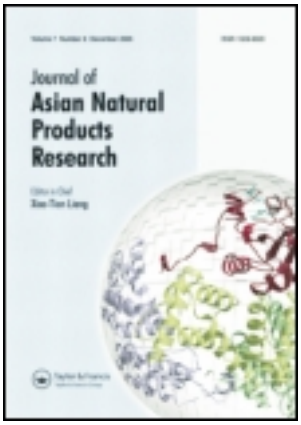

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Secondary metabolites from the roots of *Phlomis umbrosa*

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Secondary metabolites from the roots of *Phlomis umbrosa*

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The phytochemical study of the roots of *Phlomis umbrosa* Turcz afforded a new phenylethanoid glycoside, 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-[2,3-diacetyl-α-L-rhamnopyranosyl-(1 → 3)]-4-*O*-*cis*-feruloyl-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (**1**), and two new 28-noroleanane-derived spirocyclic triterpenoids, phlomishexaol C (**2**) and phlomishexaol D (**3**). Their structures were elucidated on the basis of 1D and 2D NMR analyses, in combination with high-resolution MS experiment.

Keywords: *Phlomis umbrosa*; rhizomes; phenylethanoid glycoside; nortriterpenoids

1. Introduction

Phlomis umbrosa Turcz, one of the plants of the genus *Phlomis* (Lamiaceae), is a perennial herbaceous plant found in northern China. The rhizome of *P. umbrosa* has been used in tujia medicine as a drug to treat cold, reduce swelling, and staunch bleeding in Tujia drug [1,2]. Phytochemical studies carried out by different research groups have resulted in the isolation of various triterpenoids, iridoid glycosides, and phenylethanoid glycosides [2–10]. As reported previously [11–15], we isolated some new nortriterpenes, phenylethanoid glycosides, and iridoid glycosides from the plant. In the course of our further investigation on the roots of *P. umbrosa*, a novel phenylethanoid glycoside and two new nortriterpenes were obtained from this plant. Their structures were elucidated on the basis of various 2D NMR techniques, including HSQC, HMBC, $^{1}H-^{1}H$ COSY, and NOESY spectroscopy.

2. Results and discussion

The petroleum ether (PE) and *n*-BuOH fractions from *P. umbrosa* were separated by silica gel, gel permeation chromatography, and pre-HPLC (ODS-A) to give a new phenylethanoid glycoside, 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-[2,3-diacetyl-α-L-rhamnopyranosyl-(1 → 3)]-4-*O*-*cis*-feruloyl-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (**1**), and two new nortriterpenes, phlomishexaol C (**2**) and phlomishexaol D (**3**) (Figure 1).

Compound **1** was obtained as an amorphous yellowish powder. Its HR-ESI-MS exhibited a pseudo-molecular ion at *m/z* 891.2924 $[M + Na]^{+}$, which was compatible with the molecular formula $C_{40}H_{52}O_{21}$. The IR spectrum showed absorption bands of hydroxyl (3443 cm^{-1}), α, β-unsaturated ester (1732 and 1630 cm^{-1}), and aromatic rings (1620, 1595, and 1516 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **1** (Table 1), extensively analyzed with the aid of $^{1}H-^{1}H$ COSY and HSQC experiments, exhibited proton

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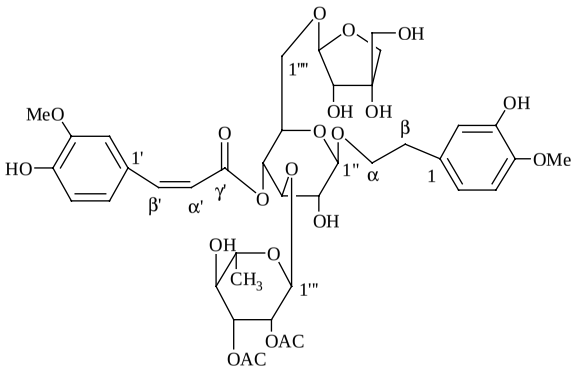

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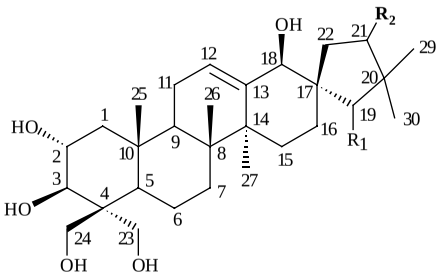

2 R_1 = OH R_2 = H

3 R_1 = H R_2 = OH

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

Table 1. ^{1}H and ^{13}C NMR spectral data of compound **1** (^{1}H, 500 MHz; ^{13}C, 125 MHz in CD_3OD; δ in ppm, J in Hz).

Position	δ (H)	δ (C)	Position	δ (H)	δ (C)
1		133.0	1″	4.32 (d, 7.7)	104.3
2	6.73 (br s)	117.2	2″	3.43–3.46 (m)	74.6
3		147.3	3″	3.82–3.87 (m)	82.8
4		147.1	4″	5.00 (m)	71.1
5	6.83 (d, 8.3)	113.0	5″	Overlap	75.8
6	6.70 (br d, 8.3)	121.6	6″	3.75–3.80 (m)	68.1
α	3.75, 4.03 (m)	72.3	1‴	5.15 (br s)	100.6
β	2.82 (t, 7.3)	36.7	2‴	5.35 (br s)	71.7
OCH_3	3.83 (s)	56.6	3‴	4.95 (m)	73.4
1′		127.8	4‴	3.41 (m)	71.5
2′	7.88 (br s)	115.6	5‴	3.75 (m)	70.7
3′		148.4	6‴	1.16 (d, 6.1)	18.3
4′		149.7	$COCH_3$	2.06	172.4/20.8
5′	6.71 (d, 8.2)	112.1		1.90	172.7/20.9
6′	7.09(br d, 8.2)	127.4	1⁗	4.92 (overlapped)	111.0
α′	5.82 (d, 12.9)	115.5	2⁗	3.88 (m)	79.5
β′	6.91 (d, 12.9)	148.4	3⁗		80.7
γ′		167.3	4⁗	3.75–3.95 (m)	75.4
OCH_3	3.91 (s)	56.6	5⁗	3.50–3.60 (m)	65.8

signals characteristic of a *Z*-feruloyl group [three aromatic protons resonating at δ 7.88 (br s), 7.09 (br d, $J = 8.2$ Hz), 6.71 (d, $J = 8.2$ Hz) as an ABX system and two *cis*-olefinic protons as an AB system at δ 5.82 and 6.91 (d, $J = 12.9$ Hz)], a 3-hydroxy-4-methoxy-phenylethanol moiety [three aromatic protons at δ 6.83 (d, $J = 8.3$ Hz), 6.73 (br s), 6.70 (d, $J = 8.3$ Hz) as an ABX system, a broad

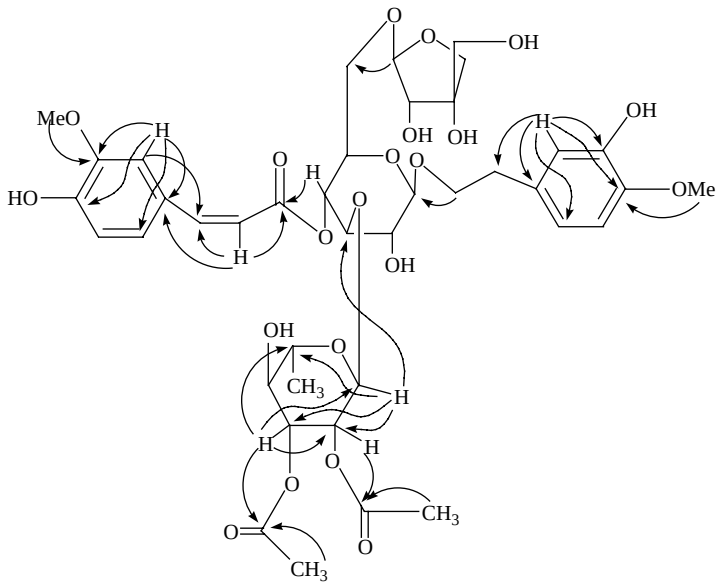

Figure 2. The key HMBC correlations of compound **1**.

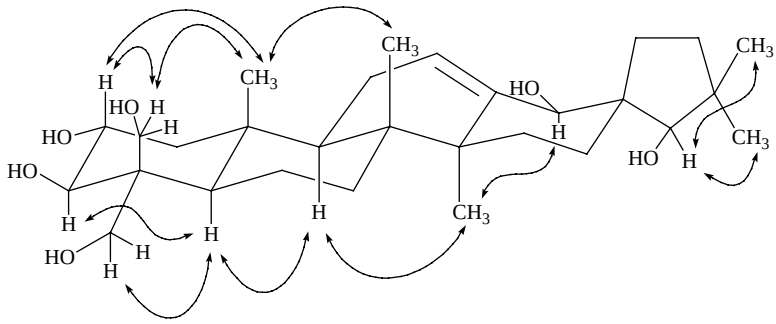

Figure 3. The key NOESY correlations of compound **2**.

triplet signal at δ 2.82 (t, $J = 7.3$ Hz), and two non-equivalent protons at δ 4.03 and 3.75 due to the side chain of the aglycone moiety], and two acetyl groups [δ 1.90 (3H, s), 2.06 (s)]. Additionally, three signals assignable to anomeric protons indicated the presence of three sugar moieties in **1**: the doublet at δ 4.32 (d, $J = 7.7$ Hz**,** H-1′ of β-glucosyl), a broad singlet at δ 5.15 (br s, H-1″ of α-rhamnosyl), and an overlapped signal at δ 4.92 (overlapped, H-1‴ of β-apiosyl). The sugars were detected after the acid hydrolysis and compared with the standard sugars on TLC. Compound **1** was a phenylethanol glycoside, with three sugar moieties and one *cis*-feruloyl group. Its ^{13}C NMR spectral data were similar to those of 2‴,3‴-diacetyl-*O*-betonyoside D [13], except for the *cis*-feruloyl group.

In the HMBC spectrum, H-4″ (δ 5.00) correlated with the ketone of *cis*-feruloyl group and H-1″ (δ 4.32, d, $J = 7.7$ Hz) with C-α (δ 72.3), which indicated that the feruloyl group and the phenylethanol moiety were linked to C-4″ and C-1″ of the glucosyl, respectively. Moreover, the clear correlations of H-1‴ (δ 5.15, br s) with C-3″ (δ 82.8) and H-1⁗ (δ 4.92) with C-6″ (δ 68.1) indicated that the rhamnosyl should be attached to C-3″ of glucosyl and the apiosyl to C-6″ of the glucosyl. The correlations between H-2‴ (δ 5.35, br s) and C-1‴, C-3‴, C-4‴, and C═O (δ 172.4) indicated that an acetyl group was attached to C-2‴ of rhamnosyl unit. In the same manner, the other acetyl group was assigned to the position C-3‴ of rhamnosyl unit (Figure 2). Therefore, the structure of **1** was elucidated as 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-[2,3-diacetyl-α-L-rhamnopyranosyl-(1 → 3)]-4-*O*-*cis*-feruloyl-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (Figure 1).

Compound **2** was obtained as an amorphous powder with $[\alpha]_D^{25} + 15.1$ (*c* 1.13, MeOH). Its HR-ESI-MS exhibited a pseudomolecular ion $[M + Na]^+$ at *m/z* 515.3338, which was compatible with the molecular formula $C_{29}H_{48}O_6$. The IR spectrum of compound **2** revealed the presence of hydroxyl groups (3415 cm^{-1}). The ^{1}H NMR spectrum of compound **2** revealed five methyl groups [δ_H 1.19, 1.14, 1.08, 1.28, 1.31 (s, each 3H)], four oxygenated methine proton signals [δ_H 4.50 (1H, m), 4.40 (d, 1H, $J = 9.6$ Hz), 4.54 (br s, 1H), 4.75 (s, 1H)], two oxygenated methylene proton signals [δ_H 4.46, 4.64 (d, each 1H, $J = 11.1$ Hz), 4.12, 4.27 (d, each 1H, $J = 11.1$ Hz)], and an olefinic proton signal [δ_H 6.29 (br s, 1H)]. Since the ^{13}C NMR spectral data of this compound (Table 1) were similar to those of the known compounds from this plant [11,13,14,16], compound **2** was assumed to be a pentacyclic nortriterpene.

The partial structure of E-ring was obtained from the HSQC and HMBC spectra and combined to the triterpene

Table 2. ^{1}H and ^{13}C NMR and HMBC spectral data of compounds **2** and **3** (at 500/125 MHz, respectively, in C_5D_5N; δ in ppm, J in Hz).

	2			**3**		
Position	δ (H)	δ (C)	HMBC	δ (H)	δ (C)	HMBC
1	1.43, 2.33 m	48.2		1.42, 2.35 m	48.3	
2	4.50 m	69.4	1, 4, 10	4.49 m	69.4	1, 4, 10
3	4.40 d, 9.6	79.9	1, 5, 23, 24	4.39 d, 9.6	80.0	1, 5, 23, 24
4		48.2			48.2	
5	2.05 m	49.0		2.05 m	48.9	
6	1.65, 1.88 m	19.5		1.66, 1.90 m	19.5	
7	1.50, 1.73 m	34.2		1.51, 1.75 m	34.1	
8		40.3			40.3	
9	1.88 m	48.5		1.86 m	48.5	
10		38.7			38.7	
11	2.07 m	24.1	10, 12, 13	2.05 m	23.9	10, 12, 13
12	6.29 br s	119.1	9, 13	6.31 br s	119.6	9, 13
13		144.1			143.3	
14		44.4			44.5	
15	1.13, 1.90 m	27.2		1.10, 1.84 m	28.1	
16	1.55, 1.98 m	28.3		1.47, 1.82 m	38.0	
17		51.4			49.6	
18	4.54 br s	73.6	12, 14, 16, 19	4.32 br s	74.5	12, 14, 16, 19
19	4.75 s	82.6	17, 18, 29, 30	2.45, 1.37 d,13.1	49.7	
20		43.0			44.8	
21	1.55, 1.77 m	38.7		3.84 m	81.3	17, 22, 29, 30
22	1.73, 2.23 m	29.2		1.82 m	39.6	
23	4.46, 4.64 d, 11.1	69.5	3, 4, 5, 24	4.45, 4.64 d, 11.0	69.6	3, 4, 5, 24
24	4.14, 4.27 d, 11.1	64.6	3, 4, 5, 23	4.11, 4.24 d, 11.0	64.6	3, 4, 5, 23
25	1.19 s	17.9	1, 5, 9, 10	1.20 s	17.8	1, 5, 9, 10
26	1.14 s	18.3	7, 8, 9, 14	1.06 s	18.1	7, 8, 9, 14
27	1.08 s	23.6	8, 14, 15	1.09 s	23.4	8, 14, 15
29	1.28 s	29.7	19, 20, 21, 30	1.07 s	29.3	19, 20, 21, 30
30	1.31 s	22.4	19, 20, 21, 29	1.31 s	24.8	19, 20, 21, 29

skeleton by a spiro-carbon atom at δ_C 51.4. According to the HMBC correlations of H-2 (δ 4.50) with C-1, C-4, and C-10; H-3 (δ 4.40) with C-1, C-5, C-23, and C-24; H_2-23 with C-3, C-4, C-5, and C-24; H-18 with C-12, C-14, C-16, and C-19; and H-19 with C-17, C-18, and C-30, the hydroxyl groups deduced from the molecular formula could be located at C-2, C-3, C-18, C-19, C-23, and C-24, respectively. The relative configuration of **2** was determined by extensive analysis of the ^{1}H NMR and NOESY spectral data. The 2-OH group should be α-orientated, considering H-2 (δ 4.50) correlated with H-24 (δ 4.14, 4.27) and H-25 (δ 1.19) in the NOESY spectrum. The obvious coupling constant ($J = 9.6$ Hz) between H-2 and H-3 and the NOESY correlation of H-3 (δ 4.40) with H-23 (δ 4.46, 4.64) revealed the β-orientation of 3-OH. The NOESY correlations of H-18 (δ 4.54) with H-27 (δ 1.08) and H-18 (δ 4.54) with H-19 (δ 4.75) indicated that both the hydroxyl groups at C-18 and C-19 should also be β-oriented (Figure 3). So, compound **2** was designated as (17R)-(2α,3β,18β,19α,23,24)-hexahydroxy-19(18 → 17)-abeo-28-norolean-12-ene (Figure 1).

Compound **3**, obtained as an amorphous powder with $[\alpha]_D^{25} + 15.6$ (*c* 1.2, MeOH), revealed an $[M + Na]^+$ ion at *m/z* 515.3336 $[M + Na]^+$ in the high-resolution positive Fourier transform mass spectrometer, consistent with a molecular formula of $C_{29}H_{46}O_6$. The ^{1}H and ^{13}C NMR spectral data of **3** (Table 2) were similar to those of compound **2**, except for the positions of the hydroxyl group in ring E. The hydroxyl groups deduced from the molecular formula and NMR spectra could be located at C-2, C-3, C-18, C-23, C-24, C-21, or C-22. The ^{1}H–^{1}H COSY spectrum of **3** revealed a separated spin–spin system (H_1-21/H_2-22) for the ring E; and in the HMBC spectrum, the signal of H-21 (δ 3.84) correlated with the carbon signals of C-22 (δ 39.6), C-17, C-29, and C-30, which indicated that the hydroxyl group was located at position C-21. The relative configuration of the three hydroxyl groups at C-2, C-3, C-18 of **3** was determined by NOESY experiment and the coupling constant. The NOESY correlations between H-2 and H-24, H-25; between H-3 and H-23; between H-18 and H-27 (δ 1.08) indicated that the hydroxyl groups should be assigned as 2α-, 3β-, and 18β-oriented, as same as compound **2**. The NOESY correlations between H-18 and H-19a (δ 1.37) and between the protons at δ_{HH} 1.37 (H-19a) and δ_H 1.31 (s, 3H, H-30) indicated that H-19a and H-30 should be α-oriented. So the NOESY correlation between the protons at δ_H 3.84 (m, 1H, H-21) and δ_H 1.31 (s, 3H, H-30) revealed that the configuration of the C-21 hydroxyl group had an α-orientation in compound **3**. Therefore, the structure of **3** was elucidated as (17R)-(2α,3β,18β,21α,23,24)-hexahydroxy-19(18 → 17)-abeo-28-norolean-12-ene (Figure 1).

3. Experimental

3.1 General experimental procedures

Optical rotation was measured with an MC 241 digital polarimeter (PerkinElmer, Waltham, MA, USA). IR spectra were recorded on a FTS3000 IR Fourier transform spectrometer (BIO-RAD, San Francisco, CA, USA). NMR spectra were performed on a Bruker Avance 500 instrument with tetramethylsilane as an internal standard (Rheinstetten, Germany). HRFTMS was obtained on a *Waters LCT Premier* instrument (Milford, MA, USA). HPLC was performed using a Waters 600 with Waters TP pump, UV-2487 detector (Milford, MA, USA), and a YMC-Pack ODS-A column (SH-343-5, Tokyo, Japan). Column chromatography (CC) was performed on silica gel (Qingdao Marine Chemical Co., Ltd, Qingdao, China) and Toyopearl HW-40 (TOSOH, Tokyo, Japan).

3.2 Plant material

The rhizomes of *P. umbrosa* Turcz. were collected in Jianshi county, Hubei Province, China, in January 2008. The plant was identified by Prof. Zhong-dong Wang, Chemical Engineering & Pharmaceutical College, Henan University of Science and Technology, China. A voucher specimen (no. N20081005) has been deposited at the Chemical Engineering and Pharmaceutical College, Henan University of Science and Technology, Luoyang, China.

3.3 Extraction and isolation

The dried rhizomes (10.6 kg) of *P. umbrosa* were crushed and then extracted with 80% aqueous EtOH (201) for 4 h at reflux (3 ×). The pooled EtOH solutions were concentrated *in vacuo*, and the resulting residue (1480 g) was suspended in H_2O and then successively extracted with PE, AcOEt, and *n*-BuOH. The PE-soluble fraction afforded, upon evaporation, a residue (75 g), which was further separated by CC (1 kg silica gel; PE/AcOEt 10:1, 8:1, 6:1, 3:1, 2:1, 1:1, 1:2, 1:3, AcOEt only, then AcOEt/MeOH 19:1, 10:1, 0:1) to yield 20 fractions (*fractions* 1–20) according to TLC. *Fraction* 15 (2800 mg) was subjected to CC (*Toyopearl HW-40*; $CHCl_3$/MeOH 2:1) to afford six subfractions (*fractions* 15.1–15.6). Subfraction 15.4 (450 mg) was purified by HPLC (ODS-A; MeOH/H_2O 9:1, 3.0 ml/min) to provide compounds **2** (18.4 mg) and **3** (12.7 mg). The *n*-BuOH-soluble fraction afforded, upon evaporation, a residue (150 g), which was further separated by CC (2 kg *silica gel*; $CHCl_3$/MeOH 9:1, 6:1, 5:1, 3:1, 2:1, MeOH only) to yield 21 fractions (*fractions* 1–21) according to TLC. Fraction 7 (2150 mg) was subjected to CC (*Toyopearl HW-40*; $CHCl_3$/MeOH 2:1) to afford six subfractions (*fractions* 7.1–7.6). Subfraction 7.4 (350 mg) was purified by HPLC (*ODS-A*; MeOH/H_2O 3:7, 3.0 ml/min) to provide compound **1** (12.3 mg).

3.3.1 3-Hydroxy-4-methoxy-β-phenylethoxy-O-[2,3-diacetyl-α-L-rhamnopyranosyl-(1 → 3)]-4-O-cis-feruloyl-[β-D-apiofuranosyl-(1 → 6)]-β-D-glucopyranoside (*1*)

Amorphous yellowish powder. $[\alpha]_D^{25}$ − 81.1 (*c* 0.64, MeOH). IR (KBr) ν_{max} cm^{-1}: 3443, 2935, 1732, 1630, 1620, 1595, 1516, 1130, 1040, 815. ^1H and ^{13}C NMR spectral data: see Table 1. HR-ESI-MS: *m/z* 891.2924 $[M + Na]^+$ (calcd for $C_{40}H_{52}O_{21}Na$, 891.2899).

3.3.2 (17R)-(2α,3β,18β,19α,23,24)-hexahydroxy-19(18 → 17)-abeo-28-norolean-12-ene

Amorphous powder. $[\alpha]_D^{25}$ + 15.1 (*c* 1.13, MeOH). IR (KBr) ν_{max} cm^{-1}: 3415, 2934, 1632, 1452, 1376, 1262, 1042. ^1H and ^{13}C NMR spectral data: see Table 2. HR-ESI-MS: *m/z* 515.3338 $[M + Na]^+$ (calcd for $C_{29}H_{48}O_6Na$, 515.3343).

3.3.3 (17R)-(2α,3β,18β,21α,23,24)-hexahydroxy-19(18 → 17)-abeo-28-norolean-12-ene (phlomishexaol D, *2*)

Amorphous powder. $[\alpha]_D^{25}$ + 15.6 (*c* 1.2, MeOH). IR (KBr) ν_{max} cm^{-1}: 3381, 2930, 1455, 1378, 1131, 1099. ^1H and ^{13}C NMR spectral data: see Table 2. HR-ESI-MS: *m/z* 515.3336 $[M + Na]^+$ (calcd for $C_{29}H_{48}O_6Na$, 515.3343).

Acknowledgements

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