Novel Triterpenoids from *Microtropis triflora* with Antitumor Activities

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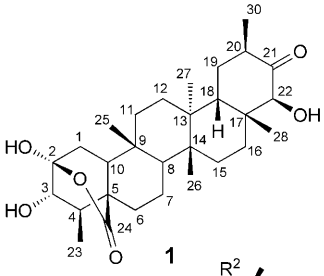
Two unprecedented norfriedelane-type triterpenoids, triflora lactone (**1**) and triptocalline B (**2**), were isolated from the MeOH extract of the stalks of *Microtropis triflora* MERR. et FREEM., together with five known compounds (**3**–**7**). Their structures were elucidated on the basis of spectroscopic and mass-spectrometric data (*Tables 1* and *2*). The new compounds were found to be cytotoxic against human cervical squamous carcinoma (Hela) cells, with IC_{50} values of 21.6 and 17.6 μg/ml, respectively.

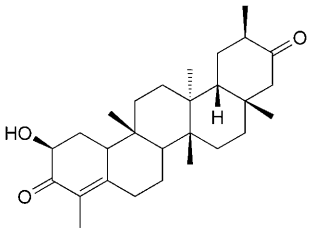
Introduction. – In our continuing search for bioactive molecules from the plants of the Celastraceae family [1], we obtained a cytotoxic MeOH extract from the stalks of the new plant species *Microtropis triflora* MERR. et FREEM. (Celastraceae), collected in Chongqing, P. R. China. This extract was selected for bioassay-guided fractionation on the basis of its cytotoxicity against human cervical squamous carcinoma (Hela) cells. In this paper, we report two novel norfriedelane-type triterpenes, triflora lactone (**1**) and triptocalline B (**2**), which were obtained together with five known compounds: friedelin (**3**), 3-oxofriedelan-28-oic acid (**4**), 29-hydroxyfriedelan-3-one (**5**), salaspermic acid (**6**), and orthosphenic acid (**7**).

Results and Discussion. – Compound **1**, isolated as a colorless powder (m.p. 256–258°), showed a positive *Liebermann–Burchard* reaction, indicating a triterpene. The molecular formula was established as $C_{29}H_{44}O_6$, with eight degrees of unsaturation, by FT-ICR-MS (*m/z* 511.3021 ($[M+Na]^+$; calc. 511.3036)) and NMR experiments. The IR spectrum showed OH, ester C=O and keto C=O absorptions at 3307, 1691, and 1730 cm^{-1}, respectively. All ^1H- and ^{13}C-NMR signals could be fully assigned by means of $^1H,^1H$-COSY, HMQC, and HMBC experiments (*Table 1*).

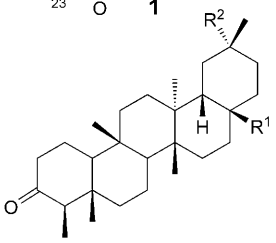
The ^{13}C-NMR (DEPT) spectrum of **1** allowed the assignment of 29 signals, including six Me, eight CH_2, and seven CH groups, as well as eight quaternary C-atoms, with two C=O resonances at δ(C) 213.9 and 175.3, and two oxygenated methines at δ(C) 77.1 and 77.4, as well as a doubly oxygenated quaternary C-atom at δ(C) 105.0. The ^1H-NMR spectrum showed four Me *singlet*s at δ(H) 0.74 (Me(28)), 0.77 (Me(25)), 0.83 (Me(26)), and 1.27 (Me(27)), two Me *doublet*s at δ(H) 0.80 (Me(23)) and 0.90 (Me(30)), and two oxygenated methine signals at δ(H) 3.01 and 4.45, all characteristic of a friedelane-type triterpene [2]. Further, taking into account the degree of unsaturation, compound **1** had to carry a lactone group.



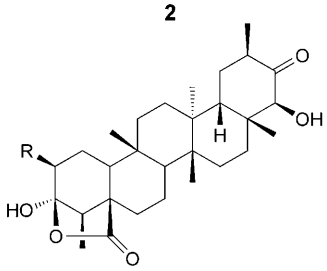
1



2



3 $R^1 = R^2 = Me$
4 $R^1 = COOH$ $R^2 = Me$
5 $R^1 = Me$ $R^2 = CH_2OH$



6 R = H
7 R = OH

The ^{13}C-NMR spectroscopic data of **1** were very similar to those of triptocalline A [3], except for the signals due to the *A*-ring. Thus, an oxo group at C(21) and an OH group at C(22) were inferred. The two *doublet* Me groups could only be connected with C(4) and C(20). One of them, that at δ(H) 0.90, was identified as Me(30) based on HMBC long-range correlations from its H-atoms to C(19), C(20), and C(21). Therefore, the other Me *doublet* at δ(H) 0.80 was assigned to Me(23) in 4-position. The HMBC correlation from Me(23) to an oxygenated methine (δ(C) 77.1) and the correlated resonance at δ(H) 3.01 suggested an OH group at C(3). Similarly, the HMBC correlation from H–C(4) to a quaternary C-atom at δ(C) 105.0 suggested that C(2) was substituted with *two* O-atoms in a hemiacetal fashion, one O-atoms belonging to a lactone. The correlation from H–C(4) to the C=O atom at δ(C) 175.3 indicated that the ester C=O group was at C(24), in accord with a 29-norfriedelan-24,2-olide. The downfield shift of C(1) at δ(C) 26.9, and the upfield shift of C(10) at δ(C) 52.1, in combination with HMBC correlations of H–C(4), H–C(10), and H–C(6) to C(24), confirmed this assignment [2].

The relative configuration of **1** was deduced from the 2D-NOESY correlations between H–C(3) and H_β–C(1), between H–C(20) and H–C(22), between H–C(22) and Me(27), and between H–C(18) and Me(30), as shown in the chemical formula. From the above data, the structure of triflora lactone (**1**) was, thus, elucidated as (2α,3α,22β)-2,3,22-trihydroxy-21-oxo-29-norfriedelan-24,2β-olide.

Compound **2**, isolated in crystalline form (m.p. 213–215°), showed a positive *Liebermann–Burchard* reaction. Its molecular formula was determined as $C_{28}H_{42}O_3$ by FT-ICR-MS (*m*/*z* 449.3018 ($[M+Na]^+$; calc. 449.3032)) and NMR spectroscopy. The IR spectrum of **2** suggested the presence of OH, C=O, and α,β-unsaturated C=O groups at 3495, 1716, 1672 and 1613 cm^{-1}, respectively. All ^{1}H- and ^{13}C-NMR signals

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**. In (D_6)DMSO at 500/125 MHz, resp.; δ in ppm, J in Hz.

Position	^{13}C	^{1}H	^{1}H,^{1}H-COSY	HMBC (H → C)
1	26.9 (*t*)	1.58, 2.19	H–C(10)	C(2), C(3), C(5), C(9), C(10)
2	105.0 (*s*)			
3	77.1 (*d*)	3.01	H–C(4)	C(2), C(23)
4	47.7 (*d*)	1.44	H–C(3), Me(23)	C(2), C(3), C(5), C(10), C(23), C(24)
5	44.6 (*s*)			
6	30.1 (*t*)	1.04, 2.11	H–C(7)	C(5), C(10), C(24)
7	18.6 (*t*)	1.38, 1.44	H–C(6)	C(5)
8	48.1 (*d*)	1.38		C(10), C(13), C(26)
9	37.5 (*s*)			
10	52.1 (*d*)	1.78	H–C(1)	C(1), C(2), C(4), C(5), C(11), C(24), C(25)
11	33.3 (*t*)	1.30	H–C(12)	C(13)
12	28.8 (*t*)	1.51	H–C(11)	C(9)
13	40.2 (*s*)			
14	39.1 (*s*)			
15	28.3 (*t*)	1.23, 1.30	H–C(16)	C(26)
16	29.6 (*t*)	1.30, 1.44	H–C(15)	C(14), C(17)
17	44.8 (*s*)			
18	45.6 (*d*)	1.65	H–C(19)	C(12), C(14), C(17), C(20), C(27), C(28)
19	31.7 (*t*)	1.53, 2.16	H–C(18), H–C(20)	C(17), C(20), C(21)
20	41.5 (*d*)	2.70	H–C(19), Me(30)	C(19), C(21), C(30)
21	213.9 (*s*)			
22	77.4 (*d*)	4.45 (*s*)		C(17), C(20), C(28)
23	16.1 (*q*)	0.80 (*d*, J=7.1)	H–C(4)	C(3), C(4), C(5)
24	175.3 (*s*)			
25	16.6 (*q*)	0.77 (*s*)		C(8), C(9), C(10), C(11)
26	15.8 (*q*)	0.83 (*s*)		C(8), C(13), C(14), C(15)
27	19.3 (*q*)	1.27 (*s*)		C(12), C(13), C(14), C(18)
28	26.1 (*q*)	0.74 (*s*)		C(16), C(17), C(18), C(22)
30	15.6 (*q*)	0.90 (*d*, J=6.1)	H–C(20)	C(19), C(20), C(21)

could be readily assigned by means of ^{1}H, ^{1}H-COSY, HMQC, and HMBC experiments (*Table 2*).

The ^{1}H-NMR spectrum of **2** showed five Me *singlet*s at δ(H) 0.82 (Me(25)), 0.96 (Me(26)), 0.98 (Me(28)), 1.36 (Me(27)), and 1.86 (Me(23)), as well as one Me *doublet* at δ(H) 1.02 (J=8.2 Hz, Me(30)). The ^{13}C-NMR (DEPT) spectrum of **2** showed six Me, nine CH_2, and five CH groups, as well as eight quaternary C-atoms, including an α,β-unsaturated C=O moiety with signals at δ(C) 127.1, 158.8, and 199.6. Also observed were a six-membered ring C=O function at δ(C) 213.7, and a oxygenated methine at δ(C) 71.0.

The location of the α,β-unsaturated C=O moiety at C(3) (δ(C) 199.6), C(4) (127.1), and C(5) (158.8) was inferred from HMBC long-range couplings from H−C(1), H−C(2), and H−C(23) to C(3), respectively, from both Me(23) and H−C(10) to both C(4) and C(5). Similarly, the HMBC correlation from the oxygenated methine at δ(H) 4.03 to both C(1) and C(3) indicated a 2-OH group, and its β-configuration was apparent from a NOESY correlation between H−C(2) and H−C(10). Because of the quaternary C(4) atom and the methine H−C(20), the Me *doublet* at δ(H) 1.02 was

Table 2. *^{1}H- and ^{13}C-NMR Data of* **2**. In (D_6)DMSO at 500/125 MHz, resp.; δ in ppm, J in Hz.

Position	^{13}C	^{1}H	^{1}H, ^{1}H-COSY	HMBC
1	28.2 (*t*)	2.34, 1.52	H–C(2), H–C(10)	C(2), C(3), C(5), C(10)
2	71.0 (*d*)	4.03 (*d*, *J* = 6.2, 14.0)	H–C(1)	C(1), C(3)
3	199.6 (*s*)			
4	127.1 (*s*)			
5	158.8 (*s*)			
6	30.4 (*t*)	2.95, 2.07	H–C(7)	C(4), C(5), C(8), C(10)
7	20.3 (*t*)	1.74, 1.53	H–C(6), H–C(8)	C(5), C(9), C(14)
8	47.3 (*d*)	1.76	H–C(7)	C(6), C(10), C(14), C(27)
9	37.2 (*s*)			
10	51.8 (*d*)	2.37	H–C(1)	C(2), C(4), C(5)
11	32.4 (*t*)	1.65, 1.42	H–C(12)	C(9), C(10), C(12), C(13)
12	28.9 (*t*)	1.68, 1.44	H–C(11)	C(9), C(14), C(18)
13	39.2 (*s*)			
14	39.5 (*s*)			
15	27.9 (*t*)	1.43	H–C(16)	C(8), C(13), C(16)
16	35.1 (*t*)	1.78, 1.32	H–C(15)	C(14), C(18), C(22)
17	37.7 (*s*)			
18	43.4 (*d*)	1.63	H–C(19)	C(13), C(14), C(17), C(20), C(22)
19	31.3 (*t*)	2.18, 1.70	H–C(18), H–C(20)	C(13), C(17), C(18), C(21), C(30)
20	41.9 (*d*)	2.59	H–C(19), H–C(21)	C(18), C(22), C(30)
21	213.7 (*s*)			
22	53.3 (*t*)	2.95, 1.86		C(17), C(18), C(20), C(21), C(28)
23	10.7 (*q*)	1.86 (*s*)		C(3), C(4), C(5)
25	17.0 (*q*)	0.82 (*s*)		C(8), C(9), C(10), C(11)
26	15.0 (*q*)	0.96 (*s*)		C(8), C(13), C(14), C(15)
27	17.9 (*q*)	1.36 (*s*)		C(12), C(13), C(14), C(18)
28	32.3 (*q*)	0.98 (*s*)		C(16), C(17), C(18), C(22)
30	14.7 (*q*)	1.02 (*d*, *J* = 8.2)	H–C(20)	C(19), C(20), C(21)

assigned to C(30), and its β-configuration was apparent by the NOESY correlation between Me(30) and both H−C(18) and Me(28).

The ^{13}C-NMR spectroscopic data of **2** (*Table 2*) were similar to those of triptocalline A [3], except for the signals due to rings *D* and *E*, with signals for an oxo group at C(21) and a CH_2(22) group. In the HMBC spectrum of **2**, the correlations from H−C(19) and H−C(30) to C(21) at δ(C) 213.7, and the correlation from H−C(22) to C(17), C(18), C(20), C(21), and Me(28) confirmed these assignments. From all these data, compound **2** was identified as (2β)-2-hydroxy-24,29-dinorfriedel-4-ene-3,21-dione.

The five known compounds, friedelin [3], 3-oxofriedelan-28-oic acid [3], 29-hydroxyfriedelan-3-one [3], salaspermic acid [4], and orthosphenic acid [5], were identified by comparison of their spectroscopic data with those reported in the literature.

The two new compounds **1** and **2** were tested for *in vitro* antitumor activity against human cervical squamous carcinoma (Hela) cells. Both compounds showed significant anti-tumor activities, with IC_{50} values of 21.6 and 17.6 μg/ml, respectively, as compared to cisplatin (IC_{50} = 5.4 μg/ml) used as positive control.

Experimental Part

General. TLC: Silica-gel plates GF_{254} (*Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, China); detection by spraying with 10% H_2SO_4 in EtOH, followed by heating at 105°. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Haiyang*). Melting points (m.p.): X_4 micro-melting-point apparatus; uncorrected. IR Spectra: *Nicolet NEXUS-470* FT-IR spectrometer, with KBr pellets; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker Avance DRX-500* spectrometer, at 500/125 MHz, resp.; δ in ppm rel. to Me_4Si, J in Hz. FT-ICR-MS: *Bruker Apex-III* mass spectrometer; in m/z. ESI-MS: *Bruker Esquire-3000^{plus}* spectrometer.

Plant Material. The stalks of *Microtropis triflora* MERR. et FREEM. were collected in Jinfuoshan, Chongqing, P. R. China, in September 2004. A voucher specimen was deposited in our laboratory, and identified by Dr. *Bin Wu* (Zhejiang University, Hangzhou, P. R. China).

Extraction and Isolation. The shade-dried powder (8.6 kg) of the stalks of *M. triflora* was extracted at r.t. with MeOH (3×). After filtration and evaporation *in vacuo*, a gummy residue (886 g) was obtained, which was taken up in H_2O and successively extracted with petroleum ether (PE), AcOEt, and BuOH (4×3 l each). This procedure yielded 83 g, 140 g, and 326 g of PE-, AcOEt-, and BuOH-soluble materials after solvent removal.

Part of the PE extract (78 g) was adsorbed on SiO_2 (100 g) and subjected to CC (1 kg SiO_2; PE/AcOEt 1:0 → 0:1): 5 main fractions (*Fr.*). From *Fr. P-1* (4.5 g), compounds **3** and **5** were obtained by eluting with PE/AcOEt 50:1 and 20:1, resp. *Fr. P-3* (5.3 g) was rechromatographed (110 g SiO_2; PE/AcOEt 3:1) to afford **2** (20.1 mg). *Fr. P-4* (3.5 g) was rechromatographed (80 g SiO_2; PE/AcOEt 2:1) to afford **7**.

Part of the original AcOEt extract (135 g) was adsorbed on SiO_2 (150 g) and chromatographed (2 kg SiO_2; $CHCl_3$/MeOH 1:0 → 0:1): 8 main fractions. *Fr. A-1* was rechromatographed (PE/acetone 5:1) to afford **4**. *Fr. A-2* (6.3 g) was rechromatographed (130 g SiO_2; PE/acetone 4:1) to afford **6**. *Fr. A-3* (5.6 g) was rechromatographed (120 g SiO_2; PE/acetone 3:1) to afford **1** (20.8 mg) and **7**.

Triflora Lactone (=*(2α,3α,22β)-2,3,22-Trihydroxy-21-oxo-29-norfriedelan-24,2β-olide*; **1**). Colorless powder. M.p. 256–258°. $[\alpha]_D^{20} = +10.32$ ($c = 0.018$, MeOH). IR (KBr): 3307, 2918, 2850, 1730, 1691, 1470, 1449, 1384, 1346, 1261, 1224, 1191, 1178, 1145, 1086, 1051, 884. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (pos.): 511 ($[M+Na]^+$). FT-ICR-MS: 511.3021 ($[M+Na]^+$, $C_{29}H_{44}NaO_6^+$; calc. 511.3036).

Triptocalline B (=*(2β)-2-Hydroxy-24,29-dinorfriedel-4-ene-3,21-dione*; **2**). Colorless powder. M.p. 213–215°. $[\alpha]_D^{20} = +15.32$ ($c = 0.032$, MeOH). IR (KBr): 3495, 2943, 2923, 2866, 2793, 1716, 1672, 1613, 1458, 1375, 1312, 1140, 1074, 1032. ESI-MS (pos.): 449 ($[M+Na]^+$). FT-ICR-MS: 449.3018 ($[M+Na]^+$, $C_{28}H_{42}NaO_3^+$; calc. 449.3032).

Antitumor Assay. The cytotoxicities of **1** and **2** towards human cervical squamous carcinoma (Hela) cells were tested as follows. The Hela cells were cultured at 37° under a humidified atmosphere of 5% CO_2 in *RPMI-1640* medium supplemented with 10% fetal calf serum, and dispersed in replicate 96-well plates (5×10^4 cells/well) for 48 h. Compounds **1** and **2** (2.5–100 μg/ml) or cisplatin (pos. control) were then added. After 48 h of exposure to the toxins, the cell viability was determined by the MTT (=3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) colorimetric assay [6] by recording the absorbance at λ_{max} 570 nm with an ELISA reader. Each test was performed in triplicate ($n = 3$).

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