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# Two New Norursane-type Triterpenoids from *Dipsacus chinensis* Collected in China

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Two new norursane-type triterpenoids were isolated from *Dipsacus chinensis* (Dipsacaceae) collected in China. The structures were determined based on the spectroscopic data to have enol forms of ene-dione and dione structures in ring A, respectively.

Plants belonging to the genus *Dipsacus* (Dipsacaceae) are widely distributed in Europe, Asia, and Africa and they are widely used as folk medicines.[1] There are about 20 species and many triterpenoid glycosides have been isolated from them.[1] However, *D. chinensis* has never been reported so far. We had a chance to collect this species in Wengshui, Yunnan at 3600 m altitude in 2009.[2] In a continuation of a systematic survey of an active substance and plant diversity, we have studied this species and found two new nortriterpenoids. Now we describe the structures of these compounds.

The root of the plant was extracted with EtOAc and the extract was separated repeatedly by silica gel column chromatography to afford two new compounds **1** and **2** (Chart 1) as well as $\beta$-sitosterol and loganin aglycon.[3]

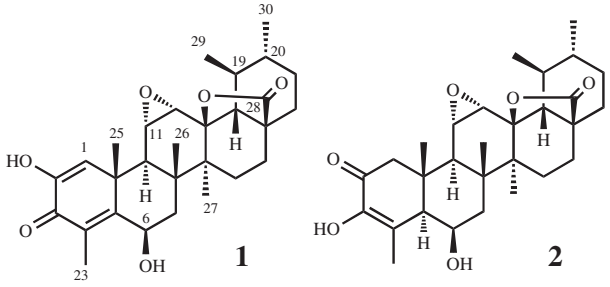


**Chart 1.**

Compound **1**[4] exhibited a quasi-molecular ion peak at $m/z$ 483 and its molecular formula was determined to be $C_{29}H_{38}O_6$ (HRMS). The IR spectrum showed the absorptions at 3447 and 1769 $cm^{-1}$ indicating the presence of a hydroxy group and a lactone. The presence of a lactone was supported by the $^{13}C$ NMR absorption at $\delta$ 177.4 (CO) (Table 1). The $^{13}C$ NMR and HSQC spectra further revealed the presence of six methyl, five methylene, eight methine, and ten quaternary carbons, with two double bonds ($\delta$ 159.2, 145.1, 130.2, and 124.5) and one more carbonyl group ($\delta$ 182.2). The degree of unsaturation was 11 and hence this molecule should be heptacyclic.

The HMBC and $^1H$–$^1H$ COSY spectra were carefully analyzed and the correlations found are shown in Figure 1. The norursane skeleton was assigned and six rings out of seven were apparent now. Because the hydrogen atom for the 2-OH group appeared at $\delta$ 6.50 and was assigned at C-2, 1,4-dien-3-

**Table 1.** $^1H$ and $^{13}C$ NMR data for compounds **1** and **2** ($\delta$ (mult), in $C_6D_6$, 500 MHz for $^1H$ and 125 MHz for $^{13}C$)

| Position | **1** | **1** | **2** | **2** |
|---|---|---|---|---|
| 1$\alpha$ | 6.43 (s) | 124.5 | 1.73 (d, $J$ = 16.1 Hz) | 52.5 |
| 1$\beta$ | | | 2.71 (d, $J$ = 16.1 Hz) | |
| 2 | — | 145.1 | — | 192.3 |
| 3 | — | 182.2 | — | 145.6 |
| 4 | — | 130.2 | — | 126.9 |
| 5 | — | 159.2 | 1.50–1.54 (m) | 50.2 |
| 6 | 4.60–4.63 (m) | 68.4 | 3.67 (br s) | 67.4 |
| 7$\alpha$ | 1.04 (dd, $J$ = 14.7, 5.4 Hz) | 37.3 | 0.95 (dd, $J$ = 15.6, 4.7 Hz) | 37.3 |
| 7$\beta$ | 1.27–1.31 (m) | | 0.83–0.87 (m) | |
| 8 | — | 41.6 | — | 40.8 |
| 9 | 1.76 (d, $J$ = 2.5 Hz) | 46.8 | 1.59 (d, $J$ = 2.2 Hz) | 48.7 |
| 10 | — | 41.4 | — | 39.7 |
| 11 | 2.97 (dd, $J$ = 3.7, 2.5 Hz) | 54.5 | 2.70 (dd, $J$ = 3.7, 2.2 Hz) | 53.7 |
| 12 | 2.79 (d, $J$ = 3.7 Hz) | 56.8 | 2.72 (d, $J$ = 3.7 Hz) | 56.2 |
| 13 | — | 87.8 | — | 87.9 |
| 14 | — | 42.1 | — | 41.8 |
| 15$\alpha$ | 0.61–0.64 (m) | 27.2 | 0.70 (dd, $J$ = 13.2, 5.2 Hz) | 27.0 |
| 15$\beta$ | 1.63–1.70 (m) | | 1.69 (td, $J$ = 13.2, 5.2 Hz) | |
| 16$\alpha$ | 1.63–1.70 (m) | 22.9 | 1.78 (td, $J$ = 13.2, 5.2 Hz) | 23.0 |
| 16$\beta$ | 1.07–1.10 (m) | | 1.18 (dd, $J$ = 13.2, 5.2 Hz) | |
| 17 | — | 44.9 | — | 44.9 |
| 18 | 1.50 (d, $J$ = 12.3 Hz) | 60.4 | 1.50 (d, $J$ = 13.0 Hz) | 60.5 |
| 19 | 1.36–1.44 (m) | 37.7 | 1.50–1.54 (m) | 37.8 |
| 20 | 0.54–0.60 (m) | 40.2 | 0.58–0.63 (m) | 40.2 |
| 21$\alpha$ | 0.94–0.99 (m) | 30.7 | 1.00–1.08 (m) | 30.8 |
| 21$\beta$ | 1.24–1.29 (m) | | 1.30–1.35 (m) | |
| 22$\alpha$ | 1.79 (dt, $J$ = 13.7, 3.7 Hz) | 31.9 | 1.83 (dt, $J$ = 13.7, 3.7 Hz) | 31.9 |
| 22$\beta$ | 1.46 (td, $J$ = 13.7, 4.4 Hz) | | 1.48 (td, $J$ = 13.7, 4.2 Hz) | |
| 23 | 1.86 (3H, s) | 11.0 | 1.90 (3H, d, $J$ = 1.9 Hz) | 12.6 |
| 25 | 1.36 (3H, s) | 23.6 | 1.16 (3H, s) | 16.6 |
| 26 | 1.64 (3H, s) | 21.0 | 1.41 (3H, s) | 21.3 |
| 27 | 0.60 (3H, s) | 16.4 | 0.86 (3H, s) | 16.7 |
| 28 | — | 177.4 | — | 177.5 |
| 29 | 1.19 (3H, d, $J$ = 6.1 Hz) | 17.4 | 1.28 (3H, d, $J$ = 5.6 Hz) | 17.5 |
| 30 | 0.77 (3H, d, $J$ = 6.6 Hz) | 19.5 | 0.83 (3H, d, $J$ = 6.4 Hz) | 19.6 |
| 2-OH | 6.50 (s) | — | — | — |
| 3-OH | — | — | 6.40 (s) | — |

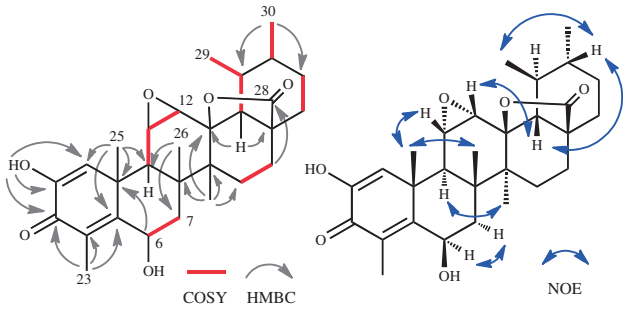


**Figure 1.** Selected COSY, HMBC, and NOESY correlations detected for compound **1**.

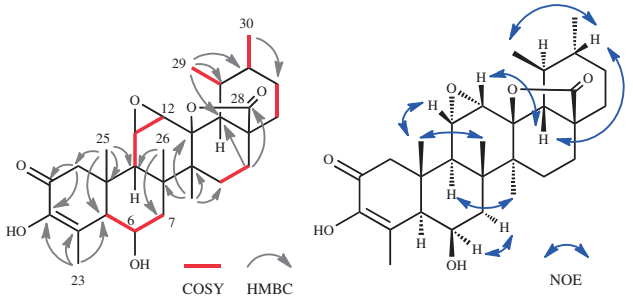


**Figure 2.** Selected COSY, HMBC, and NOESY correlations detected for compound **2**.

one partial structure was indicated for ring A. The other hydroxy group was assigned at C-6 by the correlation between H-6 and C-10. There are two carbons at $\delta$ 54.5 and 56.8, and protons attached to them resonated at $\delta$ 2.97 and 2.79 (mutually coupled with $J = 3.7$ Hz), respectively. These observations suggested the presence of an epoxide at C-11 and -12 positions (the seventh ring). The spectroscopic data of a similar partial structure further supported this assumption.[5] Thus, this compound is established as 11,12-epoxy-2,6-dihydroxy-24-norursa-1,4-dien-3-on-(28 → 13)-olide. The NOE correlation between H-25 and H-26 and H-11, between H-9 and H-27, between H-18 and H-20 and H-12, and between H-20 and H-29 were observed indicating the configuration as shown in Figure 1. The configuration of the hydroxy group at C-6 was determined as $\beta$, because H-6 had NOE with H-7$\alpha$ (axial).

The molecular formula of compound **2**[6] was determined to be $C_{29}H_{40}O_6$ (HRMS). The $^1$H NMR spectrum was very similar to that of compound **1**, except for the presence of an olefinic proton at $\delta$ 6.43 in compound **1**. The IR spectrum was almost the same as that of **1**, showing the presence of an enone and a lactone. The $^{13}$C NMR spectrum clearly indicated that two olefinic absorption peaks were absent in compound **2**, and it is assumed that one olefin was hydrogenated in the case of compound **2**. The HMBC and $^1$H–$^1$H COSY spectra were measured and their correlations are shown in Figure 2. The correlations from H-23 to C-3, -4, and -5 were observed, however, the C-3 was not a carbonyl group and C-5 was a methine group. Furthermore, correlations from H-25 to C-1, -5, -9, and -10 were observed. Further analysis shown in Figure 2 concluded that this compound had 3-en-2-one structure. An absorption at $\delta$ 6.40 (s) was assigned to a hydroxy proton at C-3, because C-3 resonated at $\delta$ 145.6. Therefore the planar structure was determined to be 11,12-epoxy-3,6-dihydroxy-24-norurs-3-en-2-on-(28 → 13)-olide. The configuration at C-6 was determined as $\beta$-OH, because H-6 had almost the same pattern as that of compound **1**, and because H-6 had NOE with H-7$\alpha$ (axial), although the chemical shift was slightly shifted to the higher field. The whole stereochemistry was established by the NOESY spectrum as shown in Figure 2.

Because these nortriterpenoids have many functional groups, biological activities are anticipated.[7] Cytotoxicities of both compounds were tested against HeLa and HL-60 cells. Compound **1** showed $IC_{50}$ 33.9 (HeLa) and 33.5 µM (HL-60), and compound **2** showed $IC_{50}$ 34.6 µM (HL-60). Compound **2** had no acitivity against HeLa (>100 µM). They had only very moderate cytotoxicities.[8]

We have isolated two new norursane-type triterpenoids and their structures were determined on the basis of spectroscopic analyses. Diosphenol structures in ring A are not so often found in nature, however, a Thailand group once reported the isolation of norursane carboxylic acid with a similar ring A structure of compound **1** from *Diospyros decandra* (Ebenaceae).[9] A New Zealand group also reported norursane carboxylic acid methyl ester with the similar ring A of compound **1** from *Pseudopanax arboreum* (Araliaceae).[10] A similar compound to **2** was reported by a Chinese group from *Isodon adenanthus* (Labiatae).[5] It is noteworthy that these types of norursane compounds were isolated from Dipsacaceae family for the first time, to the best of our knowledge.

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## References and Notes

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3 The root of *D. chinensis* (dry weight: 16.4 g) was cut into pieces and was extracted with EtOAc to afford an extract (804.7 mg). This extract was separated by silica gel column chromatography (hexane–EtOAc, in gradient) repeatedly to afford compound **1** (0.9 mg) and **2** (0.9 mg), as well as $\beta$-sitosterol (7 mg) and loganin aglycon (3.7 mg).
4 Compound **1**: $[\alpha]_D^{22}$ −2.78 (*c* 0.10, $CHCl_3$); FT-IR (KBr): 3447, 1769, 1634 cm$^{-1}$; MS (CI) *m/z*: 483 $[M + H]^+$ (base), 467, 465, 317, 166. HRMS (CI) *m/z*: $[M + H]^+$ calcd for $C_{29}H_{39}O_6$, 483.2746; found, 483.2751.
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6 Compound **2**: $[\alpha]_D^{21}$ +51.4 (*c* 0.09, $CHCl_3$); FT-IR (KBr): 3460, 1769, 1666 cm$^{-1}$; MS (CI) *m/z*: 485 $[M + H]^+$ (base), 467, 439, 249. HRMS (CI) *m/z*: $[M + H]^+$ calcd for $C_{29}H_{41}O_6$, 485.2903; found, 485.2879. CD [$\theta$] 314 nm −5686, 282 nm +14208 ($CHCl_3$).
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