Four New Nortriterpenoids from *Phlomis umbrosa*

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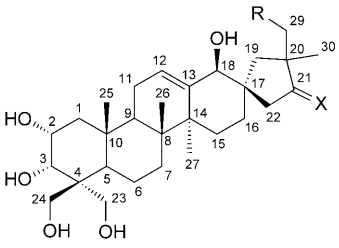
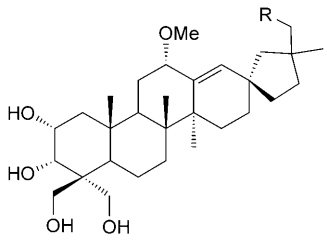
Four new 28-noroleanane-derived spirocyclic triterpenoids, compounds **1**–**4**, were isolated from the rhizomes of *Phlomis umbrosa*. Their structures were elucidated on the basis of 1D- and 2D-NMR analyses, in combination with high-resolution MS experiments.

Introduction. – *Phlomis umbrosa* TURCZ. (Labiatae) is a perennial herb growing in North China. In traditional Chinese medicine (TCM), the rhizomes of *P. umbrosa* have been used to treat cold, reduce swelling, and staunch bleeding [1][2]. Previous chemical investigations of this plant resulted in the isolation of various compounds including triterpenoids, iridoid glycosides, and phenylethanoid glycosides [3–10]. In a previous study, we already reported a new nortriterpene from *P. umbrosa*, and its structure was confirmed by X-ray analysis [11].

In the present work, we report the isolation and characterization of the new spirocyclic nortriterpenoids **1**–**4**, which were obtained from the rhizomes of *P. umbrosa*. Their structures were established by spectroscopic and mass-spectrometric methods, especially 2D-NMR and HR-MS analyses.



1 R = H X = H_2
2 R = OH X = H_2
5 R = H X = O



3 R = H
4 R = OH

Results and Discussion. – Compound **1** was isolated as a colorless, amorphous powder. HR-ESI-MS indicated the molecular formula $C_{29}H_{48}O_5$ (*m*/*z* 499.3400 ($[M+Na]^+$; calc. 499.3399), and the IR spectrum revealed OH groups (3406 cm^{-1}). In the ^{1}H-NMR

spectrum of **1** (*Table 1*), five Me *singlet*s (δ(H) 1.06, 1.09, 1.11, 1.20, 1.20), three oxygenated methines (δ(H) 4.50 (*m*), 4.88 (br. *s*), 4.22 (*m*)); two hydroxymethyl groups (δ(H) 4.43, 4.63 (2*d*, $J=11.1$ Hz each); 4.10, 4.25 (2*d*, $J=11.0$ Hz each)), and a trisubstituted C=C bond (δ(H) 6.31 (br. *s*)) were distinguished. A total of 29 signals were observed in the ^{13}C-NMR spectrum of **1** (*Table 1*). A direct comparison of the ^{13}C-NMR data of **1** with those of the reported compound (17*S*)-2α,3α,18β,23,24-pentahydroxy-19(18 → 17)-*abeo*-28-norolean-12-en-21-one (**5**) [11] indicated that the two compounds had the same skeleton, **1** being the 21-deoxo congener. In the HMBC spectrum of **1** (*Table 1*), Me(27) and CH_2(11) correlated with an olefinic quaternary C-atom at δ(C) 143.6. Further, an ^{1}H,^{1}H-COSY correlation between CH_2(11) and H−C(12) indicated that the trisubstituted C=C bond was located in 12-position. The five OH groups were located at C(2), C(3), C(18), C(23), and C(24), based on HMBC and NOE data.

The relative configuration of **1** was determined by extensive analysis of the ^{1}H-NMR and NOESY data. The 2-OH group should be α-orientated, considering the NOEs for H−C(2)/Me(25) and H−C(2)/Me(24). The small coupling constant between H−C(2) and H−C(3), and the NOE for H−C(3)/Me(24) revealed an α-OH group at C(3). The NOE for H−C(18)/Me(27) indicated that the 18-OH group was β-orientated. The quaternary C-atom C(17) (δ(C) 52.9) is a spiro center. The NOE for H−C(18)/H−C(19) (δ(H) 1.28, 2.36) suggested that CH_2(19) was α-orientated. Thus, from the above data, the structure of compound **1** was assigned as (2α,3α,17*R*,18β)-19(18 → 17)-*abeo*-28-norolean-12-ene-2,3,18,23,24-pentol.

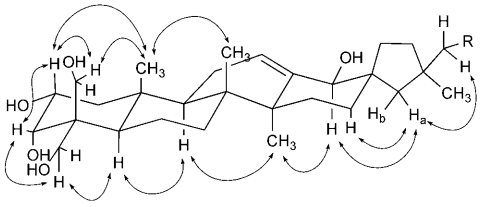


Fig. 1. *Key NOE correlations for* **1** *and* **2**

The molecular formula of **2**, $C_{29}H_{48}O_6$, determined by HR-ESI-MS (*m*/*z* 515.3334 ([$M+$Na]$^+$; calc. 515.3349)), had one O-atom more than in the case of **1**. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **1** and **2** (both recorded in C_5D_5N) indicated that they had closely related structures, the only difference being an additional OH group at C(29) in **2**. This structural deduction was confirmed by HMBC experiments (*Table 1*): both H-atoms of CH_2(29) (δ(H) 3.80, 3.89) correlated with C(19), C(20), C(21), and C(30), and Me(30) correlated with C(19), C(20), and C(29). Thus, the OH group was assigned to be at C(29). Complete ^{1}H- and ^{13}C-NMR assignments were achieved on the basis of HSQC, HMBC, and NOESY experiments. Thus, the structure of compound **2** was assigned as (2α,3α,17*R*,18β)-19(18 → 17)-*abeo*-28-norolean-12-ene-2,3,18,23,24,29-hexol.

Compound **3** was isolated as an amorphous powder. HR-ESI-MS showed the [$M+$Na]$^+$ peak at *m*/*z* 513.3550, indicating the molecular formula $C_{30}H_{50}O_5$. The IR spectrum of **3** revealed OH absorptions (3415 cm^{-1}). The ^{1}H-NMR spectrum of **3** (*Table 2*) showed five Me, three oxygenated CH, and two oxygenated CH_2 groups, a

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**, *together with HMBC (H → C) Correlations.* At 300/75 MHz, resp., in C_5D_5N; δ in ppm, J in Hz.

Position	**1**			**2**		
	δ(H)	δ(C)	HMBC	δ(H)	δ(C)	HMBC
1	1.85–1.90 (*m*)	43.8		1.85–1.90 (*m*)	43.8	
	2.05–2.10 (*m*)			2.05–2.10 (*m*)		
2	4.50 (*m*)	66.9		4.46 (*m*)	66.9	
3	4.88 (br. s)	74.2	1, 2, 23	4.90 (br. *s*)	74.2	1, 2, 23
4		48.0			48.0	
5	2.15–2.20 (*m*)	45.3	4, 6, 24	2.15–2.20 (*m*)	45.3	4, 6, 24
6	1.60–1.70 (*m*)	19.5		1.60–1.70 (*m*)	19.5	
	1.90–1.95 (*m*)			1.90–1.95 (*m*)		
7	1.45–1.50 (*m*)	35.2		1.50–1.60 (*m*)	35.2	
	1.70–1.80 (*m*)			1.70–1.80 (*m*)		
8		40.3			40.3	
9	1.85–1.90 (*m*)	48.5	8, 10, 26	1.85–1.90 (*m*)	48.5	8, 10, 26
10		38.7			38.7	
11	2.10–2.15 (*m*)	24.0	9, 12	2.10–2.15 (*m*)	24.0	9, 12
12	6.31 (br. *s*)	118.9		6.31 (br. *s*)	118.8	
13		143.6			143.4	
14		44.6			44.6	
15	1.00–1.10 (*m*)	28.1		1.05–1.10 (*m*)	28.2	
	1.80–1.85 (*m*)			1.55–1.70 (*m*)		
16	1.50–1.60 (*m*)	36.6		1.60–1.75 (*m*)	36.6	
	1.70–1.80 (*m*)					
17		50.9			50.9	
18	4.22 (br. *s*)	75.3		4.30 (br. *s*)	75.9	
19	2.36 (*d*, *J* = 12.6)	52.9	16–18, 20–22, 29, 30	2.61 (*d*, *J* = 13.2)	49.0	16–18, 20–22, 29, 30
	1.28 (*d*, *J* = 12.6)			1.35 (d, *J* = 13.2)		
20		39.6			45.2	
21	1.45–1.50 (*m*)	42.9		1.50–1.55 (*m*)	38.0	
	1.80–1.85 (*m*)			2.20–2.25 (*m*)		
22	1.40–1.50 (*m*)	29.6		1.50–1.55 (*m*)	29.4	
	2.00–2.05 (*m*)			2.10–2.15 (*m*)		
23	4.43 (*d*, *J* = 11.1)	69.6	3–5, 24	4.45 (*d*, *J* = 11.0)	69.6	3–5, 24
	4.63 (*d*, *J* = 11.1)			4.64 (*d*, *J* = 11.0)		
24	4.10 (*d*, *J* = 11.0)	64.6	3–5, 23	4.10 (*d*, *J* = 11.0)	64.6	3–5, 23
	4.25 (*d*, *J* = 11.0)			4.27 (*d*, *J* = 11.0)		
25	1.20 (*s*)	17.8	1, 5, 9, 10	1.19 (*s*)	17.9	1, 5, 9, 10
26	1.06 (*s*)	18.3	7–9, 14	1.08 (*s*)	18.2	7–9, 14
27	1.09 (*s*)	23.6	8, 13–15	1.10 (*s*)	23.6	8, 13–15
29	1.11 (*s*)	30.6	19–21, 30	3.80 (*d*, *J* = 10.1)	71.7	19–21, 30
				3.89 (*d*, *J* = 10.1)		
30	1.20 (*s*)	30.8	19–21, 29	1.32 (*s*)	26.9	19–21, 29

vinyl H-atom, and a MeO signal. The ^{13}C-NMR data of **1** (*Table 1*) and **3** (*Table 2*) were similar. For **3**, a total of 30 signals were observed, 29 being due to the basic skeleton. The major difference between the two compounds was the location of the C=C bond and the absence or presence of a MeO group.

Table 2. *^{1}H- and ^{13}C-NMR Data of* **3** *and* **4**, *together with HMBC (H → C) Correlations.* At 300/75 MHz, resp., in C_5D_5N (**3**) or CD_3OD (**4**); δ in ppm, J in Hz. Asterisks (*) mark overlapping signals.

Position	**3**			**4**		
	δ(H)	δ(C)	HMBC	δ(H)	δ(C)	HMBC
1	1.85–1.90 (*m*)	43.8		1.32 (*m*)	42.8	
	2.05–2.10 (*m*)			1.68 (*m*)		
2	4.48 (*m*)	66.9		3.83 (*m*)	67.3	
3	4.90 (br. *s*)	74.2	1, 2, 23	4.00 (br. *s*)	73.9	1, 2, 23
4		48.1			48.0	
5	2.15–2.20 (*m*)	45.4	4, 6, 24	1.60–1.65 (*m*)	45.2	4, 6, 24
6	1.65–1.75 (*m*)	19.4		1.40–1.45 (*m*)	19.5	
7	1.50–1.80 (*m*)	35.7		1.42–1.60 (*m*)	35.9	
8		41.4			41.8	
9	1.60–1.70 (*m*)	46.9	8, 10, 26	1.80–1.85 (*m*)	47.2	8, 10, 26
10		38.9			39.2	
11	2.00–2.10 (*m*)	38.9		2.00–2.10 (*m*)	39.0	9, 12
12	3.54 (br. *s*)	84.3		3.11 (br. *s*)	85.2	
13		136.3			136.8	
14		43.3			44.0	
15	1.90–2.05 (*m*)	29.5		1.05–1.10 (*m*)	30.0	
				1.65–1.70 (*m*)		
16	1.63–1.72 (*m*)	34.3		1.61–1.82 (*m*)	34.4	
17		44.9			45.7	
18	5.53 (br. *s*)	140.5	12, 14, 16, 17, 19	5.48 (br. *s*)	141.2	12, 14, 16, 17, 19
19	1.55–1.60 (*m*)	57.2	16–18, 20, 21, 29, 30	1.65*	52.9	16–18, 20, 21, 29, 30
	1.35–1.40 (*m*)			1.25*		
20		39.7			45.5	
21	1.40–1.60 (*m*)	41.1		n.v.[a])	36.3	
22	1.55–1.65 (*m*)	29.5		n.v.[a])	30.9	
	1.90–2.05 (*m*)					
23	4.44 (*d*, *J* = 10.9)	69.6	3–5, 24	3.71 (*d*, *J* = 11.0)	68.9	3–5, 24
	4.64 (*d*, *J* = 10.9)			3.91 (*d*, *J* = 11.0)		
24	4.09 (*d*, *J* = 10.8)	64.5	3–5, 23	3.56 (*d*, *J* = 11.0)	64.7	3–5, 23
	4.25 (*d*, *J* = 10.8)			3.67 (*d*, *J* = 11.0)		
25	1.13 (*s*)	18.3	1, 5, 9, 11	0.97 (*s*)	18.0	1, 5, 9, 11
26	0.94 (*s*)	18.8	7–9, 14	0.90 (*s*)	18.8	7–9, 14
27	1.22 (*s*)	22.4	8, 13–15	1.20 (*s*)	22.3	8, 13–15
29	1.07 (*s*)	31.4	19–21, 30	3.28*	71.8	19–21, 30
				3.32*		
30	1.07 (*s*)	30.8	19–21, 29	1.06 (*s*)	26.7	19–21, 29

[a]) Not visible due to overlapping signals.

The four OH groups of **3** were located at C(2), C(3), C(23), and C(24), based on HSQC and the HMBC correlations (*Table 2*). The trisubstituted C=C bond was placed between C(13) and C(18), in accord with HMBC correlations between Me(27) and C(13), and between H–C(18) and C(12), C(14), C(17), and C(19), respectively. The location of the MeO group was deduced by an HMBC correlation from the MeO H-atoms (δ(H) 3.17) to C(12) (δ(C) 84.3). The NOESY cross-peaks between H–C(2)

and both Me(25) and Me(26), and the small coupling constant between H–C(2) and H–C(3) indicated that the 2- and 3-OH groups were α-orientated. Furthermore, the NOESY cross-peaks from the MeO group to both Me(27) and H–C(9), and the small coupling constant for H–C(12) indicated an α-MeO function. From these data, the structure of compound **3** was determined as (2α,3α,12α,17*R*)-12-methoxy-19(18 → 17)-*abeo*-28-norolean-13(18)-ene-2,3,23,24-tetrol.

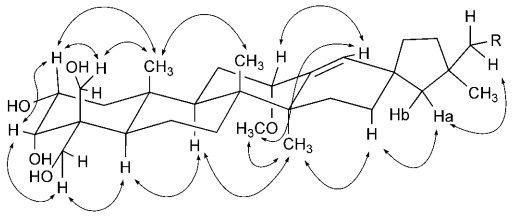


Fig. 2. *Key NOE correlations for* **3** *and* **4**

Compound **4** had the molecular formula $C_{30}H_{50}O_6$, as determined by HR-ESI-MS (*m*/*z* 529.3510 ($[M+Na]^+$, $C_{30}H_{50}NaO_6^+$; calc. 529.3505). Comparison of the ^{1}H- and ^{13}C-NMR data of **3** and **4** (*Table 2*) indicated very similar structures, compound **4** being the 29-hydroxylated congener of **3**. HMBC correlations from CH_2(29) (δ(H) 3.28, 3.32) to C(19), C(20), C(21), and C(28), respectively, supported this conclusion. The relative configuration of **4** was determined by the same method as described above. Accordingly, its structure was derived as (2α,3α,12α,17*R*)-12-methoxy-19(18 → 17)-*abeo*-28-norolean-13(18)-ene-2,3,23,24,29-pentol.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Co., Ltd.*) and *Toyopearl HW-40* (*TOSOH*). TLC: silica gel GF_{254} plates; visualization under UV light and by spraying with Ce_2SO_4, followed by heating. HPLC separations were performed on a *Jasco Gulliver* system, with a *PU-2089* pump, an *RI-2031* and *UV-2075* detector, and an *ODS* column (*YMC-Pack ODS-A*, *SH-343-5*), eluting with MeOH/H_2O. IR Spectra: *Perkin-Elmer 577* spectrometer; in cm^{-1}. Optical rotations: *Perkin-Elmer 241-MC* digital polarimeter. NMR Spectra: *Bruker AV-300* instrument; at 300 (^{1}H) and 75 MHz (^{13}C)); δ in ppm rel. to Me_4Si, *J* in Hz. HR-ESI-MS: *Waters LCT-Premier* instrument; in *m*/*z*.

Plant Material. The rhizomes of *Phlomis umbrosa* TURCZ. were collected in Jianshi County, Hubei Province, P. R. China, in January 2005. The plant was identified by Prof. *Dingrong Wan*, School of Life Sciences, South-Central University for Nationalities, China. A voucher specimen (No. D20050110) was deposited at the School of Pharmaceutical Sciences, Tianjin Medical University, Tianjin, P. R. China.

Extraction and Isolation. The dried rhizomes (3.2 kg) of *P. umbrosa* were crushed and then extracted with 95% aq. EtOH (10 l) for 6 h at reflux (3×). The pooled EtOH solns. were concentrated *in vacuo*, and the resulting residue (500 g) was suspended in H_2O, and then successively extracted with petroleum ether (PE), AcOEt, and BuOH. The PE-soluble fraction afforded, upon evaporation, a residue (20 g), which was further separated by CC (1 kg SiO_2; PE/AcOEt 10:1, 8:1, 6:1, 3:1, 2:1, 1:1, 1:2, 1:3, 0:1, then AcOEt/MeOH 19:1, 10:1, 0:1) to yield 17 fractions (*Fr. 1–17*) according to TLC. *Fr. 10* (560 mg) was subjected to CC (*Toyopearl HW-40*; $CHCl_3$/MeOH 2:1) to afford four subfractions (*Fr. 10.1–10.4*). *Fr. 10.3* was purified by HPLC (*ODS-A*; MeOH/H_2O 9:1, 3.0 ml/min) to provide **1** (25.4

mg) and **2** (21.3 mg). *Fr. 12* (520 mg) was subjected to CC (*Toyopearl HW-40*; $CHCl_3$/MeOH 2:1) to afford five subfractions (*Fr. 12.1–12.5*). *Fr. 12.3* (258 mg) was further purified by HPLC (*ODS-A*; MeOH/H_2O 9:1, 3.0 ml/min) to afford **3** (12.1 mg) and **4** (6.6 mg).

*(2α,3α,17*R*,18β)-19(18 → 17)*-Abeo-*28-norolean-12-ene-2,3,18,23,24-pentol* (**1**). Amorphous powder. $[\alpha]_D^{25} = +6.0$ ($c = 0.51$, C_5H_5N). IR (KBr): 3406, 2947, 2861, 1453, 1379, 1273, 1137, 1094, 1043, 988. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 499.3400 ($[M+Na]^+$, $C_{29}H_{48}NaO_5^+$; calc. 499.3399).

*(2α,3α,17*R*,18β)-19(18 → 17)*-Abeo-*28-norolean-12-ene-2,3,18,23,24,29-hexol* (**2**). Amorphous powder. $[\alpha]_D^{25} = +4.5$ ($c = 0.45$, C_5H_5N). IR (KBr): 3392, 2929, 2862, 1455, 1385, 1260, 1095, 1040, 804. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 515.3334 ($[M+Na]^+$, $C_{29}H_{48}NaO_6^+$; calc. 515.3349).

*(2α,3α,12α,17*R*)-12-Methoxy-19(18 → 17)*-abeo-*28-norolean-13(18)-ene-2,3,23,24-tetrol* (**3**). Amorphous powder. $[\alpha]_D^{25} = -13.1$ ($c = 0.46$, C_5H_5N). IR (KBr): 3415, 2925, 2855, 1541, 1459, 1381, 1273, 1232, 1090, 1042. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 513.3550 ($[M+Na]^+$, $C_{30}H_{50}NaO_5^+$; calc. 513.3556).

*(2α,3α,12α,17*R*)-12-Methoxy-19(18 → 17)*-abeo-*28-norolean-13(18)-ene-2,3,23,24,29-pentol* (**4**). Amorphous powder. $[\alpha]_D^{25} = -7.8$ ($c = 0.31$, C_5H_5N). IR (KBr): 3422, 2919, 2849, 1539, 1462, 1397, 1259, 1042, 801. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 529.3510 ($[M+Na]^+$, $C_{30}H_{50}NaO_6^+$; calc. 529.3505).

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