Phytochemistry Letters 36 (2020) 32–36

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Angelicosides I-IV, four undescribed furanocoumarin glycosides from *Angelica dahurica* roots and their tyrosinase inhibitory activities

Penghua Shu[a,*], Junping Li[a], Yingying Fei[a], Huiqing Zhu[a], Lingxiang Zhang[a], Haoying Niu[a], Yamin Li[a], Hao Liu[a], Zhiyu Ju[a], Xialan Wei[b], Fugang Xiao[c], Zhihong Xu[a,*]

[a] School of Chemistry and Chemical Engineering, Xuchang University, Xuchang, China
[b] School of Information Engineering, Xuchang University, Xuchang, China
[c] Food and Bioengineering College, Xuchang University, Xuchang, China

ARTICLE INFO

Keywords:
Angelica dahurica
Apiaceae
Furanocoumarin
Rhamnoside
Angelicoside
Tyrosinase

ABSTRACT

Angelica dahurica is an important medicinal plant, which has been widely used in medicines and skin-whitening products. In the investigation of phytochemicals present in *A. dahurica* roots, three unusual furanocoumarin rhamnosides, angelicosides I–III (**1**–**3**), one undescribed furanocoumarin glucoside, angelicoside IV (**4**), together with eight known compounds (**5**–**12**) were isolated. Their structures were characterized by a combination of UV, IR, NMR, HRESIMS, along with acid hydrolysis and enzymatic hydrolysis. All the isolated compounds were tested for their mushroom tyrosinase inhibitory activities, and compounds **1**, **2**, and **11** showed moderate activities.

1. Introduction

Tyrosinase, a natural copper-containing enzyme, plays a crucial role in the process of melanogenesis. Its inhibitors have good potentials to be used as skin-whitening ingredients in cosmetics (Pillaiyar et al., 2017). In the past decades, plenty of tyrosinase inhibitors have been obtained by purification of natural products and chemical synthesis, such as L-ascorbic acid, kojic acid, and arbutin (Lee et al., 2016). However, most of them have more or less drawbacks. For example, L-ascorbic acid is sensitive to air and heat, and degrades easily (Phillips et al., 2016; Caritá et al., 2020). Arbutin is chemically unstable and can be metabolized to generate benzene analogues, which might be causal factors in leukemia (McDonald et al., 2001). Therefore, it is still in great need of developing safe, stable, and effective tyrosinase inhibitors.

Angelica dahurica (Fisch. ex Hoffm.) Benth. et Hook, a plant belonging to the family Apiaceae, is widely distributed in China, Russia, Korea and Japan. *A. dahurica* roots (ADR) have been used as skin-whitening agents since ancient times (Huang et al., 2016). Recent studies revealed that the skin-whitening function of ADR might be due to the coumarins with tyrosinase inhibitory activities (Cho et al., 2006; Li et al., 2016). In order to discover potential tyrosinase inhibitors from ADR, a detailed chemical investigation was carried out, which resulted in the isolation of four new coumarin glycosides (**1**–**4**) (Fig. 1) and eight known glycosides (**5**–**12**) (Fig. 1S, Supporting Information). Angelicosides I–III (**1**–**3**) were furanocoumarin rhamnosides, whose analogues have been rarely reported from the family Apiaceae, and for the first time reported from *A. dahurica*. Herein, the isolation, identification, and tyrosinase inhibitory activities of all the isolated compounds are reported.

2. Results and discussion

Compound **1** was isolated as a colorless oil. Its molecular formula was assigned as $C_{23}H_{28}O_{11}$, on the basis of its sodium adduct ion peak at *m/z* 503.1535 $[M + Na]^+$ (calcd for $C_{23}H_{28}O_{11}Na$, 503.1529) in the HRESIMS spectrum (Fig. 2S, Supporting Information). The IR spectrum showed characteristic absorption bands for hydroxyl (3434 cm^{-1}), α-pyrone (1720, 1481 cm^{-1}) and aromatic ring (1620 cm^{-1}), respectively (Fig. 4S, Supporting Information). The ^1H and ^{13}C NMR spectral data of compound **1** were shown in Table 1. A pair of doublets [δ_H 8.26 (1H, d, J = 10.0 Hz), 6.30 (1H, d, J = 10.0 Hz)] confirmed an AB spin system, which was identified as the signals of H-3 and H-4 of the α-pyrone ring system. Another pair of doublets [δ_H 7.88 (1H, d, J = 2.4 Hz), 7.25 (1H, d, J = 2.4 Hz)], which was assigned as H-2′ and H-3′, indicated that compound **1** was a linear furanocoumarin. Two methyl singlets at δ_H 1.34 and 1.32, and a distinct singlet at δ_H 4.22 indicated the presence of a hydroxyl isopropyl and a methoxyl group. The anomeric proton of the sugar at δ_H 5.02 (1H, d, J = 1.2 Hz) and the ^{13}C NMR signal pattern

* Corresponding authors at: School of Chemistry and Chemical Engineering, Xuchang University, 88 Bayi Road, Xuchang, China.
E-mail addresses: shupenghua@yeah.net (P. Shu), xuzhihong1980@xcu.edu.cn (Z. Xu).

https://doi.org/10.1016/j.phytol.2020.01.006
Received 6 November 2019; Received in revised form 19 December 2019; Accepted 15 January 2020

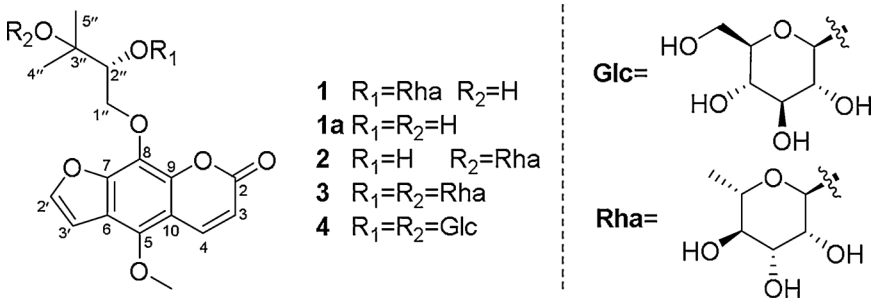

Fig. 1. Structures of compounds **1–4.**

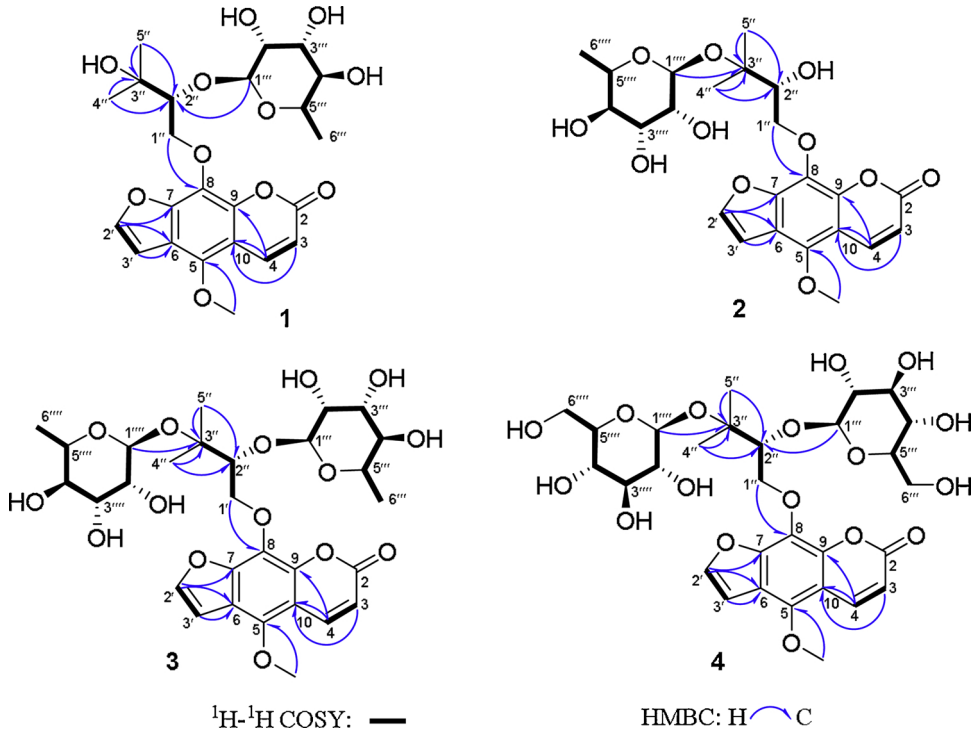

Fig. 2. Key ^{1}H-^{1}H COSY and HMBC correlations for compounds **1–4.**

showed that compound **1** contained an α-L-rhamnose. On acid hydrolysis, compound **1** afforded (+)-byakangelicin (**1a**) (Fujioka et al., 1999) (Table 1; Figs. 42S–44S, Supporting Information) as an aglycone and L-rhamnose, which confirmed its subunit and linkage. The correlations from the anomeric proton to C-2′′ suggested the *O*-glycosylation occurred at C-2′′. Further analysis of its ^{1}H NMR, ^{13}C NMR, COSY, HSQC, and HMBC spectra (Fig. 2; Figs. 5S–10S, Supporting Information) established the structure of **1** to be 2′′-O-*α*-L-rhamnosyl-(+)-byakangelicin, named as angelicoside I.

Compound **2**, a colorless oil, showed the same molecular formula of $C_{23}H_{28}O_{11}$ (calcd for $C_{23}H_{28}O_{11}Na$, 503.1529) as **1**, which was determined by the sodium adduct ion peak at *m/z* 503.1530 $[M + Na]^+$ in the HRESIMS spectrum (Fig. 12S, Supporting Information). The NMR data (Table 1) of **2** were very similar to those of **1**, except that C-2′′ in **2** (δ_C 77.9) shifted upfield compared to **1** (δ_C 86.0), while C-3′′ in **2** (δ_C 79.7) shifted downfield compared to **1** (δ_C 73.7). Thus, the *O*-glycosylation position should be at C-3′′ in **2**, instead of at C-2′′ in **1**. The key HMBC correlations from the anomeric proton of the rhamnose (δ_H 5.07, d, J = 1.2 Hz) to C-3′′ confirmed this deduction (Fig. 2). On the basis of detailed 2D NMR analysis and acid hydrolysis, the structure of **2** was determined as 3′′-O-*α*-L-rhamnosyl-(+)-byakangelicin, named as angelicoside II.

Compound **3**, a colorless oil, possessed a molecular formula $C_{29}H_{38}O_{15}$, which was suggested by the sodium adduct ion peak at *m/z* 649.2100 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_{15}Na$, 649.2108) in the HRESIMS spectrum (Fig. 22S, Supporting Information). By comparison of the molecular formulas ($C_{29}H_{38}O_{15}$ for **3**, $C_{23}H_{28}O_{11}$ for **1**) and NMR data of **1** and **3** (Table 1), it was easy to find that compound **3** contained an additional *α*-L-rhamnose than **1**. An obvious downfield of C-3′′ in **3** (δ_C 80.5) compared to **1** (δ_C 73.7) indicated the another *O*-glycosylation occurred at C-3′′ in **3**. The key HMBC correlations from H-1′′′ to C-2′′, and from H-1′′′′ to C-3′′confirmed this deduction (Fig. 2; Fig. 30S, Supporting Information). Finally, the structure of **3** was determined as 2′′,3′′-di-O-*α*-L-rhamnosyl-(+)-byakangelicin by detailed 2D NMR analysis and acid hydrolysis, named as angelicoside III.

Compound **4** was isolated as a colorless oil. Its molecular formula $C_{29}H_{38}O_{17}$ was determined by the protonated molecule ion peak at *m/z* 659.2181 $[M+H]^+$ (calcd for $C_{29}H_{39}O_{17}$, 659.2187) in the HRESIMS spectrum (Fig. 32S, Supporting Information). The NMR data of **4** were similar to those of **3** (Table 1). The main differences were the absence of two methyls in **4** compared to **3** (δ_C 17.9, 18.1) and the emergence of two oxygenated methylenes in **4** (δ_C 62.4, 62.6). The anomeric protons of the sugars at δ_H 4.62 (1H, d, J = 7.6 Hz) and 4.72 (1H, d, J = 8.0 Hz) and the ^{13}C NMR signal pattern (Table 1) showed that compound **4** contained two *β*-D-glucoses. Subsequent enzymatic hydrolysis of **4** yielded (+)-byakangelicin (**1a**) as an aglycone and D-glucose, which confirmed the above speculations. The key HMBC correlations from H-1′′′ to C-2′′, from H-1′′′′ to C-3′′ indicated that the two D-glucoses

Table 1
^{1}H and ^{13}C NMR data of **1–4**[a].

	1		**2**		**3**		**4**		**1**[a,b]	
position	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
2	162.9		162.9		163.0		162.6		159.8	
3	113.3	6.30, d (10.0)	113.3	6.29, d (9.6)	113.3	6.31, d (10.0)	113.4	6.31, d (9.6)	112.6	6.32, d (10.0)
4	141.7	8.26, d (10.0)	141.7	8.25, d (9.6)	141.8	8.26, d (10.0)	141.5	8.25, d (9.6)	139.8	8.17, d (10.0)
5	146.4		146.3		146.5		146.4		144.1	
6	116.5		116.6		116.6		116.4		114.5	
7	151.8		151.8		151.9		151.7		149.6	
8	128.5		128.5		128.4		128.3		126.9	
9	145.0		145.0		145.1		145.1		143.2	
10	108.9		108.8		108.9		108.9		107.0	
2′	147.2	7.85, d (2.4)	147.3	7.87, d (2.0)	147.3	7.88, d (2.4)	147.2	7.85, d (2.4)	146.3	8.07, d (2.0)
3′	106.6	7.24, d (2.4)	106.5	7.23, d (2.0)	106.6	7.25, d (2.4)	106.6	7.25, d (2.4)	105.7	7.36, d (2.0)
1′′a	76.0	4.48, dd (10.4, 3.6)	76.7	4.67, dd (10.4, 2.4)	75.8	4.52, dd (10.4, 2.0)	75.7	4.47, dd (10.4, 5.6) 4.64, dd (10.4, 4.0)	75.9	4.17, dd (10.0, 2.0) 4.44, dd (10.0, 2.0)
1′′b		4.42, dd (10.4, 5.6)		4.27, dd (10.4, 8.8)		4.40, dd (10.4, 5.6)				
2′′	86.0	3.93, dd (5.6, 3.6)	77.9	3.87, dd (8.8, 2.4)	85.5	4.00, dd (5.6, 2.0)	89.3	3.90, dd (5.6, 4.0)	76.7	3.64, td (2.0, 5.6)
2′′-OH										4.99, d (5.6)
3′′	73.7		79.7		80.5		73.9		70.9	
4′′	26.3	1.34, s	23.4	1.35, s	22.6	1.42, s	24.8	1.38, s	27.2	1.22, s
5′′	26.4	1.32, s	22.6	1.28, s	23.8	1.39, s	26.4	1.35, s	24.5	1.04, s
OCH_3	61.6	4.22, s	61.6	4.22, s	61.6	4.23, s	61.6	4.22, s	60.9	4.15, s
1′′′	104.0	5.02, d (1.2)			103.9	5.00, d (1.2)	106.1	4.62, d (7.6)		
2′′′	72.5	3.99, dd (1.2, 3.2)			72.6	3.94, dd (1.2, 2.8)	76.2	3.25, dd (7.6, 8.0)		
3′′′	72.7	3.78, dd (3.2, 9.6)			72.7	3.78, dd (2.8, 9.6)	78.6	3.38, overlap		
4′′′	74.3	3.38, dd (9.6, 9.6)			74.3	3.38, dd (9.6, 9.6)	71.2	3.35, overlap		
5′′′	70.3	4.10, qd (6.4, 9.6)			70.3	4.10, qd (6.4, 9.6)	77.9	3.34, overlap		
6′′′a	17.9	1.09, d (6.4)			17.9	1.09, d (6.4)	62.6	3.64, dd (12.0, 4.4)		
6′′′b								3.52, dd (12.0, 2.8)		
1′′′′			96.3	5.07, d (1.2)	96.1	5.12, d (1.2)	105.0	4.72, d (8.0)		
2′′′′			73.6	3.70, dd (1.2, 2.4)	73.7	3.75, dd (1.2, 3.2)	84.4	3.25, dd (8.0, 8.8)		
3′′′′			72.5	3.66, dd (2.4, 9.2)	72.6	3.68, dd (3.2, 9.6)	78.2	3.59, overlap		
4′′′′			74.3	3.35, dd (9.2, 9.6)	74.2	3.36, dd (9.6, 9.6)	71.2	3.35, overlap overlapoverlap, overlap		
5′′′′			70.1	3.75, qd (6.4, 9.6)	70.0	3.76, qd (6.0, 9.6)	77.8	3.33, overlap		
6′′′′a			18.1	1.20, d (6.4)	18.1	1.21, d (6.0)	62.4	3.89, dd (12.0, 1.2)		
6′′′′b								3.72, dd (12.0, 3.2)		

[a] 400 MHz for ^{1}H and 100 MHz for ^{13}C, recorded in CD_3OD.
[b] Recorded in DMSO-d_6.

Table 2
Inhibitory effects of compounds **1–12** and of kojic acid on mushroom tyrosinase[a].

Compound	Tyrosinase inhibition (%)	Compound	Tyrosinase inhibition (%)
1	9.75 ± 0.72	**8**	NI[b]
2	8.02 ± 0.60	**9**	NI[b]
3	NI[b]	**10**	NI[b]
4	NI[b]	**11**	11.79 ± 0.52
5	NI[b]	**12**	NI[b]
6	NI[b]	kojic acid (positive control)	26.01 ± 0.67
7	NI[b]		

[a] Tyrosinase inhibitions were measured at a derivative concentration of 25 μM, with L-tyrosine as the substrate. Results were expressed as means ± SEMs.
[b] NI: no inhibition.

were connected to the C-2′′ and C-3′′ positions of **4**, respectively (Fig. 2; Fig. 40S, Supporting Information). On the basis of detailed 2D NMR analysis, the structure of **4** was decided as 2′′,3′′-di-O-*β*-D-glucopyranosyl-(+)-byakangelicin, named as angelicoside IV.

According to the literatures, the two epimers at C-2′′, (*R*)-(+)-byakangelicin and (*S*)-(−)- byakangelicin, show opposite specific rotations, $[\alpha]_D^{20}$ $[\alpha]_D^{20}$ +17.7 (c 1.3, EtOH) (Baba et al., 1981), and $[\alpha]_D^{20}$ $[\alpha]_D^{20}$ –18.5 (c 0.5, CH_3OH) (Rao et al., 1991), respectively. After acid hydrolysis or enzymatic hydrolysis, all of the four glycosides **1**−**4** provided dextrorotatory aglycones, (+)-byakangelicin, suggesting the *R* configuration at C-2′′.

The known compounds **5**−**12** were identified as *tert*-O-*β*-D-apiofuranosyl-(1→6)-O-*β*-D-glucopyranosyl-byakangelicin (**5**) (Jia et al., 2008), 1′-O-*β*-D-glucopyranosyl-(2*R*,3*S*)-3-hydroxynodakenetin (**6**) (Xiao et al., 2001), xanthotoxol 8-O-*β*-D-glucopyranoside (**7**) (Zhao and Yuan, 2007), nodakenin (**8**) (Lee et al., 2003), decuroside IV (**9**) (Asahara et al., 1984), *β*-D-glucosyl-6′-(*β*-D-apiosyl) columbianetin (**10**) (VanWagenen et al., 1988), adenosine (**11**) (You et al., 2002), daucosterol (**12**) (Sura et al., 2019), repectively, by NMR data analysis and comparison with the reported data. The previous reports showed that the plants of *angelica* genus are rich in coumarins and related glycosides (Chen and Yang, 2004; Wei et al., 2016; Ma et al., 2019). The ten courmarin glycosides (**1**−**10**) isolated from ADR in this investigation are well consistent with the previous reports. Moreover, it is the first time that furanocoumarin rhamnosides have been isolated from *A. dahurica*, which would enriched our knowledge about the chemical diversity of *A. dahurica*.

The mushroom tyrosinase inhibitory activities of compounds **1–12** and kojic acid (positive control) were evaluated at a concentration of 25 μM. However, only compounds **1**, **2**, and **11** showed moderate tyrosinase inhibition activities (Table 2). According to the previous reports, entities with strong tyrosinase inhibitory activity usually contained several aromatic hydroxyl or amino groups (Pillaiyar et al., 2018). The deficiency of the key pharmacophores might be the reason why compounds **1–12** showed weak or moderate activities. Moreover, compared with the monoglycosides **1** and **2**, the diglycosides **3–5** exhibited weaker inhibition activities (inhibitory rate < 5 %). Perhaps the polarity of tested compounds could influence the bioactivity.

3. Materials and methods

3.1. General experimental procedures

Optical rotations were determined on a Rudolph Autopol IV polarimeter (589 nm, 20 °C). FT-IR and UV spectra were determined using FTIR-650 and Puxi TU-1950 instruments, respectively. NMR spectra were recorded on a Bruker AM-400 spectrometer. High-resolution electrospray ionization mass spectra (HRESIMS) were carried out on a Waters Xevo G2-XS QTof spectrometer. Column chromatography (CC) was performed using silica gel (Qingdao Marine Chemical Inc., China), ODS (50 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). TLC was performed with silica gel 60 F254 (Yantai Chemical Industry Research Institute).

3.2. Plant material

The fresh roots of *A. dahurica* were collected in Xuchang, People's Republic of China, in April 2019. The botanical identification was made by Prof. Lin Yang, School of Life Science and Engineering, Lanzhou University of Technology. A voucher specimen (SPH2019A) was deposited in the herbarium of School of Chemistry and Chemical Engineering, Xuchang University.

3.3. Extraction and isolation

The air-dried roots of *A. dahurica* (6.3 kg) were extracted with 95 % EtOH at room temperature (3 × 15 L) to afford a crude extract of 93.8 g after evaporation of the solvent under vacuum. The extract was suspended in distilled H_2O and partitioned with CH_2Cl_2 and *n*-BuOH, respectively. The *n*-BuOH soluble portion (36.5 g) was subjected to silica gel CC using CH_2Cl_2–MeOH (50:1 to 2:1) as eluent to give five fractions A–E. Fraction B (5.3 g, eluted by CH_2Cl_2–MeOH 35:1) was subsequently purified by Sephadex LH-20 column (CH_2Cl_2–MeOH 1:1) to give four subfractions (FB-1 to FB-4). Fraction FB-1 was chromatographed on a silica gel CC (CH_2Cl_2–MeOH 30:1) to give compound **12** (5.1 mg). Fraction C (8.9 g, eluted by CH_2Cl_2–MeOH 25:1) was purified on a RP-C_{18} CC (MeOH–H_2O, 50:50 to 100:0) to afford five subfractions (FC-1 to FC-5). Fraction C-1 was passed through a Sephadex LH-20 column (MeOH) to give compounds **1** (2.7 mg) and **11** (5.7 mg). Fraction C-2 was chromatographed on a silica gel CC (CH_2Cl_2–MeOH 15:1) to give compounds **6** (4.2 mg) and **8** (4.9 mg). Fraction D (11.4 g, eluted by CH_2Cl_2–MeOH, 15:1 to 4:1) was purified on a Sephadex LH-20 column (MeOH) to give four subfractions (FD-1 to FD-4). Fraction D-1 was further purified by Sephadex LH-20 column (MeOH) to give compounds **2** (3.5 mg) and **7** (5.8 mg). Fraction D-2 was further purified by RP-C18 CC eluted with MeOH-H_2O (20:80 to 50:50) to give compounds **3** (3.4 mg) and **5** (7.3 mg). Fraction D-3 was chromatographed on a silica gel CC (CH_2Cl_2–MeOH 5:1) to give compound **10** (6.7 mg). Fraction D-4 was chromatographed on a RP-C_{18} CC (MeOH–H_2O, 20:80 to 50:50) to give compounds **4** (4.1 mg) and **9** (8.0 mg).

*Angelicoside I (**1**)*: colorless oil; $[\alpha]_D^{20}$ –24.4 (c 0.09, CH_3OH); UV λ_{max} (MeOH) nm (log ε): 223 (4.0), 269 (3.7), 312 (3.7); IR (KBr) ν_{max} 3434, 2927, 1720, 1592, 1481, 1349, 1272, 1170, 1035, 821 cm^{-1}; ^1H NMR and ^{13}C NMR data (CD_3OD), see Table 1; HRESIMS *m/z* 503.1535 $[M + Na]^+$ (calcd for $C_{23}H_{28}O_{11}Na$, 503.1529).

*Angelicoside II (**2**)*: colorless oil; $[\alpha]_D^{20}$ –22.2 (c 0.12, CH_3OH); UV λ_{max} (MeOH) nm (log ε): 223 (4.2), 269 (4.0), 313 (3.8); IR (KBr) ν_{max} 3446, 2931, 1700, 1606, 1481, 1353, 1218, 1145, 1074, 831 cm^{-1}; ^1H NMR and ^{13}C NMR data (CD_3OD), see Table 1; HRESIMS *m/z* 503.1530 $[M + Na]^+$ (calcd for $C_{23}H_{28}O_{11}Na$, 503.1529).

*Angelicoside III (**3**)*: colorless oil; $[\alpha]_D^{20}$ $[\alpha]_D^{20}$ –30.0 (c 0.11, CH_3OH); UV λ_{max} (MeOH) nm (log ε): 223 (4.0), 269 (3.8), 313 (3.6); IR (KBr) ν_{max} 3432, 2927, 1689, 1481, 1353, 1253, 1172, 1037, 836 cm^{-1}; ^1H NMR and ^{13}C NMR data (CD_3OD), see Table 1; HRESIMS *m/z* 649.2100 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_{15}Na$, 649.2108).

*Angelicoside IV (**4**)*: colorless oil; $[\alpha]_D^{20}$ –11.7 (c 0.14, CH_3OH); UV λ_{max} (MeOH) nm (log ε): 222 (4.1), 268 (3.9), 313 (3.7); IR (KBr) ν_{max} 3426, 2927, 1720, 1589, 1477, 1353, 1170, 1072, 829 cm^{-1}; ^1H NMR and ^{13}C NMR data (CD_3OD), see Table 1; HRESIMS *m/z* 659.2181 $[M + H]^+$ (calcd for $C_{29}H_{39}O_{17}$, 659.2187).

3.4. Acid hydrolysis of compounds 1–3

The acid hydrolysis of compounds **1–3** were conducted according to the literature procedures (Zhao and Yang, 2018). The glycosides (each

1.5 mg) were separately dissolved in a mixture of 8.0 % HCl (1 mL) and MeOH (8 mL). The reaction mixture was refluxed for 2 h and then concentrated under vacuum to remove the solvents. The reaction residue was purified by silica gel CC to give (+)-(*R*)-byakangelicin (**1a**) and L-rhamonose. The latter was confirmed according to the positive $[\alpha]_D$ values [reported: $[\alpha]_D^{20}$ $[\alpha]_D^{20}$ +2.4 (c 1, H_2O)] (Heredia-Vieira et al., 2014) and comparison with the authentic sample.

3.5. Enzymatic hydrolysis of compound 4

Compound **4** (3.1 mg) and *β*-glucosidase (25.0 mg, CAS: 9001-22-3) were dissolved in 1.5 mL of H_2O and stirred at room temprature for 36 h. The reaction mixture was filtered and the filtrate was concentrated under vacuum to afford a residue, which was further purified by silica gel CC to give (+)-(*R*)-byakangelicin (**1a**) and D-glucose. D-glucose was identified by comparison with the positive $[\alpha]_D$ value {($[\alpha]_D^{20}$ +17.9 (c 0.05, H_2O) vs L-glucose, $[\alpha]_D^{20}$ –20 (c 0.89, H_2O)} (Liu et al., 2016) and the authentic sample.

3.6. Mushroom tyrosinase inhibition assay

The mushroom tyrosinase inhibition activities of compounds **1–12** were tested acoording to the literatures (Hyun et al., 2008). Compounds (**1–12**, 10 *μ*L, 25 *μ*M) and mushroom tyrosinase (20 *μ*L, 1000 U/mL) (Psaitong, China) in a potassium phosphate buffer (50 mM, pH 6.5) were added to 170 *μ*L of an assay mixture containing a 10 : 10 : 9 ratio of L-tyrosine solution (1 mM), potassium phosphate buffer (1 mM), and distilled water in a 96-well microplate. The reaction mixture was incubated at 37 °C for half an hour. Percentage tyrosinase inhibition was determined by measuring optical densities at 450 nm using a Multiskan FC microplate reader (Thermo Fisher Scientific, Inc.). Kojic acid (25 *μ*M) was chosen as the positive control. Three independent experiments were repeated. The % inhibition was determined by [1 − (As/Ac)] × 100, where As is the absorbance of tested compound and Ac the non-treated control. Statistical analysis was determined using GraphPad Prism 5 software, and the results were expressed as means ± SEMs. The inhibitory rate > 5 % is considered active.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

We are grateful to Lanxiang Dai for the collection of *A. dahurica* roots and Prof. Lin Yang at Lanzhou University of Technology for the authentication of the plant material. This work was financially supported by National Natural Science Foundation of China (No. 21702178), Key Scientific Research Program in Universities of Henan Province (No. 18A350010), Undergraduate Training Program for Innovation and Entrepreneurship of Henan Province (No. 201910480011, 201910480017), Distinguished Young Scholars Fund of Xuchang University (No. 2019), and Excellent Young Key Teacher Funding Project of Xuchang University (No. 2017).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2020.01.006.

References

Asahara, T., Sakakibara, I., Okuyama, T., Shibata, S., 1984. Studies on coumarins of a Chinese drug “Qian Hu.” V. Coumarin-glycosides from “Zi Hua Qian Hu”. Planta Med. 50, 488–492. https://doi.org/10.1055/s-2007-969780.

Baba, K., Hata, K., Kimura, Y., Matsuyama, Y., Kozawa, M., 1981. Chemical studies of *Angelica japonica* A. Gray. I. On the constituents of the ethyl acetate extract of the root. Chem. Pharm. Bull. 29, 2565–2570. https://doi.org/10.1248/cpb.29.2565.

Caritá, A.C., Fonseca-Santos, B., Shultz, J.D., Michniak-Kohn, B., Chorilli, M., Leonardi, G.R., 2020. Vitamin C: one compound, several uses. Advances for delivery, efficiency and stability. Nanomed. Nanotechnol. Biol. Med. 24, 102117. https://doi.org/10.1016/j.nano.2019.102117.

Cho, Y.H., Kim, J.H., Park, S.M., Lee, B.C., Pyo, H.B., Park, H.D., 2006. New cosmetic agents for skin whitening from *Angelica dahurica*. J. Cosmet. Sci. 57, 11–21.

Chen, J., Yang, C., 2004. Researches in *Angelica L.*. Nat. Prod. Res. Dev. 16, 359–365. https://doi.org/10.16333/j.1001-6880.2004.04.023.

Fujioka, T., Furumi, K., Fujii, H., Okabe, H., Mihashi, K., Nakano, Y., Matsunaga, H., Katano, M., Mori, M., 1999. Antiproliferative constituents from *Umbelliferae* plants. V. A new furanocoumarin and falcarindiol furanocoumarin ethers from the root of *Angelica japonica*. Chem. Pharm. Bull. 47, 96–100. https://doi.org/10.1248/cpb.47.96.

Hyun, S.K., Lee, W.-H., Jeong, D.M., Kim, Y., Choi, J.S., 2008. Inhibitory effects of kurarinol, kuraridinol, and trifolirhizin from *Sophora flavescens* on tyrosinase and melanin synthesis. Biol. Pharm. Bull. 31, 154–158. https://doi.org/10.1248/bpb.31.154.

Heredia-Vieira, S.C., Simonet, A.M., Vilegas, W., Macias, F.A., 2014. Unusual C, O-fused glycosylapigenins from *Serjania marginata* leaves. J. Nat. Prod. 78, 77–84. https://doi.org/10.1021/np500715x.

Huang, S., Cauty, C., Dolivet, A., Le Loir, Y., Chen, X.D., Schuck, P., Jan, G., Jeantet, R., 2016. Double use of highly concentrated sweet whey to improve the biomass production and viability of spray-dried probiotic bacteria. J. Funct. Foods 23, 453–463. https://doi.org/10.1016/j.jff.2016.02.050.

Jia, X., Zhao, X., Wang, M., Chen, Y., Dong, Y., Feng, X., 2008. Two new coumarin biosides from *Angelica dahurica*. Chem. Nat. Compd. 44, 692–695. https://doi.org/10.1007/s10600-009-9199-z.

Lee, S.Y., Baek, N., Nam, T., 2016. Natural, semisynthetic and synthetic tyrosinase inhibitors. J. Enzyme Inhib. Med. Chem. 31, 1–13. https://doi.org/10.3109/14756366.2015.1004058.

Lee, S., Shin, D.-S., Kim, J.S., Oh, K.-B., Kang, S.S., 2003. Antibacterial coumarins from *Angelica gigas* roots. Arch. Pharm. Res. 26, 449–452. https://doi.org/10.1007/BF02976860.

Li, C.-Q., Peng, L.-N., Yao, C., Lu, C.-C., Kang, W.-Y., Wang, J.-M., 2016. Effect of heating treatment on stability of two coumarins in *Angelica dahurica* and activity of tyrosinase. Zhongguo Zhong Yao Za Zhi 41, 845–849.

Liu, Z., Yoshihara, A., Jenkinson, S.F., Wormald, M.R., Estévez, Rn.J., Fleet, G.W., Izumori, K., 2016. Triacetonide of glucoheptonic acid in the scalable syntheses of D-gulose, 6-deoxy-D-gulose, L-glucose, 6-deoxy-L-glucose, and related sugars. Org. Lett. 18, 4112–4115. https://doi.org/10.1021/acs.orglett.6b02041.

Ma, J., Huang, J., Hua, S., Zhang, Y., Zhang, Y., Li, T., Dong, L., Gao, Q., Fu, X., 2019. The ethnopharmacology, phytochemistry and pharmacology of *Angelica biserrata*–A review. J. Ethnopharmacol. 231, 152–169. https://doi.org/10.1016/j.jep.2018.10.040.

McDonald, T.A., Holland, N.T., Skibola, C., Duramad, P., Smith, M.T., 2001. Hypothesis: phenol and hydroquinone derived mainly from diet and gastrointestinal flora activity are causal factors in leukemia. Leukemia 15, 10–20. https://doi.org/10.1038/sj.leu.2401981.

Phillips, K.M., Council-Troche, M., McGinty, R.C., Rasor, A.S., Tarrago-Trani, M.T., 2016. Stability of vitamin C in fruit and vegetable homogenates stored at different temperatures. J. Food Anal. 45, 147–162. https://doi.org/10.1016/j.jfca.2015.09.008.

Pillaiyar, T., Manickam, M., Namasivayam, V., 2017. Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. J. Enzyme Inhib. Med. Chem. 32, 403–425. https://doi.org/10.1080/14756366.2016.1256882.

Pillaiyar, T., Namasivayam, V., Manickam, M., Jung, S.-H., 2018. Inhibitors of melanogenesis: an updated review. J. Med. Chem. 61, 7395–7418. https://doi.org/10.1021/acs.jmedchem.7b00967.

Rao, G.X., Sun, H.D., Lin, Z.W., Hu, R.Y., 1991. Studies on the chemical constitutents of the traditional Chinese medicine “yun qian-hu” (*Peucedanum rubricaule* Shan et Shch.). Yao Xue Xue Bao 26, 30–36. https://doi.org/10.16438/j.0513-4870.1991.01.007.

Sura, M.B., Ponnapalli, M.G., Annam, S.C.V.R., Bobbili, V.P., 2019. Ipomeolides A and B, resin glycosides from *Ipomoea pes-caprae* and combination therapy of ipomeolide A with doxorubicin. J. Nat. Prod. 82, 1292–1300. https://doi.org/10.1021/acs.jnatprod.8b01100.

VanWagenen, B.C., Huddleston, J., Cardellina II, J.H., 1988. Native American food and medicinal plants, 8. Water-soluble constituents of *Lomatium dissectum*. J. Nat. Prod. 51, 136–141. https://doi.org/10.1021/np50055a021.

Wei, W.-L., Zeng, R., Gu, C.-M., Qu, Y., Huang, L.-F., 2016. *Angelica sinensis* in China-A review of botanical profile, ethnopharmacology, phytochemistry and chemical analysis. J. Ethnopharmacol. 190, 116–141. https://doi.org/10.1016/j.jep.2016.05.023.

Xiao, Y.Q., Li, L., Masahiko, T., Kimiye, B., 2001. Glucosides from *Pleurospermum rivulorum*. Yao Xue Xue Bao 36, 519–522. https://doi.org/10.16438/j.0513-4870.2001.07.010.

You, X.L., Li, L., Xiao, Y.Q., 2002. Studies on chemical constituents from the water-soluble part of root of *Angelica dahurica*. Zhongguo Zhongyao Zazhi 27, 279–280.

Zhao, Y., Yuan, Z., 2007. A new coumarin glycoside from *Glehnia littoralis*. Yao Xue Xue Bao 42, 1070–1073. https://doi.org/10.16438/j.0513-4870.2007.10.007.

Zhao, A.-H., Yang, X.-W., 2018. New coumarin glucopyranosides from roots of *Angelica dahurica*. Chin. Herb. Med. 10, 103–106. https://doi.org/10.1016/j.chmed.2018.01.008.