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WAHLENBERGIOSIDE, A PHENYLPROPANOID GLUCOSIDE FROM *WAHLENBERGIA MARGINATA*

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Key Word Index—*Wahlenbergia marginata*: Campanulaceae; wahlenbergioside; phenylpropanoid glucoside.

Abstract—Wahlenbergioside, a new hydroxymethylglutaroyl phenylpropanoid glucoside, and a known compound, lobetyolin, were isolated from the methanol extract of *Wahlenbergia marginata*. The structures were determined by spectroscopic analysis and chemical degradations.

INTRODUCTION

The whole herb of *Wahlenbergia marginata* (Thunb.) A. DC., a widely-used Chinese medicinal plant, has been employed for many years by different ethnic groups in the Yunnan Province for the treatment of coughs, pneumonia, tuberculosis and heart disease. It is also used as a tonic and haematopoietic [1]. However, due to the complexity of its constituents, little work has been done on the chemistry of the genus *Wahlenbergia*. As part of our phytochemical investigations on Chinese medicinal plants [2–8], *W. marginata* has been studied.

RESULTS AND DISCUSSION

Compound **1**, named wahlenbergioside, was obtained as a colourless gum. In its negative ion FAB mass spectrum a deprotonated molecular ion was observed as the base peak at m/z = 515 $[M-H]^-$, together with an intense dimer ion at m/z = 1031 $[2\times M-H]^-$. The molecular formula $C_{23}H_{32}O_{13}$ was deduced from the combined FAB-MS data, and ^1H and ^{13}C NMR data (Tables 1 and 2).

The UV spectrum of **1** showed absorptions at λ_{max} (MeOH) 220 and 265 nm and suggested that there was an aromatic system conjugated with an unsaturated side chain. In the ^1H NMR spectrum of **1**, the presence of a *trans*-propenyl alcohol moiety linked to a 3,4,5-trisubstituted phenyl residue was indicated by a pair of olefinic signals at δ 6.56 (1H, *br d*, J = 16.2 Hz, H-γ) and 6.23 (1H, *dt*, J = 16.2, 7.6 Hz, H-β), and a broadened methylene doublet (J = 7.6 Hz) at δ 4.72 (H-α) as well as two *meta*-coupled aromatic doublets (J = 2 Hz) at δ 6.62 and 6.59. A three-proton singlet at δ 3.84 was characteristic of a methoxyl group situated on an aromatic nucleus. Furthermore, a methyl singlet at δ 1.38 (6′-Me), a methyl ester singlet at δ 3.66 (-OMe), and a pair of methylene singlets at δ 2.71 (H-2′ and H-4′), together with two carbonyl signals at δ 173.3 (C-1′) and 176.7 (C-5′) demonstrated the presence of a methyl ester derivative of a 3-oxygenated 3-methylglutarate moiety (HMG) [9]. This diester chain was shown to be at C-α by the downfield shifted methyleneoxy doublet of H-α at δ 4.72 when compared with the shifts of analogues reported previously [9, 10]. The ^1H and ^{13}C NMR spectra also showed a set of signals of a saccharide unit. This information suggested that **1** was a 'tangshenoside type' phenylpropanoid derivative [9, 10]. Comparison of the spectral data of **1** with those of tangshenoside **I** (**T-I**) (see Fig. 1) revealed that there was only one saccharide unit and one methoxyl group in compound **1** instead of two glucoses and two methoxyl groups as in the case of **T-I**.

Acid hydrolysis of **1** with 2 M HCl, followed by a comparison with authentic sugar samples on HPTLC plates [11], indicated that glucose was the saccharide unit of **1**. However, the glucose unit could either be located on the HMG chain or on the aromatic nucleus. In order to prove the attachment position of the glucose unit, alkaline hydrolysis of **1** with 0.5 M KOH, followed by acetylation was carried out. This reaction

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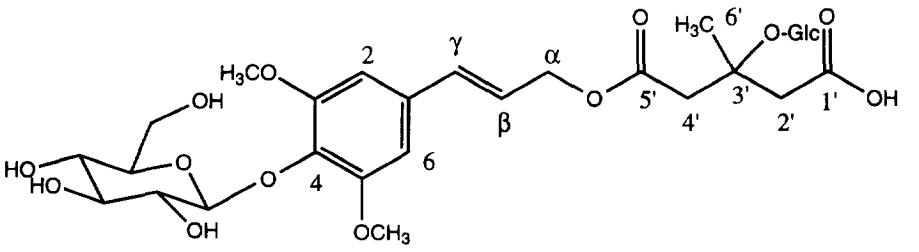
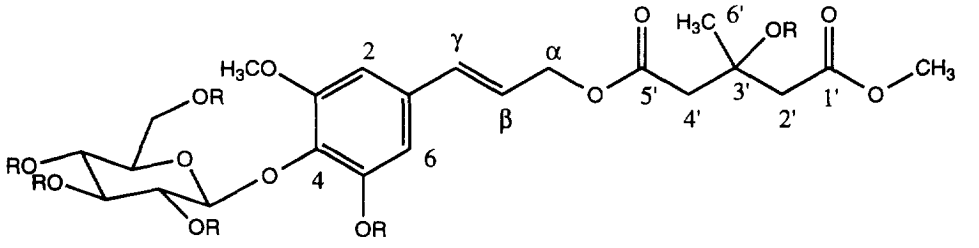
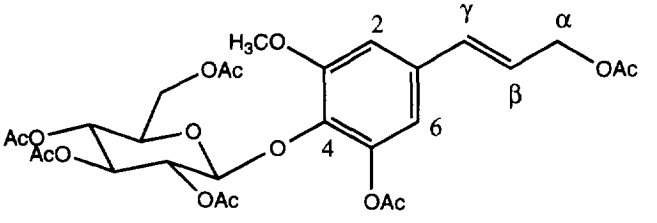


Tangshenoside I [9]

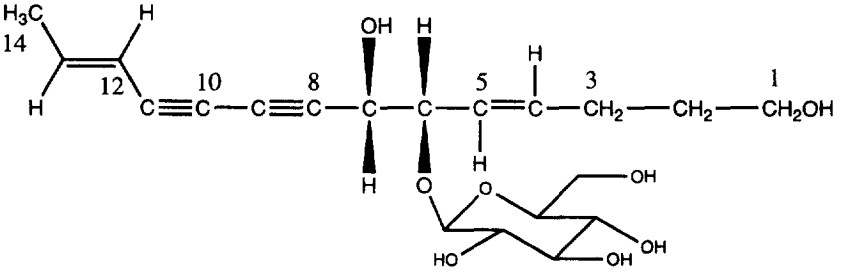


1 (Wahlenbergioside) R = H

1b R = Ac



1a



2 (Lobetyolin) [10, 13, 14]

showed that the glucose was linked on the aromatic moiety of **1**. Indeed, the signals of a peracetate of glucose, together with those of the aromatic moiety were found in the ^1H and ^{13}C NMR spectra of the acetylated product **1a** (Tables 1 and 2). The molecular formula of **1a**, $C_{28}H_{34}O_{15}$, was deduced from its TSP-MS spectrum ($[M+NH_4]^+$ at m/z 628) [12] combined with its ^1H and ^{13}C NMR data (Tables 1 and 2). In order to prove the position of the methoxyl group on the aromatic moiety of **1**, a NOE experiment was performed. An effect was observed between H-2 and the methoxyl singlet at δ 3.84 (5%) (C-3), confirming that the methoxyl group was situated on C-3 of the aromatic nucleus. A comparison of the aromatic proton signals of **1a** with those of **1** showed that the signals of H-2 and H-6 were shifted downfield (H-2 at

Table 1. ^{1}H NMR spectral data of compounds **1**, **1a**, **1b** (measured in CD_3OD) and tangshenoside **I** (T-**I**)

H	T-I [9]*	1	1b	1a
2	6.66 (*s*, 1H)	6.59 (*d*, 2, 1H)	6.78 (*d*, 2, 1H)	6.81 (*d*, 2, 1H)
6	6.66 (*s*, 1H)	6.62 (*d*, 2, 1H)	7.01 (*d*, 2, 1H)	7.04 (*d*, 2, 1H)
α	4.65 (*d*, 6.1, 2H)	4.72 (*brd*, 7.6, 2H)	4.74 (*brs*, 6.2, 2H)	4.71 (*brs*, 6.2, 2H)
β	6.17 (*dt*, 16.1, 6.1, 1H)	6.23 (*dt*, 16.2, 7.6, 1H)	6.31 (*dt*, 15.8, 5.8, 1H)	6.32 (*dt*, 16, 7.6, 1H)
γ	6.52 (*d*, 16.1, 1H)	6.56 (*brd*, 16.2, 1H)	6.63 9*brd*, 15.8, 1H)	6.61 (*brd*, 16.2, 1H)
Ar-OC$\underline{H}_3$	3.75 (*s*, 6H)	3.84 (*s*, 3H)	3.85 (*s*, 3H)	3.89 (*s*, 3H)
2′	2.47 (*d*, 15.1, 1H), 2.61 (*d*, 15.1, 1H)	2.71 (*d*, 7.6, 2H)	2.71 (*d*, 5.8, 2H)	—
4′	2.82 (*s*, 2H)	2.71 (*d*, 7.6, 2H)	2.71 (*d*, 5.8, 2H)	—
6′	1.41 (*s*, 3H)	1.38 (*s*, 3H)	1.38 (*s*, 3H)	—
$-$COOC$\underline{H}_3$		3.66 (*s*, 3H)	3.62 (*s*, 3H)	—
G-1	4.58 (*d*, 7.3)	4.69 (*d*, 8.2, 1H)	4.71 (*d*, 8.2, 1H)	4.69 (*brd*, 4.2, 1H)
2	—	3.41–3.52 (unresolved)	4.78–4.90 (unresolved)	4.79–4.92 (unresolved)
3	—	—	5.29 (*dd*, 4.3, 2.4, 1H)	5.30 (*dd*, 8.3, 7.9, 1H)
4	—	—	5.11 (*dd*, 4.3, 2.4, 1H)	5.08 (*dd*, 8.3, 7.9, 1H)
5	—	3.24 (*m*, 1H)	3.81 (*m*, 1H)	3.85 (*m*, 1H)
6_a	—	3.78 (*dd*, 12.2, 1.8, 1H)	4.34 (*dd*, 12.3, 4, 1H)	3.95 (*dd*, 6.45, 2.2, 1H)
6_b	—	3.72 (*dd*, 12.2, 4, 1H)	3.93 (*dd*, 12.3, 2, 1H)	3.72 (*dd*, 6.45, 1.9 1H)
Ar-COC$\underline{H}_3$	—	—	2.27 (*s*, 3H)	2.25 (*s*, 3H)
-COC$\underline{H}_3$-3′	—	—	2.02 (*s*, 3H)	2.03 (*s*, 3H)
G-COC$\underline{H}_3$	—	—	2, 2.07, 1.98 × 2 (*s*, 12H)	2.09 × 2, 2.0 × 2 (*s*, 12H)

* The data for the C-3′ glucosyl unit of T-**I** are not listed because **1** does not contain a C-3′ glucosyl unit.

Proton coupling constants (J in Hz), signal multiplicities and integrations are in parentheses. Assignments were aided by ^{1}H-^{1}H COSY.

Table 2. ^{13}C NMR spectral data of compounds **1**, **1b**, **1a** (measured in CD_3OD), tangshenoside **I** (T-**I**) and HMG*

Position	T-I [9]*	1	1b	1a	HMG [9]
C-1	134.2	135.1 *s*	135.0	135.0	
2	105.1	108.8 *d*	109.3	109.4	
3	153.3	154.2 *s*	154.3	154.3	
4	134.4	135.1 *s*	138.0	138.0	
5	153.3	151.9 *s*	145.8	145.8	
6	105.1	106.7 *d*	115.0	115.0	
Ar-O$\underline{C}$H$_3$	57.0	56.2 q	56.9	56.9	
α	66.3	65.7 *t*	65.7	65.8	
β	124.3	123.9 *d*	125.5	125.6	
γ	134.4	134.8 *d*	133.6	133.6	
1′	173.3	173.1 *s*‡	173.1†		175.8
2′	44.3	46.2 *t*†	46.2‡		46.0
3′	78.2	70.7 *s*	70.8		70.7
4′	47.4	46.0 *t*†	46.0‡		46.0
5′	176.7	172.4 *s*‡	172.3†		175.8
6′	24.8	27.8 *q*	27.8		27.2
-COO$\underline{C}$H$_3$		51.9 *q*	52.0		
G-1	103.8	103.4 *d*	102.4	102.4	
2	74.5	75.3 *d*	73.0§	73.1†	
3	77.0	78.3 *d*§	74.1	74.1	
4	70.3	70.7 *d*	69.5	69.5	
5	76.6	77.6 *d*§	73.1§	73.2†	
6	61.5	62.0 *t*	62.7	62.8	
-CO$\underline{C}$H$_3$ (×6)			20.5–20.9	20.5–20.9	
-$\underline{C}$OCH$_3$ (×6)			170.7–172.3	170.7–172.6	

* The data for the C-3′ glucosyl unit T-**I** are not listed because **1** contains no C-3′ glucosyl unit.

†, ‡, § Values may be interchangeable in the same column. HMG, 3-hydroxy-3-methylglutaric acid.

δ 6.81 and H-6 at δ 7.04). These downfield shifts indicated that the acetylated hydroxyl group was at C-5 position and confirmed that H-2 and H-6 were in *para* and *ortho* positions to the hydroxyl group. All this information showed that the phenyl moiety of **1** was substituted at position C-3 by a methoxyl and at C-5 by a hydroxyl group. The glucose was thus located at position C-4.

In the ^{13}C NMR spectrum of **1**, the signal due to the C-3′ of HMG moiety was overlapped by the signals of the glucosyl unit (Table 2). However, after acetylation of **1**, the signal of C-3′ was shifted to δ 70.8 (**1b**). It was easily distinguishable from the signals of the glucosyl moiety and proved the presence of a hydroxyl group at C-3′. This result also confirmed that the glucosyl unit was on the aromatic moiety.

The large coupling constant ($J = 8.2$ Hz) of the anomeric proton of the glucose in the ^1H NMR spectrum of **1** suggested a β-configuration for the saccharide moiety [11]. The chirality of the asymmetric carbon (C-3′) of the acyl moiety of **1** was not established due to the small quantity of **1**, as a consequence of chemical degradations. From the chemical and spectral data above, **1** was characterised as shown in formula **1**.

Compound **2** was identified as lobetyolin by comparison of its spectroscopic data with those of the literature [10, 13, 14]. This compound was characterised previously from *Codonopsis lanceolata*, *C. tangshen* (Campanulaceae) [10], *Lobelia inflata* [13] and *L. sessilifolia* [14] (Campanulaceae). It is noteworthy that the phenylpropanoid derivative with a diester side chain and lobetyolin isolated from *W. marginata* have been characterised previously in genera like *Codonopsis* and *Lobelia* [10, 13]. This can provide in some degree chemical evidence for the use of *W. marginata* as a substitute of *Codonopsis tangshen* in China.

EXPERIMENTAL

General. Open CC: silica gel (40–63 μm, Merck); Mps: uncorrected; IR: KBr; FAB-MS glycerol, negative ion mode) and TSP LC-MS: Finnigan MAT TSQ 700 triple stage quadrupole instrument; NMR: ^1H (200 MHz) and ^{13}C (50 MHz), CD_3OD, TMS as int. standard; TLC: Merck HPTLC RP-18 WF_{254} plates and Merck silica gel TLC plates. Saccharide identification was carried out on Merck HPTLC silica gel 60 F_{254} plates.

Extraction and isolation. The powdered air-dried whole herb (4 kg) of *Wahlenbergia marginata* was extracted with MeOH by refluxing at ca 50° × 3 (10 hr each time). Evaporation of MeOH from the combined extract gave a black gum (240 g). This gum was dissolved in MeOH–H_2O) (1:19) by agitation on a bath at 50° and the soln partitioned with petrol (60–90), $CHCl_3$, and *n*-BuOH. The BuOH-soluble part (8 g) was subsequently separated on a highly porous polymer, Diaion HP-20 (Mitsubishi Kasei Co. Ltd, Tokyo) using successively H_2O, H_2O–MeOH (8:2, 7:3, 4:6, and 2:8), MeOH. Six fractions (A: 1.9 g, B: 0.3 g, C: 0.2 g, D: 0.12 g, E: 2.2 g and F: 3.1 g) were collected. Fractions E and F contained mainly saccharides while fractions B–D were complex mixtures of minor compounds. Fraction A was separated into three parts (A-1, A-2, and A-3) by silica gel CC eluted with $CHCl_3$–MeOH (9:1, 8:2, and 6:4). Repeated gel filtration of A-1 over Sephadex LH-20 with MeOH afforded glucoside **1** (41 mg). Glucoside **2** (28 mg) was obtained from A-2 by Lobar RP-18 CC with MeOH–H_2O (1:1), followed by repeated gel filtration on Sephadex LH-20 with MeOH–H_2O (9:1).

Acid and alkaline hydrolysis of **1**. **1** (about 1 mg) in 2 M HCl–MeOH (0.5 ml) was heated at 60° for 3 hr. The reaction mixt. was taken to dryness under a stream of N_2. The residue was checked for sugars by HPTLC (comparison with authentic samples) using *n*-BuOH–i-PrOH–H_2O (10:5:4) and anisaldehyde–H_2SO_4 as detection reagent. The presence of glucose was confirmed. 10 mg of **1** in 5% KOH (MeOH) was heated at 60° for 4 hr; after cooling, the reaction mixt. was neutralised with 5% HCl and then partitioned with *n*-BuOH. The *n*-BuOH extr. was treated with (Ac_2O–pyridine (1:1) to give crude derivative **1a** which was purified over silica gel with $CHCl_3$–MeOH (49:1) to provide pure **1a** (9 mg).

Acetylation of **1**. **1** (8 mg) was added to Ac_2O–pyridine (1:1) and then kept at room temp for 16 hr. The reaction was stopped by adding ice water. The reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness *in vacuo*. The residue was purified on Sephadex LH-20 with MeOH–$CHCl_3$ (9:1) to provide derivative **1b** (10 mg) as a white powder.

Walenbergioside (**1**). White powder (28 mg), mp 42–45°, HPTLC RP-18 (MeOH–H_2O 6:4), R_f 0.4; $[\alpha]_D^{25}$D −9.6° (*c* 0.014, MeOH); UV λ_{max} (MeOH) nm (log ε): 222 (3.34), 265 (3.82); IR (KBr) ν_{max} cm^{-1} 3100–3500, 2950, 1720, 1590, 1500, 900, 850, 810; FAB-MS data (negative ion mode, glycerol) *m/z* 515 $[M-H]^-$; NMR: Tables 1 and 2.

Derivative **1a**. Yellowish amorphous powder; mp 43–45°; Silica gel TLC ($CHCl_3$–MeOH, 99:1), R_f 0.58; $[\alpha]_D^{25}$D −0.43° (*c* 0.017, MeOH); UV λ_{max} (MeOH) (log ε): 215 (4.32), 255 (3.84), 290 (3.1); IR (KBr) ν_{max} cm^{-1}: 3450, 2950, 1740, 1595, 1500, 1448, 1385, 1245, 1060 cm^{-1}; TSP-MS data (positive ion mode, NH_4OAc buffer) *m/z* 628 $[M+NH_4]^+$; NMR: Tables 1 and 2.

Derivative **1b**. White amorphous powder; mp 44–46°; ($CHCl_3$–MeOH, 98: 19:1), R_f 0.68; $[\alpha]_D^{25}$D −1.5° (*c* 0.005, MeOH); UV λ_{max} (MeOH) nm (log ε): 220 (4.31), 260 (3.82), 285 (3.25); IR (KBr) ν_{max} cm^{-1}: 3380–3450, 2950, 1740, 1580, 1500, 1440, 1375, 1200–1250, 1040–1100, 970; TSP-MS data (positive ion mode, NH_4OAc buffer) *m/z*: 786 $[M+NH_4]^+$, 744 $[M-COCH_3+H]^+$, 391 $[M\text{-}Glu(OAc)_4\text{-}CH_3\text{-}CH_3OH]^+$, 331 $[M\text{-}Glu(OAc)_4+H]^+$; NMR: Tables 1 and 2.

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