Spectral Assignments and Reference Data

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Complete assignments of ^{1}H and ^{13}C NMR spectral data for three polyhydroxylated 12-ursen-type triterpenoids from *Dischidia esquirolii*

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The complete assignments of all the ^{1}H and ^{13}C NMR signals of three polyhydroxylated 12-ursen-type triterpenes, 6β,19α,22α-trihydroxyurs-12-en-3-oxo-28-oic acid (1), 3β,6α,19α,23-tetrahydroxyurs-12-en-28- oic acid (2) and 3β,6β,19α,23-tetrahydroxyurs-12-en-28-oic acid (3), were carried out by means of homo- and hetero-nuclear two-dimensional NMR experiments. Compounds 1–3 were isolated from the aerial parts of *Dischidia esquirolii*. Of them, 1 and 2 were identified as new polyhydroxylated ursolic acid derivatives. Compound 2 is the C-6 hydroxyl epimer of 3, which was isolated first from *Adina rubella*, and its structure is revised in this paper.

Keywords: NMR; 1D/2D NMR; polyhydroxylated ursolic acids; epimer; *Dischidia esquirolii*; Asclepiadaceae

Introduction

Dischidia esquirolii Tsiang et Li (Asclepiadaceae) is widely distributed in the southwest of China. The whole plant is used as folk medicine to relieve skin itching, to treat eye disease, and to cure furuncle and acariasis. So far, no chemical constituents of this plant have been reported. As a part of our phytochemical investigation on medicinal plants, three ursane-type triterpenoids were isolated from the aerial parts of *D. esquirolii* (Fig. 1). Of them, compounds **1** and **2** were elucidated as new polyhydroxylated ursolic acid derivatives. It is noticed that the assignments of C-23 and C-24 methyl groups of this type of pentacyclic triterpenes were sometimes contradictory in different references, even for the same compound.[1–4] This paper deals with this problem on the basis of the complete ^{1}H and ^{13}C NMR assignments of these three compounds by means of 1D and 2D NMR experiments.

Results and Discussion

Compound **1** was obtained as a white amorphous powder with $[\alpha]_D^{25} = -4.26$ ($c = 0.24$, MeOH). The IR spectrum showed the presence of hydroxyl (3444 cm^{-1}) and carbonyl (1700 cm^{-1}) groups as well as an olefinic band (1641 cm^{-1}). On the basis of negative high-resolution electrospray ionization mass spectrometry (HRESIMS) data (*m/z* 501.3205 $[M-H]^-$; calcd. 501.3216) and ^{13}C NMR (DEPT) analysis, **1** possessed the molecular formula $C_{30}H_{46}O_6$ with eight degrees of unsaturation. The ^{13}C NMR (DEPT) spectra displayed the presence of 30 carbon signals due to seven methyls, seven methylenes, seven methines (including one olefinic (δ 128.1) and two oxygen-bearing (δ 68.4 and 75.1) ones) and nine quaternary carbons (including one ketone (δ 215.9), one carboxyl (δ 179.9), one olefinic (δ 139.5), and one oxygen-bearing (δ 72.6) ones) (Table 1), suggesting **1** to be a pentacyclic triterpene with three hydroxyl, one carboxyl, and one ketone groups and one double bond in the molecule. The ^{1}H NMR spectrum showed the occurrence of six singlet methyl signals at δ 1.35, 1.47, 1.67, 1.69, 1.73, and 1.76, and one doublet methyl signal at δ 1.18 ($J = 6.6$ Hz), as well as an olefinic proton at δ 5.69 (br s). These NMR features, together with the carboxylic signal at δ 179.9, two olefinic carbons at δ 128.1 and 139.5, and the oxygen-bearing quaternary carbon at δ 72.6, indicated that compound **1** was a 12-ursen-28-oic acid, whose methine at C-19 or C-20 was oxidated. A singlet proton signal at δ 3.13 assignable to C-18 (δ 55.6) pointed to the hydroxyl substituent at C-19.[1,2,5] In the HMBC spectrum of **1**, the methyl protons of H-30 (δ 1.18 d, $J = 6.6$ Hz) showed long-range correlations with carbon signals at δ 72.6 (C-19), 40.7 (C-20), and 35.9 (C-21). The corresponding proton signals at δ 2.48 (q, $J = 12.0$ Hz) and 1.89 (m) of the latter (δ 35.9) also exhibited cross peaks with the protons at δ 4.46 (dd, $J = 12.0$, 3.8 Hz) (H-22) and 1.73 (m) (H-20) in the $^{1}H-^{1}H$ COSY experiment. The proton signal of high frequency shift corresponding to the oxymethine carbon at δ 75.1 suggested a hydroxyl group was substituted at C-22, which could be further confirmed by its long-range correlations with C-20 (δ 40.7), C-21 (δ 35.9), and C-28 (δ 179.9). Another hydroxyl group was suggested to be located on the C-6 position by the $^{1}H-^{1}H$ COSY experiment, which showed correlations of the proton signal of H-6 (δ 4.70 br s) with both proton signals at δ 1.34 (m) (H-5) and 1.91 (m) (H-7), whose corresponding carbon signals were at δ 57.1 (C-5) and δ 41.3 (C-7), respectively.

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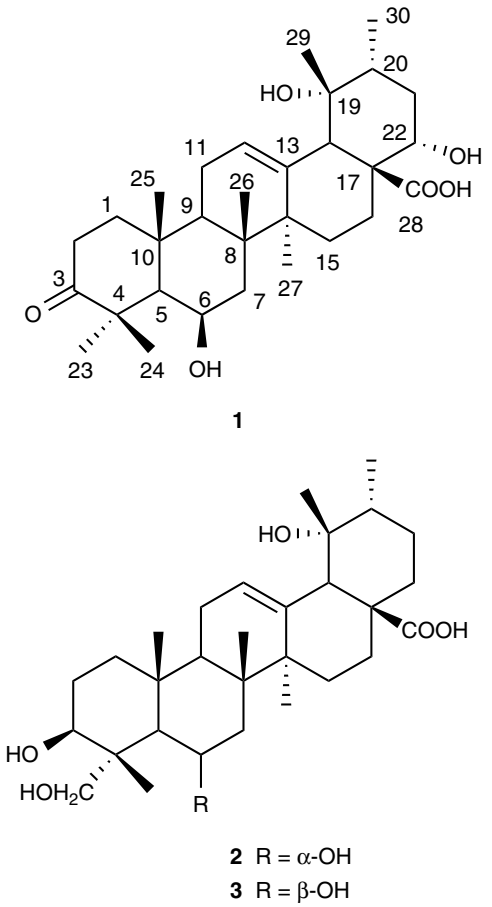

Figure 1. Structures of compounds **1**–**3**.

The distinct HMBC correlations from H-5 to C-6 (δ 68.4), C-10 (δ 36.9), and C-25 (δ 16.3), and from H-7 (δ 1.91) to C-5, C-6, C-8 (δ 39.9), C-9 (δ 47.7), and C-26 (δ 18.4) confirmed that C-6 bore a hydroxyl group. The olefinic proton at δ 5.69 (H-12) gave obvious interactions with the methylene protons at δ 2.25 (H-11α) and 2.12 (H-11β), while these two protons have another connectivity with a methine proton at δ 1.97 (dd, J = 11.0, 6.5 Hz) (H-9) in the ^{1}H–^{1}H COSY spectrum, indicating that there were no substituents in the C-ring. The ketone group was proved to be located on C-3 (δ 215.9), according to its long-range correlations with H-1 (δ 1.27, 1.78), H-2 (δ 2.30, 2.78), H-23 (δ 1.35), and H-24 (δ 1.67) in HMBC experiment and the correlations between H-1 and H-2 in the ^{1}H–^{1}H COSY spectrum. Meanwhile, the protons at H-15 (δ 1.39 and 2.64) and H-16 (δ 2.76 and 3.00) relevant to two methylene carbons at δ 28.9 (C-15) and 19.4 (C-16), which showed mutual relationships in the ^{1}H–^{1}H COSY spectrum and no HMBC correlations with the ketone carbon, were parts of the D-ring. The rotational nuclear overhauser effect spectroscopy (ROESY) interactions (Fig. 2) of H-22 (δ 4.46 dd, J = 12.0, 3.8 Hz) with H-18 (δ 3.13), H-20 (δ 1.73), and H-21β (δ 1.89), as well as H-18 with H-29 (δ 1.47) and H-20 illustrated that both of the hydroxyl substituents at C-22 and C-19 were in α-configurations. The β-orientation of the hydroxyl group at C-6 was confirmed from the small coupling constant of H-6 (δ 4.70, br s) and its correlations with H-5 (δ 1.34) and H-23 (δ 1.35) in the ROESY spectrum. On the basis of the above evidence, the structure of compound **1** was elucidated to be 6β,19α,22α-trihydroxyurs-12-en-3-oxo-28-oic acid.

Table 1. ^{1}H, ^{13}C NMR data and HMBC correlations of compound **1** (C_5D_5N)

H/C	δ H	δ C	HMBC
1α	1.27 td (13.5, 3.9)	42.0	C-2, C-3, C-5, C-9, C-10, C-25
1β	1.78 m		
2α	2.30 m	34.8	C-1, C-3, C-4, C-10
2β	2.87 td (13.5, 6.1)		
3	–	215.9	–
4	–	49.4	–
5	1.34 m (overlapped)	57.1	C-1, C-6, C-10, C-25
6	4.70 br s	68.4	–
7	1.91 m	41.3	C-5, C-6, C-8, C-9, C-26
8	–	39.9	–
9	1.97 dd (11.0, 6.5)	47.7	C-5, C-8, C-10, C-11, C-14, C-25, C-26
10	–	36.9	–
11α	2.25 m	24.2	C-8, C-9, C-10, C-12, C-13
11β	2.12 m		
12	5.69 br s	128.1	C-9, C-11, C-13, C-14, C-18, C-19, C-27
13	–	139.5	–
14	–	43.3	–
15α	1.39 br d (13.0)	28.9	C-8, C-14, C-17, C-27
15β	2.64 br t (11.5)		
16α	3.00 td (13.0, 3.7)	19.4	C-14, C-15, C-17, C-18
16β	2.76 br d (11.5)		
17	–	54.6	–
18	3.13 s	55.6	C-12, C-13, C-14, C-16, C-17, C-19, C-28, C-29
19	–	72.6	–
20	1.73 m (overlapped)	40.7	–
21α	2.48 q (12.0)	35.9	C-17, C-19, C-20, C-22, C-30
21β	1.89 m		
22	4.46 dd (12.0, 3.8)	75.1	C-16, C-20, C-21, C-28
23	1.35 s	25.8	C-3, C-4, C-5, C-24
24	1.67 s	24.1	C-3, C-4, C-5, C-23
25	1.69 s	16.3	C-1, C-5, C-9, C-10
26	1.73 s	18.4	C-8, C-9, C-14
27	1.76 s	25.1	C-8, C-13, C-14, C-15
28	–	179.9	–
29	1.47 s	26.9	C-18, C-19, C-20
30	1.18 d (6.6)	16.7	C-19, C-20, C-21

Compound **2** was obtained as a white amorphous powder. Its molecular formula was deduced to be $C_{30}H_{48}O_6$ on the basis of the negative HRESIMS (m/z 503.3374 [M − H]$^-$; calcd. 503.3372) and the ^{13}C NMR (DEPT) spectra (Table 2). The ^{13}C NMR spectral data were similar to those of **1**, except for the absence of one ketone and one methyl signals, as well as the appearance of an oxygenous methylene at δ 70.4 in **2**. In addition, three oxygen-bearing carbon signals including two methines at δ 67.2 and 74.0 and one quaternary carbon were observed. These NMR features indicated that **2** was a tetra-hydroxylated 12-ursen-28-oic acid whose C-19 was oxidized.[1,2,5] The partial structure of C-, D- and E-ring coincided with those reported in the literature by comparison of the ^{1}H and ^{13}C NMR data, suggesting that all three oxygenous groups were located at the A- and B-ring.[6–8] One of the oxymethines was confirmed to be located at C-6 (δ 67.2) through the same evidence as for compound **1** in the

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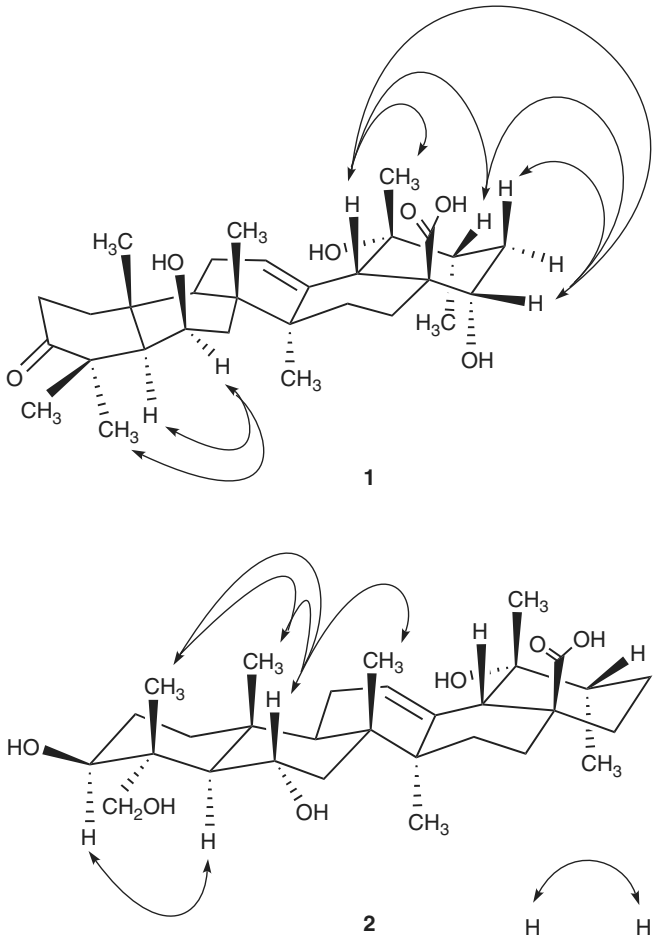

Figure 2. Key ROESY correlations of compounds **1**–**2**.

Table 2. ^{1}H, ^{13}C NMR data of compound **2** and **3** (C_5D_5N)

	2		**3**	
H/C	δ H	δ C	δ H	δ C
1	1.07 m 1.56 m	38.9	1.15 m 1.65 m	41.2
2	1.91 m 1.99 m	27.7	1.97 m 2.15 m	28.0
3	3.96 dd (11.4, 5.0)	74.0	4.27 dd (11.7, 4.4)	73.6
4	–	44.1	–	44.1
5	1.64 d (10.6)	56.0	1.77 brs	49.6
6	4.31 m	67.2	5.06 brs	67.9
7	2.05 m –	45.5	1.90 brd (13.1) 2.11 m	41.5
8	–	41.4	–	39.8
9	1.98 m	47.4	2.08 m	48.4
10	–	39.1	–	37.0
11	1.99 m 2.09 m	24.2	2.21 m 2.29 m	24.2
12	5.61 brs	128.2	5.70 m	128.5
13	–	139.8	–	139.4
14	–	42.4	–	42.7
15	1.31 m 2.42 td (11.4, 4,4)	29.4	1.29 m 2.48 td (13.7, 4.7)	29.4
16	3.09 m 2.03 m	26.5	3.11 m 2.01 m	26.5
17	–	48.4	–	48.4
18	3.05 s	54.6	3.09 s	54.8
19	–	72.8	–	72.8
20	1.51 m	42.4	1.50 m	42.5
21	1.34 m 2.03 m	27.0	1.33 m 2.07 m	27.0
22	2.07 m 2.12 m	38.5	2.08 m 2.14 m	38.6
23	4.41 d (10.5) 4.55 d (10.5)	70.4	4.05 d (10.3) 4.39 d (10.3)	67.4
24	1.37 s	13.7	1.73 s	14.7
25	1.04 s	16.8	1.69 s	17.6
26	1.21 s	18.5	1.71 s	18.4
27	1.74 s	24.7	1.71 s	24.8
28	–	180.8	–	180.8
29	1.44 s	27.2	1.47 s	27.2
30	1.11 d (6.6)	16.9	1.12 d (6.6)	16.9

$^{1}H-^{1}H$ COSY and HMBC experiments. The other oxymethine was supposed to be a usual oxidation at C-3 (δ 74.0), which could be confirmed by the correlations of H-2 (δ 1.91 and 1.99) with both H-3 (δ 3.96, dd, J = 11.4, 5.0 Hz) and H-1 (δ 1.07 and 1.56) in the $^{1}H-^{1}H$ COSY spectrum, and the long-range correlations from H-3 to C-2 (δ 27.7), C-4 (δ 44.1), C-23 (δ 70.4), and C-24 (δ 13.7) in the HMBC experiment. The oxygenous methylene is assigned as the primary alcohol located at the C-23 position (δ 70.4), whose orientation was deduced as α on the basis of the ROESY experiment (Fig. 2). Both protons of H-23 (δ 4.41 and δ 4.55 (each d, J = 10.5 Hz)) have no correlations with H-25 (δ 1.04) or H-26 (δ 1.21). However, correlations of the methyl proton signal at δ 1.37, assignable to H-24 with H-25, were distinctly observed. The large coupling constant of H-5 (δ 1.64, d, J = 10.6 Hz),[9] as well as the significant ROESY correlations of H-6 (δ 4.31) with H-24 (δ 1.37), H-25 (δ 1.04), and H-26 (δ 1.21), revealed the α hydroxy group at C-6. The β hydroxyl group at C-3 was in conformity with the peak split of H-3 and its mutual relationship between H-5 in the ROESY spectrum. Therefore, the structure of compound **2** was identified as 3β,6α,19α,23-tetrahydroxyurs-12-en-28-oic acid.

Compound **3** was the isomer of **2,** found through the quasi-molecular ion peak $[M-H]^-$ at *m/z* 503 displayed in the negative fast atom bombardment mass spectrometry (FAB-MS) and the 1D NMR spectra. The ^{1}H and ^{13}C NMR spectral data of **3** were identical to those of **2** except for some obvious differences in the chemical shifts arising from rings A and B (Table 2). Detailed 2D NMR studies ($^{1}H-^{1}H$ COSY, heteronuclear single quantum coherence (HSQC), and HMBC) revealed that **3** had the same substituted positions as **2.** The ROESY spectrum illustrated that the hydroxyl groups at C-3 and C-19 positions in **3** had the same orientations as those in compound **2**. However, H-6 (δ 5.06, br s) was supposed to be in α configuration owing to the signal split and its ROESY correlation between H-5 (δ 1.77, br s). The deshielded effects of H-24, H-25, and H-26 were also observed because of the 1,3-diaxial interactions by β and axial oriented hydroxyl group at C-6, compared with those of compound **2** (Table 2). The oxygen-bearing methylene was concluded to be at C-23 with α configuration the same as in **2**, according to the obvious cross peaks of one of the H-23 at δ 4.05 (d, J = 10.3 Hz) with H-5 (δ 1.77, br s) and H-6 (δ 5.06, br s) in the ROESY spectrum. Meanwhile, H-24 (δ 1.73, s)

showed no ROESY correlations with H-5 and H-6, confirming its β orientation. Accordingly, the structure of **3** was deduced to be 3β,6β,19α,23-tetrahydroxyurs-12- en-28-oic acid, the C-6 hydroxyl epimer of **2**.

The ^{1}H and ^{13}C NMR spectral data of **3** were exactly the same as those reported in the literature; however, it was determined as 3β,6β,19α,24-tetrahydroxyurs-12-en-28-oic acid, the C-4 epimer of **3**.[6] Accounting for the assignments of C-23 and C-24 signals, the analysis of several literature values revealed that C-23 and C-24 methyl carbon signals often appeared at *ca* δ 28 and δ 17, respectively. Since the C-24 methyl group has the same orientation as the C-25 and C-26 methyl groups, their electron clouds are close in space. They have low frequency shifts because of the shielding effect of the electron cloud density outside the carbon nucleus. The electron cloud of C-23 methyl group is relatively looser, so it is deshielded. When oxidization occurred at C-23 or C-24, the chemical shifts of C-24 or C-23 would be shielded by 3–8 ppm because of the substituent effect.[8–15] Combining with the 2D NMR spectral data, it could be concluded that the primary hydroxyl group in **3** was located at C-23 owing to the methyl carbon signals at δ 14.7. Therefore, the structure of 3β,6β,19α,24-tetrahydroxy- urs-12-en-28-oic acid as reported in the reference should be revised, since **3** is 3β,6β,19α,23-tetrahydroxyurs-12-en-28-oic acid.

According to this study, the assignments of C-23 and C-24 methyl groups of this type of pentacyclic triterpenes should be coincident with each other in different references.

Experimental

Methods

Optical rotations were obtained on a JASCO P-1020 automatic digital polarimeter. IR spectra were determined on a Bruker Tensor 27 spectrometer in KBr pellets. HRESIMS data were detected on an API QSTAR Pulsar LC-Q-TOF spectrometer. FAB-MS spectra were recorded on a VG Autospect 3000 spectrometer. Silica gel HF_{254} prepared for TLC and silica gel (200–300 mesh) for column chromatography (CC) were obtained from Qingdao Marine Chemical Company, Qingdao, China. Reversed-phase silica gel Rp-8 for CC was purchased from Merck Co., Inc. MCI gel CHP20P was the product of Mitsubishi Chemical Corporation.

^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, ROESY, HSQC, and HMBC spectra of compounds **1**–**3** were recorded on Bruker AV-400 and DRX-500 spectrometers equipped with a 5 mm inverse probe. The three compounds were dissolved separately in 0.5 ml C_5D_5N and transferred to a 5 mm NMR tube. All chemical shifts are in ppm (δ), relative to the low-field signals at δ 8.71 and δ 149.9 of the solvent for the ^{1}H and ^{13}C spectra as an internal reference, and the coupling constants (*J*) are in Hz.

The pulse conditions for 6β,19α,22α-trihydroxyurs-12-en-3-oxo-28-oic acid (**1**) were as follows: for the ^{1}H NMR spectra: transmitter frequency (SF01) 500.032 MHz, time domain data size (TD) 32 768, number of scans (NS) 2, number of dummy scans (DS) 0, acquisition time (AQ) 1.363 s, temperature (TE) 300.0 K, relaxation delay duration (D1) 1.000 s, spectral width in hertz (SWH) 12 019.2, 90° pulse width (P1) 9.20 µs; for the ^{13}C NMR spectrum: SF01 100.624 MHz, TD 32 768, NS 2400, DS 2, AQ 0.695 s, TE 292.7 K, D1 3.000 s, SWH 23 584.9 Hz, P1 9.40 µs; for the ^{1}H–^{1}H COSY spectrum: the experiment used 1024 × 128 data point matrices, SF01 500.032 MHz, NS 1, DS 16, AQ 0.114 s, TE 300.0 K, D1 2.000 s, SWH 4496.4 Hz, spectral width or sweep width (SW) 9.000 ppm, gradient pulse duration (P16) 1000 µs; for the HSQC spectrum: the experiment used 1024 × 128 data point matrices, SF01 500.032 MHz, NS 4, DS 16, AQ 0.114 s, TE 300.0 K, D1 1.500 s, SWH 4496.4 Hz, SW 153.974 ppm, P16 1000 µs; for the HMBC spectrum: the experiment used 2048 × 128 data point matrices, SF01 500.033 MHz, NS 96, DS 16, AQ 0.228 s, TE 300.0 K, D1 1.400 s, SWH 4496.4 Hz, SW 219.986 ppm, P16 1000 µs; for the ROESY spectrum: the experiment used 1024 × 180 data point matrices, SF01 500.032 MHz, NS 8, DS 16, AQ 0.093 s, TE 300.0 K, D1 2.000 s, spin-lock pulse duration (P15) 320 ms, SWH 5482.5 Hz, SW 11.000 ppm.

The pulse conditions for 3β,6α,19α,23-tetrahydroxyurs-12-en-28-oic acid (**2**) were similar to those of compound **1** except the following parameters: for the ^{1}H NMR spectra: SF01 500.134 MHz, NS 1, AQ 1.822 s, D1 2.000 s, SWH 8992.8 Hz, P1 10.00 µs; for the ^{13}C NMR spectrum: SF01 125.772 MHz, NS 465, DS 0, AQ 0.555 s, TE 300.0 K, SWH 29 498.5 Hz, P1 5.90 µs; for the HSQC spectrum: SF01 500.132 MHz, NS 1, AQ 0.102 s, D1 1.200 s, SWH 5000.0 Hz, SW 159.980 ppm; for the HMBC spectrum: SF01 500.132 MHz, NS 16, AQ 0.205 s, D1 1.300 s, SWH 5000.0 Hz, SW 235.059 ppm; for the ROESY spectrum: the experiment used 1024 × 144 data point matrices, AQ 0.114 s, P15 1200 ms, SWH 4496.4 Hz, SW 9.000 ppm.

The pulse conditions for 3β,6β,19α,23-tetrahydroxyurs-12-en-28-oic acid (**3**) were as follows: for the ^{1}H NMR spectra: the same as those of **2** except for SF01 500.133 MHz; for the ^{13}C NMR spectrum: the same as those of **2** except for NS 168; for the ^{1}H–^{1}H COSY spectrum: the same as those of **1** and **2**; for the HSQC spectrum: the same as those of **1** except for NS 2, D1 1.300 s, SW 167.958 ppm; for the HMBC spectrum: the same as those of **1** except for SF01 500.032 MHz, NS 16, D1 1.300 s, SW 200.063 ppm; for the ROESY spectrum: the same as those of **1** except for 1024 × 153 data point matrices, AQ 0.114 s, P15 1200 ms, SWH 4496.4 Hz, SW 9.000 ppm.

Plant material

The aerial parts of *D. esquirolii* were collected at Wenshan County, in the southeast of Yunnan province, China, and identified by Prof. Chongren Yang (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen is deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The fresh aerial parts of *D. esquirolii* (7.5 kg) were minced and extracted under reflux with MeOH three times (4 h–3 h–3h). The extract was concentrated and the residue (334 g) was suspended in water and partitioned successively by petrol ether, $CHCl_3$ and *n*-BuOH. After evaporation of the solvents, the corresponding petrol ether (35 g), $CHCl_3$ (72 g) and *n*-BuOH (145 g) fractions were obtained.

The $CHCl_3$ extract was subjected to silica gel (200–300 mesh) CC and eluted with $CHCl_3$–MeOH mixtures of increasing polarity to give nine crude fractions (Frs. 1–9). Fr. 4 (3.0 g) was subjected to silica gel column eluted with a gradient of $CHCl_3$–MeOH mixtures, and further purified by Rp-8 with MeOH–H_2O (20% → 100%) to yield compound **1** (7 mg). Fr. 5 (9.3 g) was submitted to MCI HP20SS eluting with MeOH–H_2O (0 → 100%) to give eight fractions (Frs. I–VIII). Fr. VI was subjected to silica gel CC repeatedly eluted with petroleum ether–acetone or $CHCl_3$–MeOH mixtures to afford compound **2** (15 mg) and compound **3** (40 mg).

6β,19α,22α-Trihydroxyurs-12-en-3-oxo-28-oic acid (**1**): white amorphous powder, $[\alpha]_D^{25} = -4.26$ ($c = 0.24$, MeOH); IR (KBr) ν_{max} cm^{-1} 3444, 1700, 1641; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz) data, see Table 1; HRESIMS (N) *m/z* 501.3205 $[M - H]^-$ (calcd for $C_{30}H_{45}O_6$, 501.3216).

3β,6α,19α,23-Tetrahydroxyurs-12-en-28-oic acid (**2**): white amorphous powder, $[\alpha]_D^{26} = +30.15$ ($c = 0.20$, MeOH); IR (KBr) ν_{max} cm^{-1} 3425, 1689, 1638; ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data, see Table 2; HRESIMS (N) *m/z* 503.3374 $[M - H]^-$ (calcd for $C_{30}H_{47}O_6$, 503.3372).

3β,6β,19α,23-Tetrahydroxyurs-12-en-28-oic acid (**3**): white amorphous powder, $[\alpha]_D^{26} = +11.85$ ($c = 0.21$, MeOH); ^1H NMR (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) data, see Table 2; FAB (N) *m/z* 503 $[M - H]^-$.

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