Three Novel Triterpenoids from the Aerial Part of *Helwingia chinensis*

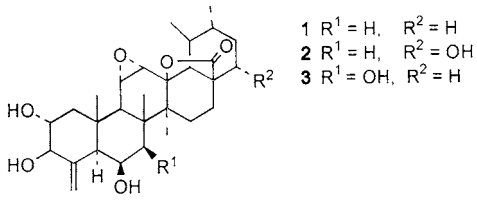
by **Guo-Fang Lai**, **Yi-Fen Wang**, **Chun-Hua Lu**, **Jian-Xin Cao**, **Yun-Long Xu**, and **Shi-De Luo***

State Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming 650204, Yunnan, P.R. China
(phone: +86-871-5223097, e-mail: shideluo@hotmail.com; laiguofang123@hotmail.com)

Three novel triterpenoids named chinenols **A**–**C** (**1**–**3**), together with two known compounds, glutin-5-en-3β-ol (**4**) and daucosterol (**5**), were isolated from the aerial part of *Helwingia chinensis* BATAL. The structures of **1**–**3** were determined on the basis of their HR-EI-MS, IR, ^{1}H- and ^{13}C-NMR (DEPT), and 2D (HMQC, HMBC, NOESY) data. Compounds **1**–**3** and **5** showed inhibition activity in an antibacterial assay.

1. Introduction. – *Helwingia chinensis* BATAL (Cornaceae) is distributed in the western and southern regions of China [1]. The aerial part of this plant has long been used to treat dysentery, hematochezia, swelling, *etc.* [2]. However, so far, extensive studies of this plant with respect to its pharmacological and chemical characteristics have not been reported. For this reason, chemical studies of this plant were undertaken. This paper mainly describes the isolation and structure identification of three novel triterpenoids from the AcOEt fraction of the aerial part of *H. chinensis.*

2. Results and Discussion. – Three novel triterpenoids and two known compounds were repeatedly chromatographed on silica gel, *Sephadex LH-20*, and *RP-18* gel to afford chinenols A–C[1]) (**1**–**3**), glutin-5-en-3β-ol (**4**), and daucosterol (**5**).



Chinenol A (**1**) was obtained as white powder. The HR-EI-MS showed a molecular-ion peak at *m*/*z* 486.2997, in accordance with the molecular formula $C_{29}H_{42}O_6$ (calc. 486.2981). Its IR (see *Exper. Part*), and ^{1}H- and ^{13}C-NMR spectral data (see *Table 1*) were very similar to those of ulmoidol [3][4], indicating that they have the same skeleton. The structure of **1** was determined to be 11α,12α-epoxy-2α,3β,6β-trihydroxy-24-norurs-4(23)-en-28,13β-olide.

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

Table 1. *^1H-* (400 MHz) *and ^{13}C-NMR* (100 MHz) *Data for Compounds* **1**–**3** (**1** in $CDCl_3$, **2** and **3** in C_5D_5N, δ in ppm, J in Hz)

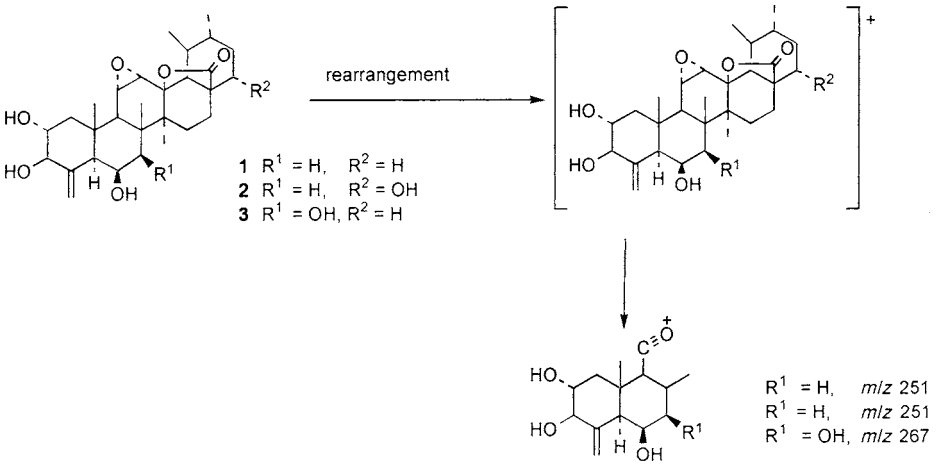
Position	**1**			**2**			**3**		
	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)	HMBC (H → C)
1α	1.12 (br. *d*, *J* = 12.6, 1 H)	47.5 (*t*)	2, 3, 5, 10	1.80 (br. *d*, *J* = 12.4, 1 H)	49.4 (*t*)	2, 3, 5, 10	1.71 (br. *d*, *J* = 12.1, 1 H)	49.4 (*t*)	2, 3, 5, 10
1β	2.03 (*dd*, *J* = 4.8, 12.6, 1 H)		2, 3	2.67 (*dd*, *J* = 4.6, 12.5, 1 H)		2, 3	2.63 (*dd*, *J* = 5.0, 12.5, 1 H)		2, 3
2β	3.42 (*m*, 1 H)	71.5 (*d*)	3	4.22 (*m*, 1 H)	72.5 (*d*)	3	4.18 (*m*, 1 H)	72.9 (*d*)	3
3α	3.58 (*d*, *J* = 9.1, 1 H)	78.1 (d)	2, 4, 23	4.39 (*d*, *J* = 8.8, 1 H)	79.4 (*d*)	2, 4, 5, 23	4.33 (*d*, *J* = 8.9, 1 H)	79.6 (*d*)	2, 4, 23
4		145.9 (*s*)			149.0 (*s*)			148.9 (*s*)	
5α	1.55 (br. *s*, 1 H)	51.5 (*d*)	3, 4, 6, 10, 23	2.0 (br. *s*, 1 H)	52.2 (*d*)	3, 4, 6, 10, 23	2.03 (br. *s*, 1 H)	51.7 (*d*)	3, 4, 6, 10, 23
6α	4.27 (br. *s*, 1 H)	68.1 (*d*)	5, 7, 8	4.76 (br. *s*, 1 H)	68.4 (*d*)	5, 7, 8	4.61 (br. *s*, 1 H)	73.9 (*d*)	5, 7, 8
7	1.33 (*d*, *J* = 2.5, 2 H)	37.4 (*t*)	6, 8	1.61 (*m*, 2 H)	38.4 (*t*)	6, 8	3.90 (*d*, *J* = 3.4, 1 H)	71.8 (*d*)	6, 8
8		40.9 (*s*)			41.7 (*s*)			47.4 (*s*)	
9α	1.63 (br. *s*, 1 H)	49.7 (*d*)	1, 5, 8, 11, 14	2.09 (br. *s*, 1 H)	50.7 (*d*)	1, 5, 8, 11, 14, 25, 26	1.88 (br. *s*, 1 H)	50.4 (*d*)	1, 5, 8, 11, 14, 25
10		37.0 (*s*)			37.8 (*s*)			37.8 (*s*)	
11β	3.12 (br. *s*, 1 H)	54.4 (*d*)	9, 10	3.49 (*d*, *J* = 1.5, 1 H)	54.8 (*d*)	9, 10	3.48 (br. *s*, 1 H)	55.0 (*d*)	9, 10
12β	2.83 (*d*, *J* = 3.8, 1 H)	55.8 (*d*)	13, 14	3.17 (*d*, *J* = 3.5, 1 H)	56.2 (*d*)	13, 14	3.15 (*d*, *J* = 3.8, 1 H)	56.9 (*d*)	11, 14
13		89.4 (*s*)			88.4 (*s*)			89.6 (*s*)	
14		41.2 (*s*)			42.8 (*s*)			43.7 (*s*)	
15α	1.59 (*m*, 1 H)	26.4 (*t*)	27	1.81 (*m*, 1 H)	26.2 (*t*)	27	2.13 (*m*, 1 H)		27
15β	1.00 (*dd*, *J* = 5.3, 14.4, 1 H)		8, 14, 16, 17, 27	1.08 (*dd*, *J* = 3.8, 13.3, 1 H)		8, 14, 16, 17, 27	1.31 (*m*, 1 H)	30.8 (*t*)	8, 14, 16, 17, 27
16α	1.22 (*ddd*, *J* = 4.8, 11.1, 14.1, 1 H)	22.4 (*t*)	14, 15, 17, 18, 22	2.29 (*ddd*, *J* = 3.8, 10.2, 13.1, 1 H)	16.2 (*t*)	13, 14, 16, 17	2.42 (*ddd*, *J* = 5.6, 11.8, 13.7, 1 H)		13, 14, 16, 18
16β	1.97 (*dd*, *J* = 4.8, 14.1, 1 H)		15, 17, 18	1.93 (br. *s*, 1 H)		15, 17, 18	1.89 (*dd*, *J* = 5.6 13.7, 1 H)	23.4 (*t*)	15, 17, 18
17		45.0 (*s*)			49.8 (*s*)			45.7 (*s*)	

Table 1 (cont.)

Position	**1**			**2**			**3**		
	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)	HMBC (H → C)
18β	1.65 (br. *s*, 1 H)	60.4 (*d*)	12, 13, 14, 16, 17, 19, 22	1.93 (br. *s*, 1 H)	59.9 (*d*)	12,13,14,16,17, 19, 22	1.83 (br. *d*, *J* = 11.4, 1 H)	60.8 (*d*)	12, 13, 14, 17, 19, 22, 28, 29
19α	1.55 (br. *d*, *J* = 10.3, 1 H)	37.2 (*d*)	13, 17, 18, 20, 21	1.90 (br. *s*, 1 H)	36.7 (*d*)	13, 17, 18, 20, 21, 29	1.88 (br. *s*, 1 H)	38.1 (*d*)	13, 17, 18, 20, 21
20β	0.83 (*m*, 1 H)	39.2 (*d*)	19, 21, 30	1.63 (*m*, 1 H)	38.6 (*d*)	19, 21, 30	0.90 (*m*, 1 H)	40.5 (*d*)	19, 30
21α	1.47 (*m*, 2 H)	30.8 (*t*)	22	1.92 (*m*, 1 H)	39.6 (*t*)	20, 22	1.26 (*m*, 2 H)	31.0 (*t*)	17
21β				1.64 (*m*, 1 H)		20, 22, 30			
22	1.63 (*m*, 2 H)	30.9 (*t*)	16, 17, 18, 20, 21	4.07 (*dd*, *J* = 4.5, 11.6, 1 H)	69.4 (*d*)	16, 17, 21, 28	1.65 (*m*, 2 H)	32.0 (*t*)	16, 17, 18, 20
23	5.31 (br. *s*, 1 H)	107.8 (*t*)	3, 4, 5	6.26 (br. *s*, 1 H)	108.0 (*t*)	3, 4, 5	6.13 (br. *s*, 1 H)	108.0 (*t*)	3, 4, 5
	5.26 (br. *s*, 1 H)		3, 5	6.20 (br. *s*, 1 H)		3, 5	6.12 (br. *s*, 1 H)		3, 5
25	1.06 (*s*, 3 H)	17.2 (*q*)	1, 5, 9, 10	1.57 (*s*, 3 H)	17.9 (*q*)	1, 5, 9, 10	1.52 (*s*, 3 H)	17.8 (*q*)	1, 5, 9, 10
26	1.31 (*s*, 3 H)	20.7 (*q*)	7, 8, 9, 14	1.89 (*s*, 3 H)	21.3 (*q*)	7, 8, 9, 14	1.88 (*s*, 3 H)	17.0 (*q*)	7, 8, 9, 14
27	0.92 (*s*, 3 H)	16.5 (*q*)	8, 13, 14, 15	1.20 (*s*, 3 H)	16.5 (*q*)	8, 13, 14, 15	1.29 (*s*, 3 H)	17.5 (*q*)	8, 13, 14, 15
28		179.8 (*s*)			178.2 (*s*)			179.1 (*s*)	
29	1.05 (*d*, *J* = 5.8, 3 H)	16.9 (*q*)	18, 19, 20	1.34 (*d*, *J* = 5.8, 3 H)	16.8 (*q*)	18, 19, 20	1.33 (*d*, *J* = 5.8, 3 H)	18.0 (*q*)	18, 19, 20
30	0.83 (*d*, *J* = 5.7, 3 H)	19.0 (*q*)	19, 20, 21	0.90 (*d*, *J* = 6.3, 3 H)	19.2 (*q*)	19, 20, 21	0.89 (*d*, *J* = 5.7, 3 H)	19.7 (*q*)	19, 20, 21

The EI-MS of **1** gave a molecular ion at *m/z* 486, indicating an increase of 16 mass units compared to that of ulmoidol. A base peak at *m/z* 251 originated from decomposition accompanied by rearrangement (see *Scheme*). The ^{1}H-NMR spectrum indicated the presence of five Me groups (1.31 (*s*, 3 H), 1.06 (*s*, 3 H), 1.05 (*d*, *J* = 5.8, 3 H), 0.92 (*s*, 3 H), and 0.83 (*d*, *J* = 5.7, 3 H)), five CH_2O groups (4.27 (br. *s*, 1 H), 3.58 (*d*, *J* = 9.1, 1 H), 3.42 (*m*, 1 H), 3.15 (*d*, *J* = 3.8, 1 H), 3.12 (br. *s*, 1 H)), and two exo-CH_2 H-atoms (5.31 (br. *s*, 1 H), 5.26 (br. *s*, 1 H). The ^{13}C-NMR spectrum of **1** exhibited the signals due to one ester C=O C-atom at δ 179.8, one O-bearing quaternary C-atom at δ 89.4, five O-bearing methine C-atoms at δ 78.1, 71.5, 68.1, 55.8, and 54.4, and two *exo*-CH_2 C-atoms at δ 145.9, 107.8. Comparison of the ^{1}H- and ^{13}C-NMR spectral data of **1**, and ulmoidol showed that the presence of signals corresponding to an O-bearing methine group at δ 4.27 (^{1}H-NMR) and δ 68.1 (^{13}C-NMR) was the main difference. The ^{1}H,^{1}H COSY experiment showed that the signal at δ 4.27 correlated with the signals at δ 1.55 (H–C(5)), and 1.33 (H–C(7)); the HMBC experiment displayed correlations of the signal at δ 4.27 with those at δ 51.5 (C(5)), 37.4 (C(7)), 40.9 (C(8)). So, the additional OH group should be placed at C(6); moreover, the ^{1}H signal at δ 4.27 (br. *s*) indicates the β-OH substitution.

Scheme. *Mass Fragmentation of Compounds* **1**–**3**



Chinenol B (**2**) was obtained as white powder. The HR-EI-MS showed a molecular-ion peak at *m/z* 502.2926, in accordance with the molecular formula $C_{29}H_{42}O_7$ (calc. 502.2930). Its IR (see *Exper. Part*), and ^{1}H- and ^{13}C-NMR data (see *Table 1*) were very similar to those of ulmoidol [3][4], indicating that they have the same skeleton. The structure of **2** was determined to be 11α,12α-epoxy-2α,3α,6β,22α-tetrahydroxy-24-norurs-4(23)-en-28,13β-olide.

The EI-MS of **2** gave a molecular ion at *m/z* 502, suggesting an increase of 16 mass units compared to that of compound **1**. The base peak was observed at *m/z* 251, originating from decomposition accompanied by rearrangement (see *Scheme*), as for **1**. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** and **1** showed that the presence of a further methine group (δ 4.07 (br. *s*) in the ^{1}H-NMR and δ 69.4 in the ^{13}C-NMR) was

the main difference. The ^{1}H,^{1}H-COSY experiment showed that the signal at δ 4.07 correlated with the signal at δ 1.63 (H–C(21)), and the NOESY experiment indicated that the signal at δ 4.07 correlates with the signal at δ 1.93 (H–C(18)), and the HMBC experiment showed that the signal at δ 4.07 correlates with the C-signals at δ 16.2 (C(16)), 49.8 (C(17)), 178.2 (C(28)). Based on these results, the additional OH group should be at C(22); moreover, the signal at δ 4.07 (*dd*, $J = 4.5$, 11.6) indicated a β-H-atom.

Chinenol C (**3**) was obtained as white powder. The HR-EI-MS showed a molecular-ion peak at *m*/*z* 502.2931, in accordance with the molecular formula $C_{29}H_{42}O_7$ (calc. 502.2930). Its IR (see *Exper. Part*), and ^{1}H- and ^{13}C-NMR spectral data (see *Table 1*) were very similar to those of ulmoidol [3][4], indicating that they have the same skeleton. The structure of **3** was determined to be 11α,12α-epoxy-2α,3β,6β,7β-tetrahydroxy-24-norurs-4(23)-en-28,13β-olide.

The EI-MS of **3** gave a molecular ion at *m*/*z* 502, suggesting an increase of 16 mass units compared to that of compound **1**. A fragment peak at *m*/*z* 267 originated from decomposition accompanied with rearrangement (see the *Scheme*). Comparison of the ^{1}H- and ^{13}C-NMR spectral data of compound **3** and **1** indicated the same OH substitution at C(6), which was also supported by the ^{1}H,^{1}HCOSY, NOESY, and HMBC experiments, and an additional OH group in compound **3**. The ^{1}H,^{1}H-COSY and NOESY experiments showed that the signal at δ 3.90 correlated with the signal at δ 4.61 (H–C(6)). The HMBC data indicated that the signal at δ 3.90 correlated with the C-signals at δ 47.4 (C(8)), 43.7 (C(14)), and 17.0 (C(26)). So, the additional OH group should be at C(7). The signal at δ 3.90 (*d*, $J = 3.8$) corresponds to a α-H-atom.

Compounds **4** and **5** were identified by comparison of the spectral data with reported data as *glutin-5-en-3β-ol* (**4**) [5] and *daucosterol* (**5**) [6], isolated from the aerial part of *H. chinensis* BATAL. The compounds **1**–**3** and **5** were assayed for their activities against *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and *Streptococcus pneumonia* (see *Table 2*). Among them, compound **1** showed potent antibacterial activity (*Staphylococcus aureus*); no activities against *Pencillium avellaneum* UC-4376, were detected.

Table 2. *Antibacterial Activities of Compounds* **1**–**3** *and* **5**

	1	**2**	**3**	**5**
Staphylococcus aureus	++	–	+	+
Mycobacterium tuberculosis	+	+	+	+
Streptococcus pneumonia	+	+	+	–

The plant *H. chinensis* is widely distributed in China and has been of important pharmacological value in folk medicine, such as treating dysentery, swelling, and hematochezia [2]. Its AcOEt fraction in primary screening displayed anti-HIV activity. At present, five compounds from this plant showed antibacterial activity, indicating that further research in this area is justified.

Experimental Part

General. Column chromatography (CC): *Qingdao* silica gel (200–300 mesh) and *MCI* gel *CHP-20P*. TLC: *Qingdao* precoated plates, silica gel GF_{254} and *Merck RP-18* F_{254} plates; eluents: MeOH/$CHCl_3$ 1:30 (*A*), 5:95; H_2O/MeOH 50:50, 35:65 (*B*). M.p.: *XPC*-1 apparatus. IR Spectra: *Bio-Rad* FTS spectrometer; in cm^{-1}. NMR Spectra: *Bruker AM-400* or *DRX-500* spectrometer; C_5D_5N solns.; δ values (with ref. to the signal of C_5D_5N with Me_4Si as internal standard; δ in ppm, *J* in Hz. MS: *Autospec 3000* spectrometer; in *m*/*z* (rel. %).

Plant Material. The aerial parts of *Helwingia chinensis* BATAL were collected in Songming, Yunnan, P.R. China, in July 2002. The identity of the plant was established by Dr. *Yang Zeng-hong*, A voucher (No. 101258) was deposited in the herbarium of Kunming Institute of Botany, Kunming, P.R. China.

Extraction and Isolation. The air-dried aerial parts (40 kg) were extracted twice with 95% EtOH/H_2O at r. t.. The solvent was evaporated at $< 50^\circ$ to give a deep-brown waxy residue, which was suspended in H_2O and extracted with AcOEt (3×2000 ml). The AcOEt extract (374 g) was fractionated by CC (silica gel (1500 g, 200–300 mesh; $CHCl_3$/MeOH 100:1, 50:1, 20:1, 10:1) to afford several fractions. A 10-g amount of the fraction (47 g) obtained from $CHCl_3$/MeOH 50:1 was rechromatographed (silica gel (200–300 mesh), $CHCl_3$/Me_2CO 50:1) to afford three fractions. The first fraction (1.1 g) was purified by repeated CC (silica gel; $CHCl_3$/MeOH 50:1, 30:1) and then *Sephadex LH-20* ($CHCl_3$/MeOH 50:50) to give pure **4** (32 mg). The second fraction (0.93 g) was purified by CC (silica gel; $CHCl_3$/MeOH 30:1) and recrystallized (Me_2CO) to give pure **5** (123 mg). The third fraction (5.20 g) was purified by CC (silica gel; $CHCl_3$/MeOH 50:1, 30:1, 20:1), and then *Sephadex LH-20* ($CHCl_3$/MeOH 50:50) and *RP-18* (MeOH/H_2O 50:50, 60:40) to give pure **1** (31 mg), **2** (54 mg), and **3** (75 mg).

Chinenol A (=*11α,12α-Epoxy-2α,3β,6β-trihydroxy-24-norurs-4(23)-en-28,13β-olide;* **1**). White powder. M. p. 256–258°. $[\alpha]_D^{25} = +25.0$ ($c = 0.20$, MeOH). IR (KBr): 3443, 2930, 2867, 2364, 2339, 1772, 1634, 1458, 1386, 1055. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS (70 eV): 486 (22, M^+), 251 (100).

Chinenol B (=*11α,12α-Epoxy-2α,3β,6β,22α-tetrahydroxy-24-norurs-4(23)-en-28,13β-olide;* **2**). White powder. M. p. 212–215°. $[\alpha]_D^{24.8} = +20.0$ ($c = 0.30$, MeOH). IR (KBr): 3443, 2930, 2874, 2363, 2339, 1762, 1635, 1457, 1053. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS (70 eV): 502 (20, M^+), 251 (100).

Chinenol C (=*11α,12α-epoxy-2α,3β,6β,7β-tetrahydroxy-24-norurs-4(23)-en-28,13β-olide;* **3**). White powder. M. p. 204–206°. $[\alpha]_D^{24.8} = +30.0$ ($c = 0.70$, MeOH). IR (KBr): 3443, 2929, 1766, 1651, 1457, 1387, 1072, 1055. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS (70 eV): 502 (20, M^+), 267 (65), 95 (100).

Antibacterial-Activity Assay. Antibacterial activity of compounds **1**–**5** were determined against fungi, *Gram*-positive, and *Gram*-negative bacteria by the paper diffusion method (5-mm diameter). The samples were dissolved in $CHCl_3$ and applied to each paper disk. The disks were dried and applied to the inoculated agar media plates inoculated with the test organisms. Inhibition zones were observed after incubation at 42° and 24 hours for fungi and 42° and 10 h for bacteria.

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