.8*. *Fr. 2.2* (10 g) was decolored over *MCI CHP-20P* gel (MeOH/H_2O 9:1), and then subjected to CC (1. *Sephadex LH-20*, MeOH; 2. C_{18}, MeOH/H_2O 50:50→100:0), followed by vacuum liquid chromatography (VLC) to afford **2** (10 mg), **3** (21 mg), and **4** (30 mg). *Fr. 2.3* (5 g) was subjected to CC on *MCI CHP-20P* gel (MeOH/H_2O 9:1), *Sephadex LH-20* gel (MeOH), C_{18} silica gel (MeOH/H_2O 50:50→100:0), and repeated VLC to provide **1** (9 mg) and **5** (12 mg).

4,5β-Dihydroxy-2β-(isobutyryloxy)-10βH-guai-11(13)-en-12,8β-olide (=*(3a*R*,4a*R*,5*R*,7*R*,7a*R*,8*R*,9a*R*)-Dodecahydro-4a,5-dihydroxy-5,8-dimethyl-3-methylidene-2-oxoazuleno[6,5-*b*]furan-7-yl 2-Methylpropanoate*; **1**). Colorless oil. UV (MeOH): 240. $[\alpha]_D^{20.6} = +76.0$ ($c = 0.13$, $CHCl_3$). IR (KBr): 3448, 1747. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 375 ($[M+Na]^+$). HR-ESI-MS: 375.1780 ($[M+Na]^+$, $C_{19}H_{28}NaO_6^+$; calc. 375.1784).

4β-Hydroxyguaia-9,11(13)-dien-12,8β-olide (=*(3a*R*,4a*R*,5*S*,7a*R*,9a*R*)-3a,4,4a,5,6,7,7a,9a-Octahydro-5-hydroxy-5,8-dimethyl-3-methylideneazuleno[6,5-*b*]furan-2(3*H*)-one*; **2**). Colorless oil. UV ($CHCl_3$): 275, 239. $[\alpha]_D^{21.0} = -13.90$ ($c = 0.24$, $CHCl_3$). IR (KBr): 3427, 1761, 1635. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 271 ($[M+Na]^+$). HR-ESI-MS: 271.1321 ($[M+Na]^+$, $C_{15}H_{20}NaO_3^+$; calc. 271.1310).

Bacterial Strains. From the *National Center for Medical Culture Collections* (*CMCC*; Beijing, China), the following bacterial strains were employed: *Staphylococcus aureus* (CMCC 26001), *Escherichia coli* (CMCC 44103), *Salmonella typhimurium* (CMCC 80087), and *Shigella flexneri* (CMCC 51335). Further, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Salmonella paratyphi* (strains A and B) were clinically isolated.

Antimicrobial Assay. The antibacterial properties of **1**–**5** were tested by the agar dilution method [18]. The isolates, stored at −70°, were streaked onto tryptone-soy-agar (TSA; *Oxoid*) plates, and then incubated for 18–24 h at 37°. The inoculum was prepared by culturing each isolated bacterial colony in brain-heart infusion broth (*Oxoid*) at 37° to a turbidity equivalent to *McFarland* 0.5 standard (1.0×10^8 CFU/ml). Subsequently, the organism was diluted to 1.0×10^6 CFU/ml for susceptibility testing. For the agar dilution tests, the appropriate compound (2 mg) was first dissolved in DMSO (0.2 ml), and serially diluted (ten twofold dilutions per compound), as described by the *Clinical and Laboratory Standards Institute* (*CLSI*; Wayne, PA, USA; formerly *National Committee for Clinical Laboratory Standards*) [19]. Molten (48°) *Mueller–Hinton* agar (9 ml) was then added per milliliter of diluted compound, mixed completely, and put into plates. With a *Steers* replicator, an organism density of 10^4 CFU/spot was inoculated onto the appropriate plate, with various final concentrations of test compound in the range 0.195–100 μg/ml. The plates were incubated in ambient air at a temp. of 37° for 24 h. *MIC* Values were calculated as the lowest concentration that inhibited visible growth after incubation for 24 h. Cefradine and gentamycin were used as pos. controls. Plates containing agar or agar plus 1% DMSO served as negative and solvent controls, resp. All tests were performed in triplicate and repeated once.

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