



Original Article

Seven new 3,4-dihydro-furanocoumarin derivatives from *Angelica dahurica*

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(4S, 2R)-angelicadin A

(4R, 2S)-angelicadin A

(4S, 2S)-angelicadin A

(4R, 2R)-angelicadin A

(4S, 2S)-secoangelicadin A

(4R, 2R)-secoangelicadin A

(4R, 2R)-secoangelicadin A methyl ester

ABSTRACT

Objective: To study the chemical constituents of the roots of *Angelica dahurica*, a well-known Chinese herbal medicine named Baizhi in Chinese.

Methods: Compounds were separated by various chromatographies, and the structures of new compounds were elucidated based on the analysis of their spectroscopic and spectrometric data (1D, 2D NMR, HRESI MS, IR, and UV). The absolute configurations of new compounds were determined by the calculated electronic circular dichroism and chemical derivatization. The inhibitory activities of all isolates against nitric oxide (NO) production were evaluated using lipopolysaccharide-activated RAW 264.7 macrophage cells.

Results: Seven new 3,4-dihydro-furanocoumarin derivatives (**1a/1b**, **2a/2b**, **3a/3b**, **4**) together with a known furanocoumarin (**5**) were isolated from the roots of *A. dahurica*. The new compounds included three pairs of enantiomers, (4S, 2''R)-angelicadin A (**1a**)/(4R, 2''S)-angelicadin A (**1b**), (4S, 2''S)-angelicadin A (**2a**)/(4R, 2''R)-angelicadin A (**2b**), and (4S, 2''S)-secoangelicadin A (**3a**)/(4R, 2''R)-secoangelicadin A (**3b**), together with (4R, 2''R)-secoangelicadin A methyl ester (**4**). The known xanthotoxol (**5**) inhibited the NO production with the half-maximal inhibitory concentration (IC₅₀) value of (32.8 ± 0.8) μmol/L, but all the new compounds showed no inhibitory activities at the concentration of 100 μmol/L.

Conclusion: This is the first report of the discovery of 3,4-dihydro-furanocoumarins from *A. dahurica*. The results are not only meaningful for the understanding of the chemical constituents of *A. dahurica*, but also enrich the reservoir of natural products.

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1. Introduction

Angelica dahurica (Fisch. ex Hoffm.) Benth. et Hook. f. is a perennial medicinal plant widely distributed in China, Korea, Japan, and Russia (Yang et al., 2015). As a well-known herbal medicine, the roots of *A. dahurica* are called Baizhi in Chinese and commonly used for the treatment of headache, abscess, toothache, glabella pain, runny nose, and acne (Chinese Pharmacopoeia Committee, 2020). Previous investigations revealed that coumarins are the major components of *A. dahurica* and mainly contribute to its diverse and promising pharmacological activities such as anti-inflammatory, analgesic, antibacterial, and antioxidant activities

(Ji, Ma & Zhang, 2020; Sun et al., 2017; Wang, Jeong, Guo, & Park, 2016; Wang, Liu, Yang, & Lv, 2020; Zhao et al., 2022).

By using an off-line two-dimensional HPLC-MS approach, we previously revealed that there were diverse structurally complex dimeric coumarins and other unknown non-coumarin components in *A. dahurica* (Li et al., 2013). This promoted us to perform further investigations on the chemical constituents of this useful herbal medicine, which resulted in the determination of 11 new dimeric coumarins with significant anti-inflammatory activities and many pyrrole 2-carbaldehyde derived alkaloids (Yang et al., 2017; Qi et al., 2019). As a continuous project, we herein report the isolation and structural elucidation of seven 3,4-dihydro-furanocoumarin derivatives, (4S, 2''R)-angelicadin A (**1a**)/(4R, 2''S)-angelicadin A (**1b**), (4S, 2''S)-angelicadin A (**2a**)/(4R, 2''R)-angelicadin A (**2b**), and (4S, 2''S)-secoangelicadin A (**3a**)/(4R, 2''R)-secoangelicadin A (**3b**), together with (4R, 2''R)-secoangelicadin A methyl ester (**4**) (Fig. 1). Remarkably, the seven new compounds include three pairs

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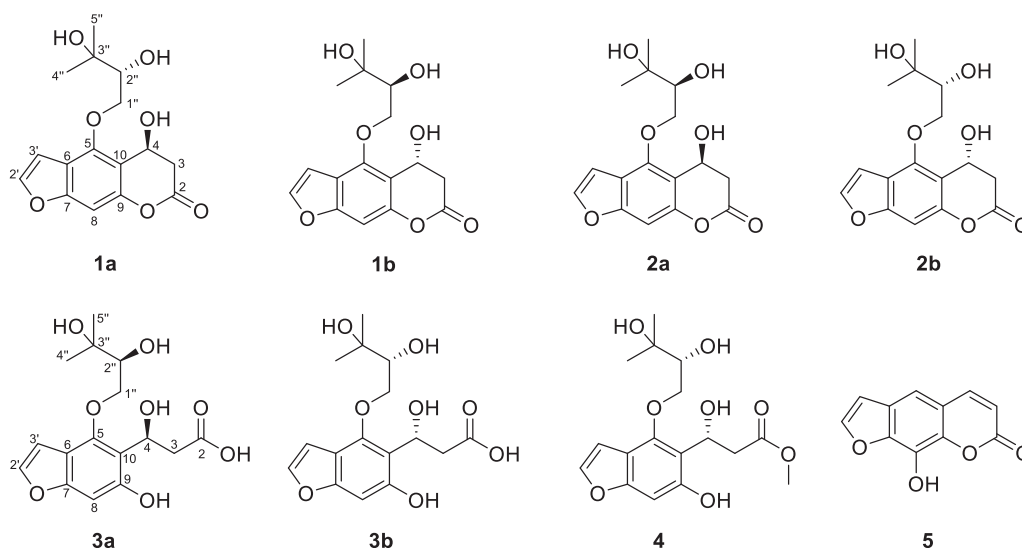


Fig. 1. Compounds isolated from roots of *A. dahurica*.

of enantiomers (**1a/1b**, **2a/2b**, **3a/3b**) which were successfully separated by chiral resolution using CHIRALPAK IC chiral column. In addition, the inhibitory activities of all isolates against nitric oxide (NO) production in lipopolysaccharide-activated RAW 264.7 macrophage cells were also evaluated.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were obtained on a Rudolph Autopol IV automatic polarimeter (Rudolph Research Analytical, NJ, USA). IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrophotometer (Thermo Nicolet Corporation, MA, USA) with KBr pellets. UV spectra were obtained using a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). NMR spectra were recorded on Bruker AVANCE III 400 M or AVANCE III 700 M spectrometer (Bruker Corporation, Billerica, USA) operating at 400 MHz or 700 MHz for ^1H NMR and 100 MHz or 175 MHz for ^{13}C NMR. HRESI MS was recorded on an LCMS-IT-TOF system, fitted with a Prominence UFLC system and an ESI interface (Shimadzu, Kyoto, Japan). Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), LiChroprep RP- C_{18} gel (40–63 μm , Merck, Darmstadt, Germany), D101 macroporous adsorption resin (Qingdao Marine Chemical Inc., Qingdao, China), and Sephadex LH-20 (Pharmacia, Qingdao Marine Chemical Inc., Qingdao, China) were used for open-column chromatography (CC). HPLC was performed on a Shimadzu LC-20AT pump system (Shimadzu Corporation, Tokyo, Japan), equipped with a SPD-M20A photodiode array detector. A semi-preparative HPLC column (YMCPack C_{18} , 10 mm \times 250 mm, 5 μm) was utilized for compound separation and purification. Chiral resolution was performed on CHIRALPAK IC chiral column (4.6 mm \times 150 mm, 5 μm). TLC was performed using GF $_{254}$ plates (Qingdao Marine Chemical Inc., Qingdao, China).

2.2. Plant materials

The roots of *A. dahurica* were collected in Anguo, Hebei Province, China, in December 2019. Plant authentication was performed by one of the authors (Shepo Shi). A voucher specimen (SPSHI-ADB-201912) was deposited in the Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine.

2.3. Extraction and isolation

The air-dried roots of *A. dahurica* (50 kg) were refluxed with 95% EtOH (2 \times 300 L, 3 h each) and 70% EtOH (300 L, 3 h). After removal of the solvent under reduced pressure, the residue (4.2 kg) was suspended in H_2O (5 L) and partitioned with petroleum ether (4 \times 5 L) and EtOAc (4 \times 5 L), successively. The H_2O extract (2.24 kg) was subjected to D101 macroporous adsorption resin chromatography and eluted with H_2O -EtOH (10:90, 20:80, 90:10, 100:0, volume percent) to yield four fractions. The 90% EtOH fraction (113 g) was subjected to silica gel chromatography and eluted with a gradient of EtOAc-MeOH (10:1 to 1:1, volume percent) to yield four fractions (Fr. A–D). Fr. A (33 g) was subjected to silica gel chromatography and eluted with a gradient of petroleum ether-EtOAc (5:1 to 1:1, volume percent) and CH_2Cl_2 -MeOH (20:1 to 1:1, volume percent) to give 14 subfractions (Fr. A1–A14). Fr. A3 (5 g) was subjected to Sephadex LH-20 and eluted with CH_2Cl_2 -MeOH (1:1, volume percent) to give seven subfractions (Fr. A3a–A3g). Fr. A3c (230 mg) was purified by semipreparative HPLC eluted with 20% aqueous MeCN to afford compound **5** (6.0 mg, t_{R} = 14.0 min). Fr. B (7.5 g) was subjected to ODS silica gel column chromatography eluted with a gradient of MeOH- H_2O from 5% to 60% to give seven subfractions (Fr. B1–B7). Fr. B2 (2.3 g) was purified by semipreparative HPLC eluted with 20% aqueous MeOH to afford compounds **3** (1.7 mg, t_{R} = 14.0 min) and **4** (4.5 mg, t_{R} = 18.0 min). Compound **3** was further separated by CHIRALPAK IC chiral column (4.6 mm \times 150 mm, 5 μm) eluted with EtOH-*n*-hexane (20:80, volume percent) to afford a pair of enantiomers **3a** (1.8 mg, t_{R} = 11.5 min) and **3b** (1.6 mg, t_{R} = 6.0 min). Fr. B4 (2.3 g) was purified by semipreparative HPLC eluted with 20% aqueous MeOH to afford compounds **1** (18.0 mg, t_{R} = 19 min) and **2** (16.0 mg, t_{R} = 26 min). Compound **1** was further separated by CHIRALPAK IC chiral column (4.6 mm \times 150 mm, 5 μm) eluted with EtOH-*n*-hexane (20:80, volume percent) to yield a pair of enantiomers **1a** (8.6 mg, t_{R} = 20.0 min) and **1b** (7.3 mg, t_{R} = 27.5 min). Compound **2** was further separated by purified by CHIRALPAK IC chiral column (4.6 mm \times 150 mm, 5 μm) eluted with EtOH-*n*-hexane (20:80, volume percent) to furnish a pair of enantiomers **2a** (5.0 mg, t_{R} = 20.0 min) and **2b** (5.0 mg, t_{R} = 27.0 min).

(4*S*,2'*R*)-angelicadin A (**1a**)/(4*R*,2'*S*)-angelicadin A (**1b**): amorphous white powder; IR (KBr) $_{\text{max}}$: 3310, 1765, 1621, 1597, 1455, 1438, 1360, 1325, 1249, 1233, 1182, 1151, 1124, 1076, 1046, 1022, 952, 937, 827, 793, 775, 759, 734, 692, 647, 595, 573, 528, 510,

436 cm^{-1} ; UV (MeOH) $\lambda_{\text{MeOH max}} (\log \epsilon)$: 255 (0.17), 222 (0.45); ^1H and ^{13}C NMR data, as shown in Table 1; positive-ion HRESIMS m/z 305.1030 $[\text{M}-\text{H}_2\text{O} + \text{H}]^+$ (calcd for $\text{C}_{16}\text{H}_{16}\text{O}_6$, 305.1020). (4*S*, 2''*R*)-angelicadin A (**1a**): $[\alpha]_{\text{D}}^{25} + 72$ (c 0.1, MeOH); ECD (MeOH), $\lambda_{\text{max}} (\Delta\epsilon)$ 220 (–16.0), 238 (+6.0), 255 (+5.0) nm; (4*R*, 2''*S*)-angelicadin A (**1b**): $[\alpha]_{\text{D}}^{25} - 72$ (c 0.1, MeOH); ECD (MeOH), $\lambda_{\text{max}} (\Delta\epsilon)$ 220 (+2.3), 238 (–1.6), 255 (–1.1) nm.

(4*S*, 2''*S*)-angelicadin A (**2a**)/ (4*R*, 2''*R*)-angelicadin A (**2b**): amorphous white powder; IR (KBr) ν_{max} : 3418, 2974, 1728, 1625, 1599, 1458, 1348, 1159, 1120, 1085, 1044, 734, 605 cm^{-1} ; UV (MeOH) $\lambda_{\text{MeOH max}} (\log \epsilon)$: 255 (0.51), 223 (1.42); ^1H and ^{13}C NMR data, as shown in Table 1; positive-ion HRESIMS m/z 305.1030 $[\text{M}-\text{H}_2\text{O} + \text{H}]^+$ (calcd for $\text{C}_{16}\text{H}_{16}\text{O}_6$, 305.1020). (4*S*, 2''*S*)-angelicadin A (**2a**): $[\alpha]_{\text{D}}^{25} + 16$ (c 0.1, MeOH); ECD (MeOH), $\lambda_{\text{max}} (\Delta\epsilon)$ 220 (–13.0), 235 (+7.0), 255 (+8.0) nm; (4*R*, 2''*R*)-angelicadin A (**2b**): $[\alpha]_{\text{D}}^{25} - 16$ (c 0.1, MeOH); ECD (MeOH), $\lambda_{\text{max}} (\Delta\epsilon)$ 220 (+1.2), 235 (–0.5), 255 (–0.4) nm.

(4*S*, 2''*S*)-secoangelicadin A (**3a**)/ (4*R*, 2''*R*)-secoangelicadin A (**3b**): white powder; IR (KBr) ν_{max} : 3377, 2969, 2924, 2853, 1731, 1625, 1597, 1457, 1378, 1348, 1216, 1159, 1116, 1086, 1044, 952, 733, 606, 526, 474 cm^{-1} ; UV (MeOH) $\lambda_{\text{MeOH max}} (\log \epsilon)$: 217 (0.36); ^1H and ^{13}C NMR data, as shown in Table 1; positive-ion HRESIMS m/z 341.1236 $[\text{M}-\text{H}_2\text{O} + \text{H}]^+$ (calcd for $\text{C}_{16}\text{H}_{16}\text{O}_6$, 341.1231). (4*S*, 2''*S*)-secoangelicadin A (**3a**): $[\alpha]_{\text{D}}^{25} + 20$ (c 0.1, MeOH); ECD (MeOH), $\lambda_{\text{max}} (\Delta\epsilon)$ 218 (–5.8), 230 (+1.3); (4*R*, 2''*R*)-secoangelicadin A (**3b**): $[\alpha]_{\text{D}}^{25} - 20$ (c 0.1, MeOH); ECD (MeOH), $\lambda_{\text{max}} (\Delta\epsilon)$ 218 (+1.6), 230 (–1.0).

(4*R*, 2''*R*)-secoangelicadin A methyl ester (**4**): white powder; $[\alpha]_{\text{D}}^{25} - 20$ (c 0.06, MeOH); UV (MeOH) $\lambda_{\text{MeOH max}} (\log \epsilon)$: 218 (0.36); ECD (c 7.0×10^{-4} M, MeOH), $\lambda_{\text{max}} (\Delta\epsilon)$ 220 (+0.6), 240 (–0.5), 255 (–0.2) nm; IR (KBr) ν_{max} : 3378, 2971, 1735, 1625, 1596, 1456, 1438, 1351, 1215, 1156, 1119, 1041, 1005, 953, 880, 824, 733, 691, 606, 506 cm^{-1} ; ^1H and ^{13}C NMR data, as shown in Table 1; positive-ion HRESIMS m/z 337.1261 $[\text{M}-\text{H}_2\text{O} + \text{H}]^+$ (calcd for $\text{C}_{17}\text{H}_{20}\text{O}_7$, 337.1282).

2.4. ECD calculations

The electronic circular dichroism (ECD) calculating experiments mainly include conformation analysis, spectrum calculation and spectrum fitting (Qi et al., 2017). Firstly, Chemdraw 3D was used to minimize the energy of the compounds, and then a preliminary conformational analysis was performed with the SYBYL 2.0 software package using the random search method. The conformers were further optimized by using the TDDFT method at the B3LYP/6-31G(d) level, and the frequency was calculated at the same level of theory. The stable conformers without imaginary frequencies were subjected to ECD calculation by the TDDFT method at the B3LYP/6-31G(d) level with the CPCM model in MeOH. ECD spectra of different conformers were simulated using SpecDis v1.5139 with a half-bandwidth of 0.3 eV, and the final ECD spectra were obtained according to the Boltzmann-calculated contribution of each conformer. The calculated ECD spectra were compared with the experimental data. All calculations were performed with the Gaussian 09 program package.

2.5. Mosher reaction

The (*R*)- and (*S*)-MTPA ester derivatives of compound **1a** were prepared as previously described. In brief, 5 μL (*R*)-(-)- α -methoxy- α -(trifluoromethyl) phenylacetyl chloride was added into 50 μL deuterated pyridine solutions of sample **1a** (3.0 mg). The reaction mixture was homogenized and kept at room temperature for 12 h to get (*S*)-MTPA ester of compounds **1**. Similarly, the (*R*)-MTPA ester of compounds **1a** was prepared (Su et al., 2002; Li, Du, Kelly, Zhou, & Nagle, 2013; Yang et al., 2015).

(*R*)-MTPA Ester of **1a**: ^1H NMR (Pyridine d_5) δ_{H} 5.39 (1H, d, $J = 9.0$ Hz, H-3a), 4.37 (1H, d, $J = 7.8$ Hz, H-3b), 4.78 (1H, t, $J = 9.0$ Hz, H-4), 5.27 (1H, d, $J = 7.2$ Hz, H-1''a), 4.04 (1H, d, $J = 16.8$ Hz, H-1''b), 3.24 (1H, dd, $J = 16.8, 7.2$ Hz, H-2''), 1.65 (1H, s, H-4''), 1.59 (1H, s, H-5'').

(*S*)-MTPA Ester of **1a**: ^1H NMR (Pyridine d_5) δ_{H} 6.10 (1H, dd, $J = 7.2, 2.4$ Hz, H-3a), 5.10 (1H, dd, $J = 11.4, 2.4$ Hz, H-3b), 5.43 (1H, dd, $J = 11.4, 7.2$ Hz, H-4), 5.12 (1H, d, $J = 6.6$ Hz, H-1''a), 3.93 (1H, d, $J = 17.4$ Hz, H-1''b), 3.16 (1H, dd, $J = 16.8, 7.2$ Hz, H-2''), 1.68 (1H, s, H-4''), 1.67 (1H, s, H-5'').

2.6. Biological assay

RAW264.7 cells in logarithmic phase were digested with trypsin and then terminated with DMEM medium containing 10% fetal bovine serum (FBS). The cells were diluted to 3.5×10^5 cells/mL, inoculated into 96-well plate, 35 000 cells per well, and cultured in a 37 °C incubator with 95% air and 5% CO_2 for 24 h. The compounds were prepared into 25 mmol/L stock solutions, then diluted with the medium and added to the 96-well culture plate to the final concentration of 100, 20, 4 $\mu\text{mol/L}$. After the plate was incubated in the incubator for 1 h, the lipopolysaccharide (LPS) was added into wells until its final concentration was 1 $\mu\text{g/mL}$. After the plate was incubated for another 24 h, the supernatant (100 μL) from each well of 96-well plate was absorbed into the new plate, 50 μL Griess R1 was added to each well, then 50 μL R2 was added and kept away from light at room temperature for 5 min. The absorbance was measured at 540 nm. The inhibition rate of each compound on NO secretion was calculated, and the half-maximal inhibitory concentration (IC_{50}) was calculated when the inhibition rate was more than 50%. Subsequently, 10 μL CCK8 was added to the 96-well plate and the plate was incubated in the incubator for 45 min. The absorbance at 450 nm was then determined and the inhibition rate of the compounds on the growth of RAW264.7 cells was calculated (Zhao & Yang, 2018).

3. Results

Compound **1** was obtained as an amorphous white powder. Its molecular formula was assigned as $\text{C}_{16}\text{H}_{18}\text{O}_7$ based on the observation of the $[\text{M}-\text{H}_2\text{O} + \text{H}]^+$ ion peak at m/z 305.1030 (calcd for $\text{C}_{16}\text{H}_{17}\text{O}_6$, 305.1020) in its HRESI MS spectrum and ^{13}C NMR data (Table 1). The IR spectrum of **1** exhibited the absorption characteristic bands of benzene ring (1621 cm^{-1}) and lactone ring (1765 cm^{-1}). The ^1H NMR spectrum of **1** showed the resonances for two methyls [δ_{H} 1.18 (3H, s, H-4''), 1.08 (3H, H-5'')], one methylene [δ_{H} 3.08 (1H, dd, $J = 16.8, 1.8$ Hz, H-3a), 3.00 (1H, dd, $J = 16.8, 6.5$ Hz, H-3b)], one oxygenated methylene [δ_{H} 4.88 (1H, br.d, $J = 9.2$ Hz, H-1''a), 4.06 (1H, t, $J = 9.2$ Hz, H-1''b)], two oxygenated methines [δ_{H} 3.62 (1H, br.d, $J = 9.2$ Hz, H-2''), 4.38 (1H, dd, $J = 6.5, 1.8$ Hz, H-4)], three aromatic protons [δ_{H} 7.91 (1H, d, $J = 2.3$ Hz, H-2'), 7.17 (1H, d, $J = 2.3$ Hz, H-3'), 6.96 (1H, s, H-8)]. The ^{13}C NMR spectrum of **1** showed sixteen carbon resonances comprising two methyl (δ_{C} 27.7, 24.0), one sp^3 methylene (δ_{C} 31.1), one sp^3 oxygenated methylene (δ_{C} 74.3), two sp^3 oxygenated methine (δ_{C} 50.2, 76.5), one sp^3 oxygenated tertiary (δ_{C} 70.3), two sp^2 methine (δ_{C} 105.6, 93.1), one sp^2 oxygenated methine (δ_{C} 144.7), five sp^2 disubstituted olefinic (δ_{C} 150.1, 112.6, 155.3, 150.1, 105.6), and one carbonyl (δ_{C} 166.7) carbons. The ^{13}C NMR data of **1** are comparable to those of oxypeucedanin hydrate, a known furanocoumarin previously isolated from *A. dahurica* (Thanh, Jin, Song, Bae, & Kang, 2004), except for the significantly upfield carbon chemical shifts of C-2 (δ_{C} 166.7, $\Delta\delta_{\text{C}} + 5.1$), C-3 (δ_{C} 31.1, $\Delta\delta_{\text{C}} - 82.2$), and C-4 (δ_{C} 50.2, $\Delta\delta_{\text{C}} - 89.3$), suggesting that the *cis*-double bond between C-3 and C-4 in oxypeucedanin hydrate was reduced to a single bond in **1**,

Table 1
NMR data of compounds **1a/1b**, **2a/2b**, **3a/3b**, and **4**.

No.	1a/1b ^a		2a/2b ^b		3a/3b ^c		4 ^a	
	δ_{H} (J, Hz)	δ_{C}	δ_{H} (J, Hz)	δ_{C}	δ_{H} (J, Hz)	δ_{C}	δ_{H} (J, Hz)	δ_{C}
2		166.7		169.3		175.1		171.5
3a	3.08, dd (16.8, 1.8)	31.1	3.40, m [#]	32.3	2.81, dd (15.2, 4.2)	38.6	3.20, dd (15.2, 4.8)	34.3
3b	3.00, dd (16.8, 6.5)		3.13, dd (17.2, 6.4)		2.68, dd (15.2, 9.8)		3.08, dd (15.2, 10.4)	
4	4.38, dd (6.5, 1.8)	50.2	4.89, m [#]	52.5	5.18, dd (9.8, 4.2)	55.6	5.06, dd (10.4, 4.8)	53.6
5		150.1		151.8		151.0		151.7
6		112.6		115.5		111.0		111.6
7		155.3		157.8		155.3		154.8
8	6.96, s	93.1	6.97, s	95.2	6.55, s	95.1	6.65, s	95.8
9		150.1		151.7		155.4		155.6
10		105.6		106.2		114.6		112.2
2'	7.91, d (2.3)	144.7	7.73, d (2.0)	145.7	7.65, d (2.1)	142.4	7.72, d (2.0)	143.0
3'	7.17, d (2.3)	105.4	7.18, d (2.0)	106.3	6.93, d (2.1)	105.2	7.01, d (2.0)	105.1
1''a	4.88, br.d (9.2)	74.3	4.79, dd (10.0, 2.6)	74.7	4.51, dd (10.7, 2.0)	74.5	4.49, br.d (9.6)	75.7
1''b	4.06, t (9.2)		4.45, dd (10.0, 7.6)		4.10, dd (10.7, 7.8)		4.01, t (9.6)	
2''	3.62, br.d (9.2)	76.5	3.91, dd (7.5, 2.6)	77.7	3.54, dd (7.8, 2.0)	75.7	3.65, br.d (9.6)	76.8
3''		70.3		72.8		70.8		70.7
4''-CH ₃	1.18, s	27.7	1.34, s	27.1	1.12, s	28.1	1.14, s	27.4
5''-CH ₃	1.08, s	24.0	1.32, s	25.4	1.03, s	24.2	1.04, s	24.5
2-OCH ₃							3.44, s	51.4

Note: ^a: Measured in DMSO *d*₆, 400 MHz. ^b: Measured in CD₃OD, 400 MHz. ^c: Measured in DMSO *d*₆, 700 MHz. [#]: Overlapped with solvent signals.

which was also supported by the observation of one methylene resonated at δ_{H} 3.08 (1H, dd, $J = 16.8, 1.8$ Hz, H-3a), 3.00 (1H, dd, $J = 16.8, 6.4$ Hz, H-3b), and one oxygenated methine at 4.39 (1H, dd, $J = 6.4, 2.0$ Hz, H-4). The above deduction was further confirmed by the ¹H-¹H COSY correlation of H₂-3/H-4 and the HMBC correlations between H₂-3 and C-2/C-4/C-10, H-4 and C-2/C-3/C-5/C-9/C-10 (Fig. 2). Determination of the planar structure of **1** was achieved by unambiguous assignments of all protons and carbons by HSQC, ¹H-¹H COSY, and HMBC experiments. However, the near zero value of the optical rotation of **1** suggested that it might be a racemic mixture. Further separation of **1** by HPLC using a chiral column expectedly afforded a pair of enantiomers **1a** ($[\alpha]_{\text{D}}^{25} + 2$) and **1b** ($[\alpha]_{\text{D}}^{25} - 72$).

Compound **2** was also obtained as an amorphous white powder, with a molecular formula C₁₆H₁₈O₇ determined by the presence of a $[M - \text{H}_2\text{O} + \text{H}]^+$ ion peak at m/z 305.103 0 in the HRESI MS spectrum. The IR spectrum exhibited the absorption bands of benzene ring (1625 cm⁻¹) and lactone ring (1728 cm⁻¹). The NMR data of **2** highly resembled to those of **1** (Table 1), suggesting that **2** shares the same skeleton to **1**. Further analysis the 2D NMR spectra (HSQC, ¹H-¹H COSY, and HMBC) of **2** allowed the unambiguous assignment of the planar structure of **2**, revealing that compound **2** shares the same planar structure to **1**. However, when com-

pounds **1** and **2** were analyzed by HPLC under the same condition, the retention time (R_t) of these two compounds were significantly different, suggesting that **2** was the stereoisomer of **1**. Further separation of **2** using chiral column afforded a pair of enantiomers **2a** ($[\alpha]_{\text{D}}^{25} + 16$) and **2b** ($[\alpha]_{\text{D}}^{25} - 16$).

As we all know, there are totally four possible stereoisomers for a compound containing two chiral centers. To determine the absolute configurations of the four stereoisomers **1a/1b** and **2a/2b**, the ECD spectra of the four possible isomers (4*S*, 2''*R*), (4*R*, 2''*S*), (4*R*, 2''*R*), and (4*S*, 2''*S*) were calculated using time-dependent density functional theory (TDDFT) at the B3LYP/6-31G level with the CPCM model. Based on the calculated ECD spectra (Fig. S1), the presence of positive Cotton effect around 215 nm implies the *R* configuration of C-4. In contrast, the observation of negative Cotton effect around 215 nm was consistent with the *S* configuration of C-4. However, the absolute configuration of C-2'' on the flexible isopentenyl substituent could not be determined by the calculated ECD method. To unambiguously resolve the absolute configurations of **1a/1b** and **2a/2b**, Mosher esters of **1a** were prepared according to our previous report (Yang et al., 2015), and the ¹H NMR data of the (*R*)-MTPA and (*S*)-MTPA esters of **1a** were carefully analyzed to establish the absolute configurations of **1a** as (4*S*, 2''*R*) and its enantiomer **1b** as (4*R*, 2''*S*). The assigned configurations of C-4 of **1a**

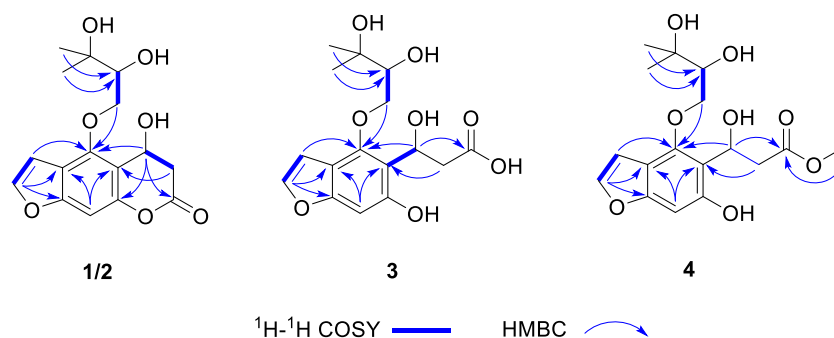


Fig. 2. Key HMBC and ¹H-¹H COSY correlations of new compounds from *A. dahurica*.

and **1b** using calculated ECD and Mosher methods were completely consistent with each other. Accordingly, the absolute configurations of **2a** must be the other two possible isomers (4*R*, 2''*R*) or (4*S*, 2''*S*). In the calculated ECD spectrum, negative Cotton effect around 215 nm was observed, suggesting the *S* configuration of C-4, which led to the assignment of the absolute configurations of **2a** and its enantiomer **2b** as (4*S*, 2''*S*) and (4*R*, 2''*R*), respectively. Therefore, the structures of **1a/1b** and **2a/2b** were unambiguously assigned as shown in Fig. 1, named as (4*S*, 2''*R*)-angelicadin A (**1a**)/(4*R*, 2''*S*)-angelicadin A (**1b**), (4*S*, 2''*S*)-angelicadin A (**2a**)/(4*R*, 2''*R*)-angelicadin A (**2b**), respectively.

Compound **3** was obtained as white powder. Its molecular formula was assigned as C₁₆H₂₀O₈ by the presence of a [M–H₂O + H]⁺ ion peak at *m/z* 341.1236 in the HRESI MS spectrum. The IR spectrum exhibited the absorption bands of benzene ring (1625 cm⁻¹) and carboxyl group (1731 cm⁻¹). The NMR data of **3** was highly similar to that of **1** (Table 1), the significant downfield chemical shift of C-2 (δ_C 175.1, $\Delta\delta_C$ + 9.6) suggested that the six-member lactone ring in compound **1** might be opened to form a free carboxyl in compound **3**. The 18 Da larger of the molecular weight of compound **3** than that of compound **1** also supported the above deduction. The planar structure of compound **3** was further confirmed by comprehensive analysis of its 1D and 2D NMR spectra (Fig. 1, Table 1). However, the optical rotation value of compound **3** was also near zero, which suggested that compound **3** was also a racemic mixture. Further chiral resolution of **3** obtained a pair of enantiomers **3a** ($[\alpha]_D^{25} + 20$) and **3b** ($[\alpha]_D^{25} - 20$). The calculated ECD and experimental CD spectra of **3a** indicated negative Cotton effect around 215 nm (Fig. S2), which suggested the *S* configuration of C-4 in **3a**. Considering the similar optical rotation value of **3a** ($[\alpha]_D^{25} + 20$) to that of **2a** ($[\alpha]_D^{25} + 16$), the absolute configuration of C-2'' of **3a** was presumably determined as *S*. Accordingly, the structure of **3a** was identified as (4*S*, 2''*S*)-secoangelicadin A, and its enantiomer **3b** was identified as (4*R*, 2''*R*)-secoangelicadin A.

Compound **4** was obtained as white powder, with a molecular formula of C₁₇H₂₀O₇ deduced by the presence of a [M–H₂O + H]⁺ ion peak at *m/z* 337.1261 in the HRESI MS spectrum. The IR spectrum exhibited the absorption bands of benzene ring (1624 cm⁻¹) and carboxyl ester group (1735 cm⁻¹). The NMR data of **4** highly resembled to those of **3** except for the additional observation of a methoxyl at δ_H 3.40 (3H, s) and the upfield chemical shifts of C-2 (δ_C 171.5, $\Delta\delta_C$ – 3.6), which suggested that **4** was the methyl ester of **3**. The long range correlation between the methoxyl and C-2 in the HMBC spectrum also supported the above deduction. Further analysis of the 2D NMR spectrum of **4** allowed the establishment of the planar structure of **4** (Fig. 1). The similar calculated ECD and experimental CD spectra (Fig. S3) as well as the optical rotation value of **4** to those of **3b** allowed the identification of **4** as (4*R*, 2''*R*)-secoangelicadin A methyl ester.

By comparing the HRESI MS and NMR data with those reported in literature, the remaining known compound **5** was identified as xanthotoxol (Shinsuke, M. & Mitsuo Miyazawa, 2010). In addition, all compounds were evaluated for their inhibitory effects on the NO production in LPS-activated RAW 264.7 macrophage cells. Indomethacin was used as a positive control [IC₅₀ = (34.28 ± 4.10) μmol/L]. The known xanthotoxol (**5**) inhibited the NO production with an IC₅₀ value of [(32.8 ± 0.8) μmol/L], but compounds **1a/1b**, **2a/2b**, **3a/3b**, and **4** showed no inhibitory activity at the concentration of 100 μmol/L.

4. Discussion and conclusion

In the present study, we have isolated seven new 3,4-dihydrofuranocoumarin derivatives (**1a/1b**, **2a/2b**, **3a/3b**, and **4**) and a known furanocoumarin xanthotoxol (**5**) from the roots of

A. dahurica. 3,4-Dihydrofuranocoumarins are very rare in nature, and this is the first report that the isolation of this kind of compounds from *A. dahurica*. The results are not only meaningful for the understanding of the chemical constituents of *A. dahurica*, but also enrich the reservoir of natural products. Another point needs to be discussed is the possible biosynthetic mechanism of the 3,4-dihydrofuranocoumarin derivatives. Based on their structures, they might be produced from the reduction of the double bonds between C-3 and C-4 and the following oxidation of C-4 to hydroxyl. However, it is generally known that enzymatic reactions in plants are always stereoselective, while the C-4 hydroxyl of the 3,4-dihydrofuranocoumarin derivatives reported here are *R* and *S* configurations coexistence, suggesting the biosynthesis of this kind of products might involve other complex steps or enzymes that need to be clarified.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chmed.2023.02.001>.

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