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Iridoids from *Phlomis umbrosa*

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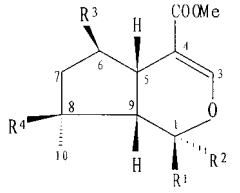
Two epimeric pairs of iridoid aglycones, named shanzhigenin methyl ester and 1-epishanzhigenin methyl ester, and 8-acetylshanzhigenin methyl ester and 8-acetyl-1-epishanzhigenin methyl ester, were isolated from *Phlomis umbrosa* roots, along with five known iridoid glucosides. The four iridoid aglycones are reported for the first time from a natural source. Their structures were established by spectroscopic methods, mainly 1D- and 2D-NMR spectroscopic experiments, and chemical methods.

1. Introduction

Phlomis umbrosa Turcz grows in northern China and is used in Chinese traditional medicine to reduce swelling and staunch bleeding, and has anti-inflammatory and detoxification properties [1]. Phytochemical studies of *P. umbrosa* were reported previously [2–7]. Further studies on the chemical constituents of the roots of this plant afforded two epimeric pairs of iridoid aglycones **1a**–**1b** and **1c**–**1d**, which are reported for the first time from natural sources, together with five known iridoid glycosides, sesamoside (**2**) [8], shanzhiside methyl ester (**3**) [9–12], barlerin (**4**) [9–11] phloyoside II (**5**) [11], phloyoside I (**6**) [11] and forsythoside B (**7**) [13–15]. This paper describes the isolation of these compounds and the structural elucidations of the four iridoidal aglycones.



	R^1	R^2	R^3	R^4
1a	OH	H	OH	OH
1b	H	OH	OH	OH
1c	OH	H	OH	OAc
1d	H	OH	OH	OAc
1e	OAc	H	OAc	OH
1f	OAc	H	OAc	OAc
3	Glc	H	OH	OH
4	Glc	H	OH	OAc

2. Investigations, results and discussion

The extract mixture of *P. umbrosa* obtained by extraction with different concentrations of aqueous methanol (from 70% to 95%) was suspended in water and subjected to sequential extraction with petrol, chloroform and n-BuOH. The n-BuOH extract was repeatedly chromatographed to give five known iridoid glycosides (**2**–**6**) and one phenylethanoid glycoside (**7**). Compounds **2**–**7** were shown to have spectroscopic (IR, UV, FABMS, ^{1}H and ^{13}C NMR) properties identical to those reported for sesamoside, shanzhiside methyl ester, barlerin, phloyoside II and I, and forsythoside B [8–15], respectively.

The chloroform extract was partitioned between petrol ether and methanol. The petrol ether fraction was chromatographed on a silica gel column to provide two epimeric pairs of non-glycosidic iridoids **1a**–**1b** and **1c**–**1d**.

The molecular formula of **1a** and **1b** was determined as $C_{11}H_{16}O_6$ by analysis of NMR and EIMS spectra and elemental analysis. The IR spectrum of the epimeric mixture **1a** and **1b** displayed absorption bands for a hydroxy group (3435–3352 cm^{-1}) and an enol ether conjugated with a methyl ester unit (1707 and 1633 cm^{-1}). Examination of the ^{1}H and ^{13}C NMR spectra of **1a** and **1b** led to the suggestion that they are non-glycosidic iridoids with one carbomethoxy (δ_C 170.2/170.2 and 53.2/53.0, δ_H 3.72/3.74) at C-4 of each. ^{1}H-^{1}H COSY and HMQC was employed to assign all resonance signals (Tables 1 and 2) in the NMR spectra. According to 2D NMR resonance signals, it was deduced that two secondary hydroxyl groups (δ_C 80.3/78.2) were linked to the C-6 positions of **1a** and **1b** and that two tertiary hydroxyl groups (δ_C 81.9/79.5) were attached to the C-8 positions of **1a** and **1b**. Comparison of ^{13}C NMR data for **1a** and **1b** with those for **3** indicated that eleven resonances matched those of shanzhiside methyl ester (**3**) with the exception of the glucopyranosyl moiety present in **3**. Two pairs of oxygenated carbon signals above were assigned to C-6 and C-8. Thus it was proposed that one of the isomers corresponded to the aglycone of **3** and the other would be its epimer at C-1. Acetylation (Ac_2O/pyridine) of **1a** and **1b** confirmed the position of epimerization. The β-configuration assigned to the acetyl group at C-1 of **1e** and **1f** was deduced through analysis of the H-1 chemical shift and $J_{1,9}$ values in the ^{1}H NMR spectra of these derivatives [10, 16]. Cross peaks between H-1 (δ 6.70), H-6 (δ 5.32) and H-10 (δ 1.52) in the NOESY spectrum of **1f** supported the above assignments at C-1. On the basis of the Karplus rule for dihedral angles between H-1 and H-9, the substituent at C-1 was in the pseudoequational position, since dihedral angles of ca. 109° were observed for **1a** ($J_{1,9} = 2.5$ Hz), **1f** ($J_{1,9} = 2.5$ Hz) and **3** ($J_{1,9} = 2.5$ Hz). This assumption is consistent with previously reported considerations of change in the different hemi-chair conformations of the flexible dihydropyran ring of an iridoidal system [17]. Considering the criteria for distinguishing α and β-epimeric forms of iridoid aglycones [18], **1a** and **1b** were elucidated as shanzhigenin methyl ester and 1-epishanzhigenin methyl ester.

Enzymatic cleavage of **3** with β-glucosidase afforded the isomers **1a** and **1b** as an equilibrium mixture of two tautomeric (anomeric) forms at C-1 [12]. The integral trace of the corresponding signals of **1a** and **1b** in the ^{1}H NMR spectra showed that they existed in a ratio of about 1 : 1.1 in solution [18]. In addition, the facile acetylation of tertiary OH in **1a** and **1b** could possibly be due to a transacylating effect of the *peri*-OH group at C-1.

1c and **1d**, $C_{13}H_{18}O_7$, showed similar absorption in IR spectra (except 1733 cm^{-1} for ester carbonyl) and UV spectra to **1a** and **1b**. Analysis of the ^{1}H NMR and ^{13}C

Table 1: ^{1}H NMR spectral data (400 MHz) for 1a–1f, 3 and 4

H	1a*	1b*	1c#	1d#	1e#	1f#	3+	4+
1	5.42 d (2.5)	5.18 d (6.1)	5.51 d (3.0)	5.12 d (6.0)	6.25 d (3.4)	6.70 d (2.5)	5.53 d (2.5)	5.70 d (2.0)
3	7.42 s	7.42 s	7.38 s	7.43 s	7.41 s	7.42 s	7.38 s	7.40 s
5	3.07 m	3.03 m (9.9)	2.90 dd (6.0; 8.2)	2.95 dd (6.4; 9.0)	3.31 brd (9.4)	3.20 brd (9.0)	2.92 m (10.0)	2.98 m
6	4.22 m (9.9)	3.99 m (3.1; 6.0; 9.2)	4.27 ddd (6.2; 6.3; 6.6)	3.98 ddd (3.2; 8.4)	5.26 m	5.32 m	4.04 m	4.28 brd (5.0)
7α	1.67 brd (14.0)	1.79 dd (5.0; 13.2)	2.20 dd (3.2; 15.2)	2.17 dd (6.1; 13.3)	1.90 dd (3.4; 14.6)	2.05 dd (5.2; 15.5)	1.75 dd (15.4; 6.0)	1.98 dd (15.7; 5.0)
7β	2.29 dd (14.0; 9.0)	2.00 dd (6.9; 13.2)	2.32 dd (9.1; 15.2)	2.34 dd (6.6; 13.3)	2.13 dd (8.4; 14.7)	2.33 brd (15.3)	2.03 dd (15.3; 6.1)	2.10 d (15.7)
9	2.14 dd (2.5; 5.8)	2.37 dd (6.1; 9.1)	2.86 dd (3.1; 8.2)	2.77 dd (6.0; 9.0)	2.63 dd (3.3; 9.3)	2.98 dd (1.5; 9.0)	2.61 brd (10.3)	2.99 m
10	1.31 s	1.36 s	1.64 s	1.55 s	1.34 s	1.52 s	1.18 s	1.41 s
OMe	3.72 s	3.74 s	3.73 s	3.73 s	3.73 s	3.73 s	3.67 s	3.66 s
CH_3CO	–	–	1.99 s	2.01 s	2.11 s	1.99 s	–	–
CH_3CO	–	–	–	–	2.17 s	2.09 s	–	–
CH_3CO	–	–	–	–	–	2.06 s	–	–
Glc-1	–	–	–	–	–	–	4.68 d (7.5)	4.69 d (6.6)
Glc-6	–	–	–	–	–	–	3.63 dd (12.4; 5.8)	3.63 dd (12.4; 5.8)
Glc-6	–	–	–	–	–	–	3.83 d (12.4)	3.83 d (12.4)

* and # Assignments by ^{1}H–^{1}H COSY experiments. Coupling constants (Hz) are given in parentheses.
# Recorded in $CDCl_3$. * Recorded in CD_3OD. + Recorded in D_2O.

NMR spectral data indicated that **1c** and **1d** are also iridoidal aglycones whose skeleton must be the same as that of **1a** and **1b**. The main differences observed in the ^{13}C NMR spectra corresponded to the tertiary acetyl groups at δ 90.7/87.2, 171.1/171.1 and 22.5/22.5, and to the methyl groups at δ 19.3/21.5. To elucidate the differences in the substitution pattern of **1c** and **1d**, ^{1}H-^{1}H COSY and HMQC were used to assign all resonance signals (Tables 1 and 2) of the NMR spectra. Comparison of the ^{13}C NMR spectral data of **1c** and **1d** with those of **4** suggested that **1c** or **1d** could be the aglycone of **4** (barlerin); the acetyl was assigned to C-8. The upfield shifts at C-7 (Δδ: −2.4/−2.5), C-9 (Δδ: −3.9/−2.5) and C-10 (Δδ: −5.7/−2.5) and downfield shift at C-8 (Δδ: +9.7/+7.7) in the ^{13}C NMR spectra of **1c** and **1d**, in contrast to the chemical shifts of corresponding carbons of **1a** and **1b**, were consistent with the above deductions. Epimerization at C-1 was confirmed by acetylation of **1c** and **1d** as in **1a** and **1b** [10, 16]. By comparison of their $J_{1,9}$ values (**1c**, $J_{1,9} = 3.0$ Hz; **1d**, $J_{1,9} = 6.0$ Hz) with those of **4** ($J_{1,9} = 2.0$ Hz) and **1f**, **1c** and **1d** were identified as 8-acetylshanzhigenin methyl ester and 8-acetyl-1-epishanzhigenin methyl ester, respectively [18].

Although most iridoidal aglycones are unstable, compounds **1a–1d** were stable for several months at low temperature (< 10 °C).

Table 2: ^{13}C NMR spectral data (100 MHz) of 1a–1f, 3 and 4

C	1a*	1b*	1c+	1d+	1e+	1f+	3**	4**	DEPT
1	92.4	94.2	90.4	93.3	89.1	89.1	95.1	95.4	CH
3	153.0	153.8	151.3	152.8	151.8	152.4	153.1	153.4	CH
4	110.0	110.0	108.7	108.3	108.3	107.3	111.1	109.5	C
5	43.3	42.6	42.2	41.7	37.7	37.9	40.2	41.8	CH
6	80.3	78.2	77.8	76.3	77.7	76.9	77.5	77.7	CH
7	49.2	48.7	46.8	46.2	46.5	44.5	49.0	46.9	CH_2
8	81.0	79.5	90.7	87.2	78.7	87.0	79.3	89.3	C
9	51.9	51.9	48.0	49.4	49.9	47.7	50.9	49.7	CH
10	25.0	24.0	19.3	21.5	24.5	21.4	24.7	21.2	CH_3
11	170.2	170.2	169.7	169.2	166.4	166.1	170.8	168.8	C
COOMe	53.2	53.0	516	51.6	51.5	51.3	53.2	51.9	CH_3
CH_3CO	–	–	171.1	171.1	169.1	168.6	–	172.9	C
				–	169.8	169.9	–	–	C
				–	170.3	–			C
CH_3CO	–	–	22.5	22.2	21.2	21.8	–	22.3	CH_3
					20.9	21.0	–	–	CH_3
Glc-1							99.5	99.9	CH
2							73.8	74.2	CH
3, 5							76.8	75.7	CH
							76.6	75.7	CH
4							70.8	71.2	CH
6							62.0	62.7	CH_2

* and + Assignments by HMQC and HMBC experiments. * Recorded in CD_3OD. + Recorded in $CDCl_3$
** Recorded in D_2O

3. Experimental

3.1. General

Mps: Uncorr. Optical rotation values were obtained on a J-20c Spectropolarimeter (Jasco) in MeOH or $CHCl_3$. IR spectra were recorded on a Nicolet-5DX Infrared spectrometer. UV spectra were obtained using a Shimadzu UV 240 Spectrometer. 1D-, 2D- ^1H and ^{13}C NMR spectra and NOESY experiments in $CDCl_3$ (CD_3OD or D_2O) were performed on a Bruker AM-400, using TMS as internal standard. EIMS and FABMS were recorded on a ZAB-HS mass spectrometer. For spectral data of the compounds see Tables 1 and 2.

3.2. Plant material

The roots of *Phlomis umbrosa* were collected in Zhang Xian County of Gansu Province in September 1996 and identified by Prof. Ru-Nen Zhao, Faculty of Pharmacy, Lanzhou Medical College. Voucher specimen No. 97010 was deposited in the Herbarium of the Pharmacy Department, Lanzhou Medical College and in the Laboratory of Natural Products, Lanzhou University, P.R. China.

3.3. Extraction and isolation

Air-dried and powdered roots of *Phlomis umbrosa* (4.32 kg) were exhaustively extracted with aqueous methanol of different concentrations (from 70% to 95%) at room temperature and the mixed extract concentrated under reduced pressure. The residue was suspended in water and subjected to sequential extraction with petrol, $CHCl_3$ and n-BuOH. The chloroform extract (50 g) was partitioned between petrol ether and methanol. The petrol ether extract (7 g) was chromatographed on silica gel (50 g, 300–400 mesh), eluting with $CHCl_3$–MeOH (from 80 : 1 to 20 : 1), to give four fractions (Fr_I–Fr_{IV}). Fr_I(775 mg) was subjected to CC eluting with benzene-methanol (150 : 1) on silica gel (11 g, 300–400 mesh) to afford a crystalline mixture of **1c** and **1d** (110 mg). Fr_w (2 g) was rechromatographed on silica gel (30 g, 300–400 mesh) developing with benzene-acetone (30 : 1) to afford **1a** and **1b** (80 mg) as a crystalline mixture of β- and α-epimers. The n-BuOH extract (20 g) was chromatographed on silica gel (200 g, 200–300 mesh) and eight fractions (Fr_1–Fr_8) were obtained. Fr_2 was submitted to silica gel CC with $CHCl_3$–MeOH (10 : 1) to provide **4** (50 mg). Compound **2** (60 mg) was isolated from Fr_3 after silica gel CC with $CHCl_3$–MeOH (8 : 1). Fr_5 after repeated silica gel CC with $CHCl_3$–MeOH–H_2O (18 : 1:0.015) yielded **3** (70 mg) and **5** (45 mg). Fr_6 was rechromatographed with $CHCl_3$–MeOH–H_2O (18 : 1 : 0.03) on silica gel to give **6** (35 mg). Fr_7 was chromatographed repeatedly on silica gel CC with EtOAc–MeOH–H_2O (20 : 1:0.5) to afford **7** (78 mg).

3.3.1. Shanzhigenin methyl ester and 1-epishanzhigenin methyl ester (1a and 1b)

Colorless needles (found: C, 54.5, H, 6.2%, $C_{11}H_{16}O_6$ requires C, 54.1, H, 6.6%); m.p. 153–155 °C (from benzene and acetone); $[\alpha]_D^{20}$: +4.1° (MeOH; C 0.67); UV λ_{max}^{MeOH} nm (log ε): 249 (4.01); IR ν_{max}^{KBr} cm^{-1}: 3435, 3352, 1674, 1636; EIMS (Probe) 70 ev, *m/z* (rel. int.): 244 $[M]^+$ (5), 226 $[M-H_2O]^+$ (14), 208 $[M-2H_2O]^+$ (8), 198 (6), 182 (12), 165 (13), 158 (10), 148 (26), 140 (43), 139 (61), 125 (49), 109 (22), 97 (43), 87 (30), 43 (100).

3.3.2. 8-Acetylshanzhigenin methyl ester and 8-acetyl-1-epishanzhigenin methyl ester (1c and 1d)

Colorless needles (found: C, 54.8 H, 6.0%, $C_{13}H_{18}O_7$ requires C, 54.5, H, 6.3%; m.p. 69–71 °C (from benzene and acetone); $[\alpha]_D^{20}$: +3.6° (CH_3COCH_3; C 0.25); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 258 (3.76); IR ν_{max}^{KBr} cm^{-1}: 3479, 3333, 1732, 1682, 1638; EIMS (probe) 70 ev, *m/z* (rel. int.): 226 $[M-OAc]^+$ (4), 208 $[M-COOMe-H_2O]^+$ (28), 197 (16), 179 (22), 176 (25), 165 (27), 152 (30), 148 (7), 139 (28), 125 (21), 95 (25), 43 (100).

3.3.3. Sesamoside (2)

Needles, m.p. 125–127 °C (from aq. methanol); $[\alpha]_D^{20}$: −36.0° (MeOH; c 0.45) lit. $[\alpha]_D^{20}$: −79.5° (MeOH; c 0.45); [11]) UV λ_{max}^{MeOH} nm: 235; IR ν_{max}^{KBr} cm^{-1}: 3329, 1635, 1076, 1046.

3.3.4. Shanzhiside methyl ester (3)

Amorphous powder (from aq. methanol); $[\alpha]_D^{20}$: −118.4° (MeOH, c 0.55) lit. $[\alpha]_D^{20}$: −115° (MeOH; c 0.9); IR ν_{max}^{KBr} cm^{-1}: 3361, 1689, 1647; FABMS *m/z*: 429 $[M + Na]^+$, 413 $[M + Li]^+$.

3.3.5. Barlerin (8-acetylshanzhiside methyl ester) (4)

Needles (from aq. methanol), m.p. 135–137 °C; $[\alpha]_D^{20}$: −105° (MeOH; c 1.10) lit. $[\alpha]_D^{20}$: −85.0° (MeOH; c 0.99); IR ν_{max}^{KBr} cm^{-1}: 3343, 2977, 1707, 1637; FABMS *m/z*: 471 $[M + Na]^+$, 455 $[M + Li]^+$.

3.3.6. Phloyoside II (5)

Amorphous powder (from aq. methanol), $[\alpha]_D^{20}$: −126.5° (MeOH; c 0.56) lit. $[\alpha]_D^{20}$: −134.4° (MeOH; c 0.17); HR-FABMS *m/z*: 455.0989, $C_{17}H_{24}O_{12}Cl$ requires 455.0957; IR ν_{max}^{KBr} cm^{-1}: 3388, 3305, 1696, 1637.

3.3.7. Phloyoside I (6)

Amorphous powder (from aq. methanol), $[\alpha]_D^{20}$: −162.3° (MeOH; C 0.55) lit. $[\alpha]_D^{20}$: −151.1° (MeOH; c 0.41); HR-FABMS *m/z*: 437.1278, $C_{17}H_{25}O_{13}$ requires 437.1295; IR ν_{max}^{KBr} cm^{-1}: 3413, 1687, 1639.

3.3.8. Forsythoside B (7)

Amorphous powder (from aq. methanol), $[\alpha]_D^{20}$: −78.8° (MeOH; C 1.0) lit. $[\alpha]_D^{20}$: −94.2° (MeOH; c 0.80); IR ν_{max}^{KBr} cm^{-1}: 3425, 1632, 1604, 1521, 1446, 1280, 1075, 1042; UV λ_{max}^{MeOH} nm: 224, 292, 336; FABMS *m/z*: 779 $[M + Na]^+$, 763 $[M + Li]^+$.

3.4. Acetylation

The epimeric mixture **1a** + **1b** was treated with Ac_2O/pyridine (1 : 1) for 12 h to yield a mixture of diacetate (**1e**) and triacetate (**1f**). TLC analysis showed that only diacetate (**1e**) was produced initially, and that triacetate (**1f**) was produced if excessive Ac_2O/pyridine was added to the reaction medium and the reaction time was longer. Acetylation of **1c** and **1d** was performed under the same conditions to yield **1f**.

3.4.1. Diacetate (1e)

Needles, m.p. 151–153 °C (from petrol ether and acetone); $[\alpha]_D^{20}$: −29.5° ($CHCl_3$; C 0.83); UV $\lambda_{max}^{CHCl_3}$ nm: 246; IR ν_{max}^{KBr} cm^{-1}: 3546, 1742, 1690, 1641; EIMS (probe) 70 ev, *m/z* (rel. int.): 297 $[M-OMe]^+$ (8), 268 $[M-HOAc]^+$ (5), 226 $[M-OAc-COMe]^+$ (4), 208 (100), 190 (18), 165 (58), 148 (54), 139 (79), 125 (21), 87 (11), 43 (56).

3.4.2. Triacetate (1f)

Cubes, m.p. 181–183 °C (from petrol ether and acetone); $[\alpha]_D^{20}$: −74.3° ($CHCl_3$, C 1.0); UV $\lambda_{max}^{CHCl_3}$ nm: 248; IR ν_{max}^{KBr} cm^{-1}: 3008, 1758, 1725, 1698, 1641; EIMS (probe) 70 ev, *m/z* (rel. int.): 339 $[M-OMe]^+$ (4), 268 $[M-OAc-COMe]^+$ (3), 250 (5), 191 (29), 190 (100), 176 (21), 148 (69), 139 (26), 126 (8), 91 (10), 43 (30).

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