Three New Triterpenoids from *Dracocephalum forrestii*

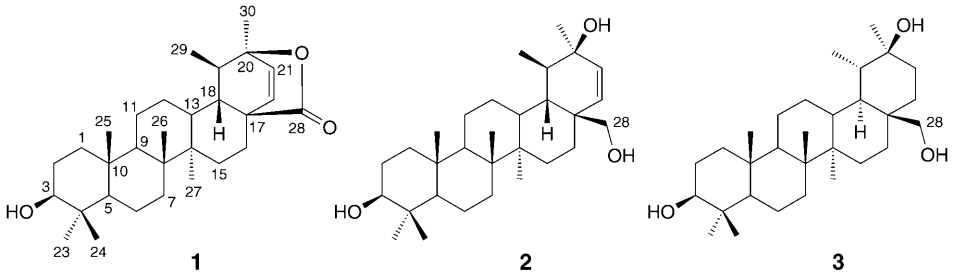
by **Gan-Peng Li**[a])[b]), **Jing-Feng Zhao**[a]), **Li-Juan Yang** [b]), **Xiao-Dong Yang**[a]), and **Liang Li***[a])

[a]) Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, P. R. China (phone: +86-871-5031119; fax: +86-871-5035538; e-mail: liliang5758@sina.com)

[b]) School of Chemistry and Biotechnology, Yunnan Nationalities University, Kunming 650031, P. R. China

From the whole plant of *Dracocephalum forrestii* (Labiatae), three new and nine known triterpenoids were isolated. The new compounds were identified as (3β,18β)-20,28-epoxy-3-hydroxyurs-21-en-28-one (**1**), (3β,18β)-urs-21-ene-3,20,28-triol (**2**), and (3β,18α,19α)-ursane-3,20,28-triol (**3**) by means of in-depth spectroscopic analysis.

Introduction. – *Dracocephalum forrestii* (Labiatae) is a wild perennial plant growing in the regions of Lijiang and Diqing, Yunnan Province, China [1]. It has been used as astringent, diuretic, and antipyretic in traditional Tibetan medicine [2]. No phytochemical study of *D. forrestii* has been reported yet. As part of our investigation on the chemical constituents of this medicine, we isolated twelve triterpenoids from the whole plant of *D. forrestii*, including three new compounds: (3β,18β)-20,28-epoxy-3-hydroxyurs-21-en-28-one (**1**), (3β,18β)-ursane-3,20,28-triol (**2**), and (3β,18α,19α)-ursane-3,20,28-triol (**3**). Their structures were elucidated by means of spectroscopic techniques. The following known triterpenoids were identified, based on comparison of their spectroscopic data and/or by co-eluting TLC using authentic samples: 28-norlup-20(29)-ene-3β,7β-diol [3], 3β,28-dihydroxylup-20(29)-ene [4], 3β-hydroxylup-20(29)-en-28-oic acid [5], 3β-hydroxyurs-11-en-28,13β-olide [6], 28-norurs-12-en-3β-ol [7], 12,13-epoxy-28-norursane-3β,17β-diol [8], 3β-hydroxyurs-12-ene-28-al [8], 3β-hydroxy-11α,12α-epoxyolean-28,13β-olide [9], and oleanolic acid.



1 **2** **3**

Results and Discussion. – The dried plant material of *D. forrestii* was extracted with 70% EtOH. The extract was filtered, concentrated, suspended in H_2O, and then succes-

sively extracted with petroleum ether, AcOEt, and BuOH. The AcOEt-soluble part was purified by successive column chromatography on silica gel, *RP-18* gel, and *Sephadex LH-20* to afford the twelve triterpenoids.

Compound **1** was obtained as a colorless, amorphous powder. In the FAB mass spectrum, the $[M+H]^+$ peak was observed at *m/z* 455, in accord with the molecular formula $C_{30}H_{46}O_3$, as confirmed by HR-ESI-MS and NMR analyses. The IR spectrum revealed the presence of OH groups (3433), a δ-lactone (1729), and a C=C bond (1622 cm^{-1}). The ^{13}C-NMR (DEPT) spectrum of **1** (*Table*) revealed 30 signals: seven Me, eight CH_2, and eight CH groups (one oxygenated and two olefinic C-atoms), and seven quaternary C-atoms (including a C=O group and an oxygenated C-atom). These data suggested that **1** was an ursane-type triterpenoid [6][10]. HMBC and HMQC experiments allowed the assignment of all H- and C-atoms. The ^{1}H-NMR spectrum of **1** further showed a secondary OH function [δ(H) 3.18 (*dd*, *J* = 4.9, 11.4 Hz)], whose chemical shift and splitting pattern indicated an equatorial 3β-OH group. The presence of a CH=CH moiety was inferred from an *AB*-type signal at δ(H) 6.09 (*d*, *J* = 7.5 Hz, 1 H) and 6.06 (*d*, *J* = 7.5 Hz, 1 H).

Table. *^{13}C-NMR Spectroscopic Data of* **1**–**3**. Recorded at 125 MHz in $CDCl_3$; δ in ppm.

Atom	**1**	**2**	**3**	Atom	**1**	**2**	**3**
C(1)	38.9 (*t*)	38.9 (*t*)	38.9 (*t*)	C(16)	25.4 (*t*)	27.3 (*t*)	25.5 (*t*)
C(2)	27.4 (*t*)	27.5 (*t*)	27.9 (*t*)	C(17)	48.1 (*s*)	37.6 (*s*)	31.8 (*s*)
C(3)	79.5 (*d*)	79.4 (*d*)	79.0 (*d*)	C(18)	47.3 (*d*)	45.4 (*d*)	47.2 (*d*)
C(4)	38.8 (*s*)	38.8 (*s*)	38.9 (*s*)	C(19)	44.6 (*d*)	44.2 (*d*)	42.4 (*d*)
C(5)	55.4 (*d*)	55.4 (*d*)	55.5 (*d*)	C(20)	83.7 (*s*)	74.2 (*s*)	72.2 (*s*)
C(6)	18.2 (*t*)	18.2 (*t*)	18.3 (*t*)	C(21)	133.7 (*d*)	133.0 (*d*)	27.5 (*t*)
C(7)	34.0 (*t*)	34.0 (*t*)	34.0 (*t*)	C(22)	138.4 (*d*)	140.6 (*d*)	35.0 (*t*)
C(8)	40.6 (*s*)	40.6 (*s*)	40.8 (*s*)	C(23)	28.0 (*q*)	28.0 (*q*)	28.0 (*q*)
C(9)	50.4 (*d*)	50.6 (*d*)	50.8 (*d*)	C(24)	15.3 (*q*)	15.3 (*q*)	15.4 (*q*)
C(10)	37.1 (*s*)	37.2 (*s*)	37.2 (*s*)	C(25)	16.2 (*q*)	16.3 (*q*)	16.4 (*q*)
C(11)	21.1 (*t*)	21.3 (*t*)	21.3 (*t*)	C(26)	15.7 (*q*)	15.7 (*q*)	15.8 (*q*)
C(12)	27.3 (*t*)	27.3 (*t*)	29.9 (*t*)	C(27)	14.1 (*q*)	14.3 (*q*)	14.3 (*q*)
C(13)	42.2 (*d*)	38.9 (*d*)	39.7 (*t*)	C(28)	175.5 (*s*)	65.9 (*t*)	69.0 (*t*)
C(14)	41.2 (*s*)	41.6 (*s*)	41.4 (*s*)	C(29)	19.7 (*q*)	21.9 (*q*)	20.2 (*q*)
C(15)	26.8 (*t*)	26.5 (*t*)	26.6 (*t*)	C(30)	21.0 (*q*)	22.2 (*q*)	24.9 (*q*)

In the HMBC spectrum of **1** (*Fig.*), the signal at δ(H) 1.53 (Me(30)) was correlated with that at δ(C) 133.7 (C(21)); the olefinic signal at δ(H) 6.06 (H–C(21)) was correlated with δ(C) 21.0 (C(30)) and 48.1 (C(17)); the olefinic signal at δ(H) 6.09 (H–C(22)) was correlated with δ(C) 83.7 (C(20)), 47.3 (C(18)), and 175.5 (C(28)), which revealed that the C=C bond was between C(21) and C(22). The observed HMBC correlations between δ(C) 175.5 (C(28)) and δ(H) 1.01 (H–C(18)), 1.62 (H_b–C(16)), and 6.09 (H–C(22)) confirmed an oxo (=O) group at C(28).

The relative configuration of **1** was deduced by a ROESY experiment. NOEs between the signal for Me(30) and H–C(19), and between the signal for Me(29) and H–C(18) indicated a β-oriented Me(29) and an α-oriented Me(30) group.

Compound **2** was obtained as a colorless, amorphous powder. Absorptions for OH (3425) and C=C (1708 cm^{-1}) functions were observed in its IR spectrum. The EI and FAB mass spectra showed a peak at *m/z* 440 for $[M-H_2O]^+$. Positive HR-ESI-MS showed the $[M-H_2O+H]^+$ signal at *m/z* 441.3719, in accord with the molecular formula $C_{30}H_{50}O_3$. The ^{13}C-NMR (DEPT) spectrum of **2** (*Table*) revealed 30 signals: seven Me, nine CH_2 (one oxygenated), and eight CH groups (one oxygenated and two olefinic C-atoms), as well as six quaternary C-atoms (one oxygenated). Comparison of the ^{1}H- and ^{13}C-NMR data of **1** and **2** indicated that the latter was also an ursane-type triterpenoid, with the same *A*-, *B*-, and *C*-rings as in **1**.

In the ^{13}C-NMR spectrum of **2**, the absence of a δ-lactone C=O group was inferred from upfield shifts for C(20) and C(28) at δ(C) 74.2 and 65.9, respectively. A C=C bond between C(21) and C(22) was established by the HMBC correlations of δ(H) 1.30 (Me(30)) with δ(C) 133.0 (C(21)), of δ(H) 5.98 (H–C(21)) with δ(C) 22.2 (C(30)) and 37.6 (C(17)), and of δ(H) 6.11 (H–C(22)) with δ(C) 74.2 (C(20)), 45.4 (C(18)), and 65.9 (C(28)). The ROESY correlation between Me(30) and H–C(19) indicated that the OH group at C(20) was β-oriented.

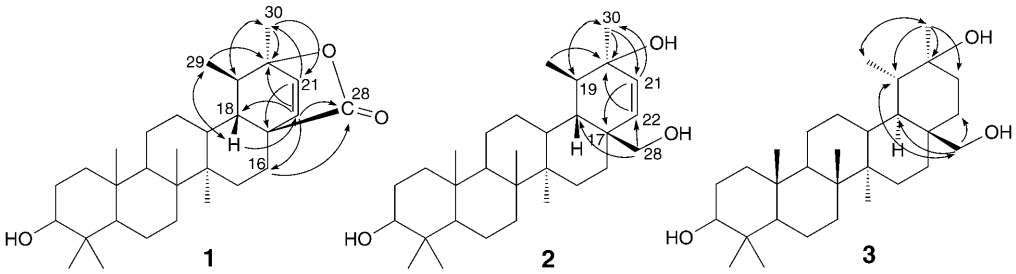


Figure. *Key HMBC* (→) *and ROESY* (↔) *correlations for* **1**–**3**

Compound **3** was obtained as a colorless, amorphous powder. In the IR spectrum, absorptions for OH groups were observed at 3449 cm^{-1}. The molecular formula $C_{30}H_{50}O_3$ was deduced by ESI-MS and NMR analyses. The ESI mass spectrum showed a peak at *m/z* 443 ($[M-H_2O+H]^+$). The ^{13}C-NMR (DEPT) spectrum of **3** (*Table*) revealed 30 signals: seven Me, eleven CH_2 (one oxygenated), and six CH groups (one oxygenated), as well as six quaternary C-atoms (one oxygenated). The ^{1}H- and ^{13}C-NMR data of **3** resembled those of **2**, except for ring *E*. Due to the absence of a C=C bond, **3** contained two CH_2 groups in positions 21 and 22, as confirmed by the HMBC correlations of δ(H) 1.03 (Me(30)) with δ(C) 27.5 (C(21)), and of both δ(H) 3.34 (H_b–C(28)) and 4.14 (H_a–C(28)) with δ(C) 35.0 (C(22)). In the ROESY spectrum, the signal at δ(H) 1.47 (H–C(19)) was correlated with both H_a–C(28) and H_b–C(28), and the signal at δ(H) 0.86 (Me(29)) was correlated with both Me(30) and H–C(18). This indicated that the OH group at C(20) was β-oriented, and that both Me(29) and Me(30) were α-oriented [11].

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh, 10–40 μm; *Qingdao Marine Chemical, Inc.*), *Sephadex LH-20* (*Amersham Pharmacia Biotech.*), or *Lichroprep RP-18* (43–63 μm; *Merck*). TLC: Silica gel GF_{254} (*Qingdao Marine Chemical, Inc.*). Optical rotations: *Perkin-Elmer-341* polarimeter. IR Spectra: *Nicolet AVATAR-360* spectrometer; KBr pellets; in cm^{-1}. ^1H and ^{13}C-NMR Spectra: *Bruker AM-400* and *Bruker DRX-500* instruments; at 400/100 or 500/125 MHz, resp.; δ in ppm rel. to Me_4Si, *J* in Hz. FAB-MS: *VG Autospec-3000* mass spectrometer; in *m*/*z* (rel. %). ESI- and HR-ESI-MS: *API Qstar-Pulsar* spectrometer.

Plant Material. The plant was collected in Xianggelila County, Yunnan Province, P. R. China, in September 2002, and identified by Mr. *A. Dou* (Diqing Tibetan Hospital). A voucher specimen was deposited at the Key Laboratory of Medicinal Chemistry for Natural Resource, Yunnan University, China.

Extraction and Isolation. The air-dried and powered plant material (4.5 kg) was repeatedly extracted with 70% aq. EtOH (4 × 10 l; 48 h each) at ambient temperature. After concentration *in vacuo*, the residue was suspended in H_2O, and then successively extracted with petroleum ether (PE), AcOEt, and BuOH. The AcOEt-soluble extract (270 g) was subjected to CC (2 kg SiO_2; $CHCl_3$/MeOH 50:1 → 50:10) to afford five fractions (Fr.). *Fr.* 2 (3.6 g) was subjected to repeated CC (SiO_2; PE/acetone 10:1 and 5:1) to afford the four known compounds 28-norlup-20(29)-ene-3β,7β-diol (24 mg) [3], lup-20(29)-ene-3β,28-diol (16 mg) [4], 3β-hydroxylup-20(29)-en-28-oic acid (36 mg) [5], and 11α,12α-epoxy-3β-hydroxyolean-28,13β-olide (28 mg) [9]. *Fr.* 3 (2.2 g) was subjected to repeated CC (1. SiO_2, PE/acetone 5:1 and 2:1; 2. *RP-18*, MeOH/H_2O 7:3) to afford the three known compounds 3β-hydroxyurs-11-en-28,13β-olide (14 mg) [6], 28-norurs-12-en-3β-ol (8 mg) [7], and 12,13-epoxy-28-norursane-3β,17β-diol (11 mg) [8]. *Fr.* 4 (9.8 g) was subjected to repeated CC (1. SiO_2, AcOEt/acetone 20:1 and 10:1; 2. *RP-18*, MeOH/H_2O 6:4) to afford **1** (85 mg), **2** (10 mg), and the known compound 3β-hydroxyurs-12-ene-28-al (16 mg) [8]. *Fr.* 5 (1.4 g) was subjected to repeated CC (1. SiO_2, AcOEt/acetone 10:1 and 2:1; 2. *Sephadex LH-20*, MeOH) to afford **3** (2 mg) and the known compound oleanolic acid (40 mg).

(3β,18β)-20,28-Epoxy-3-hydroxyurs-21-en-28-one (**1**). Amorphous powder. $[\alpha]_D^{17} = +18.0$ ($c = 0.75$, $CHCl_3$). IR (KBr): 3433, 2936, 1729, 1622, 1451, 1384, 1073, 1049. ^1H-NMR (500 MHz, $CDCl_3$)[1]): 6.09 (*d*, *J* = 7.5, H–C(22)); 6.06 (*d*, *J* = 7.5, H–C(21)); 3.18 (*dd*, *J* = 4.9, 11.4, H–C(3)); 1.53 (*s*, Me(30)); 0.96 (*s*, Me(23)); 0.93 (*s*, Me(27)); 0.93 (*s*, Me(26)); 0.84 (*d*, *J* = 6.9, Me(29)); 0.82 (*s*, Me(25)); 0.75 (*s*, Me(24)). ^{13}C-NMR: see *Table*. FAB-MS: 455 (100, $[M+H]^+$). HR-ESI-MS: 477.3356 ($[M+Na]^+$, $C_{30}H_{46}NaO_3^+$; calc. 477.3344).

(3β,18β)-Urs-21-ene-3,20,28-triol (**2**). Amorphous powder. $[\alpha]_D^{17} = +29.2$ ($c = 0.12$, $CHCl_3$). IR (KBr): 3425, 2919, 2850, 1708, 1467. ^1H-NMR (500 MHz, $CDCl_3$)[1]): 6.11 (*d*, *J* = 8.1, H–C(22)); 5.98 (*d*, *J* = 8.1, H–C(21)); 4.18 (*d*, *J* = 7.7, H_a–C(28)); 3.20 (*dd*, *J* = 5.0, 10.9, H–C(3)); 2.81 (*dd*, *J* = 1.4, 7.7, H_b–C(28)); 1.30 (*s*, Me(30)); 1.00 (*s*, Me(26)); 0.97 (*s*, Me(23)); 0.95 (*s*, Me(27)); 0.85 (*s*, Me(25)); 0.77 (*s*, Me(24)); 0.69 (*d*, *J* = 6.9, Me(29)). ^{13}C-NMR: see *Table*. FAB-MS: 441 (8, $[M-H_2O+H]^+$). HR-ESI-MS: 441.3719 ($[M-H_2O+H]^+$, $C_{30}H_{49}O_2^+$; calc. 441.3727).

(3β,18α,19α)-Ursane-3,20,28-triol (**3**). Amorphous powder. $[\alpha]_D^{17} = +15.6$ ($c = 0.04$, $CHCl_3$). IR (KBr): 3449, 2927, 2856, 1627, 1460, 1379, 1258, 1077, 1051, 868. ^1H-NMR (500 MHz, $CDCl_3$)[1]): 4.14 (*dd*, *J* = 3.0, 8.5, H_a–C(28)); 3.34 (*d*, *J* = 8.5, H_b–C(28)); 3.20 (*dd*, *J* = 4.8, 11.4, H–C(3)); 1.03 (*s*, Me(30)); 0.98 (*s*, Me(26)); 0.97 (*s*, Me(23)); 0.90 (*s*, Me(27)); 0.86 (*d*, *J* = 7.0, Me(29)); 0.84 (*s*, Me(25)); 0.76 (*s*, Me(24)). ^{13}C-NMR: see *Table*. ESI-MS: 443 (56, $[M-H_2O+H]^+$), 230 (100).

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Received August 31, 2006