New Isocourmarin and Phthalide Derivatives from the Rhizomes of *Matteuccia orientalis*

Peng SHAO,[a,b,#] Xue ZHANG,[a,b,#] Bo LI,[a,b] Wei-hua JIAO,[a,b] Li-jun WU,[a,b] and Xin-Sheng YAO*,[a,c]

[a] *College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University;* [b] *Key Laboratory of Structure-Based Drug Design & Discovery of Ministry of Education, Shenyang Pharmaceutical University; Shenyang 110016, P.R. China: and* [c] *Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University; Guangzhou 510632, P.R. China.*

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Five new compounds (1—5), were isolated from the rhizomes of *Matteuccia orientalis* (HOOK.) TREV. The structures of new compounds were elucidated on the basis of their 1D-, 2D-NMR, MS, IR and circular dichroism (CD) data.

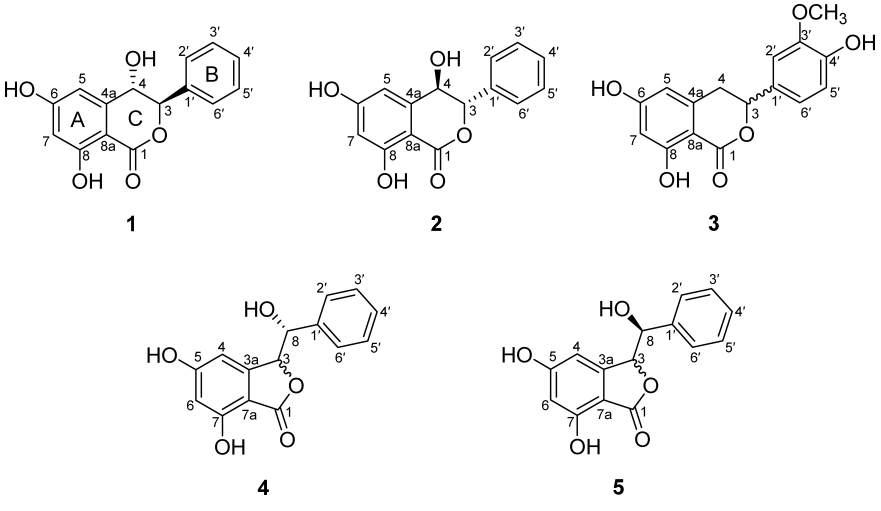
Key words *Matteuccia orientalis*; isocoumarin derivative; phthalide derivative; enantiomer

Matteuccia orientalis (HOOK.) TREV. (Onocleaceae), mainly distributed in Southern China, is a Chinese medicinal herb for the treatment of hemostatics and reliving ostalgia.[1,2] A series of flavanone derivatives[3—6] and bibenzyls[7] have been previously identified from *M. orientalis*. Some of them possessed aldose reductase inhibitory activity.[5—7] Our further investigation on searching for new bioactive constituents from the rhizomes of this plant led to the isolation of three new isocourmarin derivatives, (−)-matteucen A (**1**), (+)-matteucen A (**2**), (±)-matteucen B (**3**) and two new phthalide derivatives, (±)-matteucen C (**4**), (±)-matteucen D (**5**). The two enantiomers (**1**, **2**) were separated from the new racemic (±)-matteucen A by chiral HPLC. Herein, we report the isolation and structural elucidation of these five compounds.

Results and Discussion

The 60% (v/v) EtOH extract of dried rhizomes of *M. orientalis* was subjected to column chromatography over Diaion HP20 macroporous adsorptive resins, silica gel, Sephadex LH-20, Octadecylsilane (ODS), and further purified by HPLC to afford four new racemic compounds, (±)-matteucen A—D. Futhermore, the two enantiomers (−)-matteucen A (**1**) and (+)-matteucen A (**2**) were separated from the new racemic (±)-matteucen A by chiral HPLC. Structural elucidations of the five compounds were achieved by 1D-, 2D-NMR, MS, IR and circular dichroism (CD).

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D^{25}$ −38.2 (c=0.5, MeOH). Its molecular formula was determined as $C_{15}H_{12}O_5$ by high resolution-electrospray ionization mass spectrum (HR-ESI-MS) analysis, which showed a quasi-molecular ion peak at m/z 295.0572 $[M+Na]^+$. The UV spectrum was suggestive of a 6,8-dihydroxylated dihydroisocoumarin derivative with absorption maxima at 211, 271 and 304 nm.[8] Its IR spectrum exhibited characteristic absorption bands for hydroxyl (3430 cm^{-1}) and carbonyl (1669 cm^{-1}) groups. The ^{1}H-NMR spectrum in DMSO-d_6 (Table 1) displayed a set of five protons (δ 7.40—7.50, 5H, m) of a monosubstituted benzene ring (B-ring), two *meta*-coupled aromatic protons [δ 6.53 (1H, d, J=2.0 Hz, H-5) and δ 6.26 (1H, d, J=2.0 Hz, H-7)], together with three hydroxy protons [δ 6.00 (1H, d, 4-OH), δ 10.77 (1H, s, 6-OH) and δ 11.00 (1H, s, 8-OH)]. The correlation spectroscopy (COSY) spectrum showed correlations between δ 4.90 (H-4) and δ 5.36 (H-3), δ 6.00 (4-OH), indicating the presence of HO–CH–CH fragment. In the heteronuclear multiple bond correlation (HMBC) spectrum, correlations were observed at H-4/C-4a, C-5, C-8a, and H-5/C-4, revealing the attachment between C-4 and C-4a. Similarly, the linkage site of carbonyl



* To whom correspondence should be addressed. e-mail: yaoxinsheng@vip.tom.com
# These authors contributed equally to this work.

group (C-1) to the A-ring was established at C-8a by the upfield chemical shift of C-8a (δ 98.7). The presence of a lactone ring was implied by ^{13}C–^{1}H long range correlation from H-3 to C-1. Additionally, the connectivity of the B-ring to the benzopyranone unit was determined by the correlations between H-3 with C-1′ (δ 136.9), C-2′, C-6′ (each δ 127.7) in the HMBC spectrum (Fig. 1). The relative configuration was determined by comparison of the coupling constant ($J_{H\text{-}3/H\text{-}4}$=9.3 Hz, *anti*) with those reported in the literature.[9] The CD spectrum of **1** showed positive Cotton effects at 217 and 253 nm and a negative Cotton effect at 233 nm (Chart 1), suggesting the absolute configuration at C-3 was *R*.[10—13] Based on the foregoing evidence, the structure of **1** was elucidated as (3*R*,4*S*)-3,4-dihydro-4,6,8-trihydroxy-3-phenylisochromen-1-one, named (−)-matteucen A.

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D^{25}$ 37.9 (*c*=0.5, MeOH). It was assigned the same molecular $C_{15}H_{12}O_5$ as **1** by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR data of **2** (Table 1) were consistent with those of **1** com-

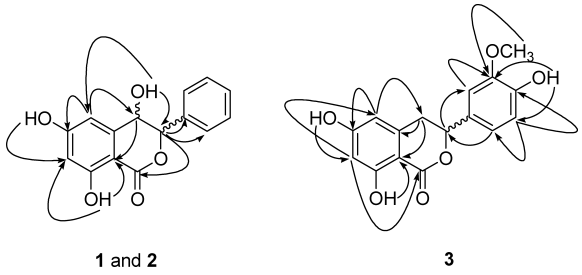


Fig. 1. Key HMBC (→) Correlations of **1**—**3**

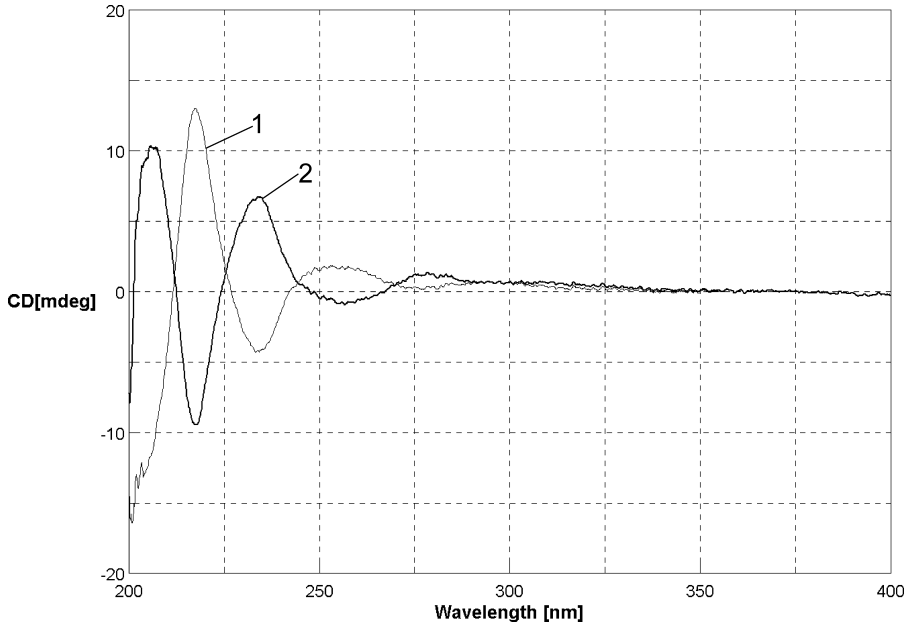


Chart 1. CD Spectra of **1** and **2**

Table 1. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) Data for Compounds **1**—**3** in DMSO-d_6

No.	**1** and **2**		No.	**3**	
	δ_C	δ_H (*J* in Hz)		δ_C	δ_H (*J* in Hz)
1	168.2		1	169.5	
3	83.7	5.36 (1H, d, 9.3 Hz)	3	80.1	5.54 (1H, dd, 12.3, 2.7 Hz)
4	67.1	4.90 (1H, pseudo t, 9.3, 8.0 Hz)	4	33.8	3.03 (1H, dd, 16.5, 2.7 Hz)
					3.32 (1H, dd, 16.5, 12.3 Hz)
4a	146.2		4a	142.5	
5	104.9	6.53 (1H, d, 2.0 Hz)	5	106.9	6.31 (1H, d, 1.8 Hz)
6	165.1		6	164.7	
7	101.3	6.26 (1H, d, 2.0 Hz)	7	101.0	6.24 (1H, d, 1.8 Hz)
8	163.1		8	163.4	
8a	98.7		8a	100.3	
1′	136.9		1′	129.2	
2′	127.7	7.40—7.50 (1H, m)	2′	111.0	7.10 (1H, d, 1.5 Hz)
3′	128.3	7.40—7.50 (1H, m)	3′	147.6	
4′	128.6	7.40—7.50 (1H, m)	4′	147.0	
5′	128.3	7.40—7.50 (1H, m)	5′	115.2	6.80 (1H, d, 8.1 Hz)
6′	127.7	7.40—7.50 (1H, m)	6′	119.6	6.90 (1H, dd, 8.1, 1.5 Hz)
4-OH		6.00 (1H, d, 8.0 Hz)	3′-OMe	55.7	3.79 (3H, s)
6-OH		10.77 (1H, s)	4′-OH		9.21 (1H, s)
8-OH		11.00 (1H, s)	6-OH		10.78 (1H, s)
			8-OH		11.11 (1H, s)

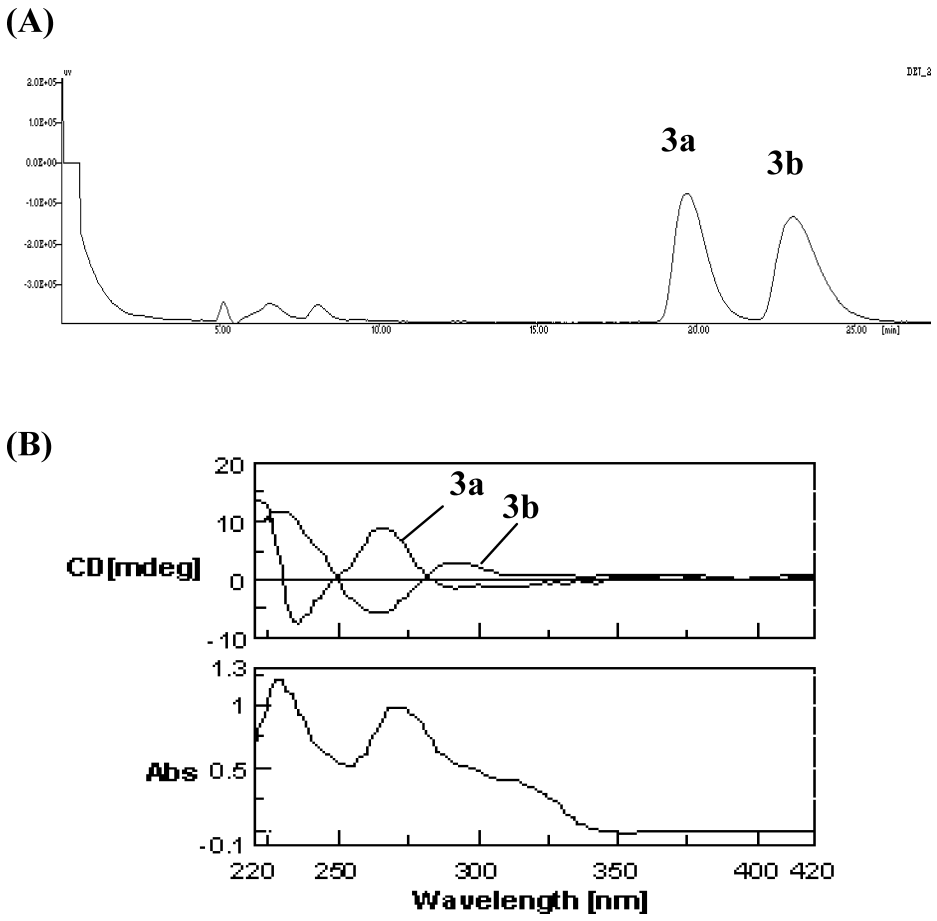


Chart 2. Chromatograms of the Separation and Full on-Line HPLC-CD Spectra of **3**

(A) HPLC chromatogram of **3**. Column: Chiral CD-Ph S5 column (5 μm, 4.6×250 mm; Shiseido); mobile phase: MeOH–H_2O (70 : 30, v/v, 0.5‰ TFA); flow rate: 0.6 ml/min; UV wavelength: 220 nm. (B) CD (upper) and UV (lower) spectra of the stereoisomers of **3**. Mobile phase: MeOH–H_2O (70 : 30, v/v, 0.5‰ TFA); flow rate: 0.6 ml/min; UV wavelength: 220—420 nm.

Table 2. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) Data for Compounds **4** and **5** in DMSO-d_6

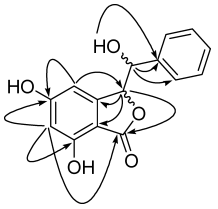
No.	**4**		**5**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	167.7		167.7	
3	81.7	5.44(1H, d, 4.0 Hz)	81.7	5.45(1H, d, 4.8 Hz)
3a	151.4		151.2	
4	101.2	6.23 (1H, d, 1.8 Hz)	101.6	6.00 (1H, d, 1.8 Hz)
5	163.9		163.8	
6	102.3	6.26 (1H, d, 1.8 Hz)	102.5	6.27 (1H, d, 1.8 Hz)
7	157.6		157.7	
7a	104.3		104.0	
8	72.7	4.94 (1H, pseudo t, 4.8, 4.0 Hz)	73.1	4.86 (1H, pseudo t, 4.8 Hz)
1′	140.7		140.4	
2′	126.9	7.34 (1H, d, 7.2 Hz)	126.9	7.35 (1H, d, 7.2 Hz)
3′	127.5	7.30 (1H, t, 7.2 Hz)	127.7	7.32 (1H, t, 7.2 Hz)
4′	127.2	7.24 (1H, t, 7.2 Hz)	127.3	7.27 (1H, t, 7.2 Hz)
5′	127.5	7.30 (1H, t, 7.2 Hz)	127.7	7.32 (1H, t, 7.2 Hz)
6′	126.9	7.34 (1H, d, 7.2 Hz)	126.9	7.35 (1H, d, 7.2 Hz)
5-OH		10.30 (1H, s)		10.31 (1H, s)
7-OH		10.30 (1H, s)		10.31 (1H, s)
8-OH		5.71 (1H, d, 4.8 Hz)		5.90 (1H, d, 4.8 Hz)

pletely, suggesting that **2** was the enantiomer of **1**. The configuration at C-3 was estimated as *S* from the CD spectrum, which exhibited negative Cotton effects at 217 and 253 nm and a positive Cotton effect at 234 nm (Chart 1).[13] Therefore, the structure of **2** was identified as (3*S*,4*R*)-3,4-dihydro-4,6,8-trihydroxy-3-phenylisochromen-1-one, named (+)-matteucen A.

Compound **3** was isolated as a brown amorphous powder,

$[\alpha]_D^{25}$ −0.5 (c=0.5, MeOH). HR-ESI-MS (m/z 325.0682, $[M+Na]^+$) gave the molecular formula $C_{16}H_{14}O_6$. Compound **3** showed typical dihydroisocoumarin derivatives UV absorptions similar to those of **1**. The ^{1}H-NMR spectrum of **3** (Table 1) exhibited an ABX system at δ 7.10 (1H, d, J=1.5 Hz, H-2′), δ 6.90 (1H, dd, J=1.5, 8.1 Hz, H-6′) and δ 6.80 (1H, d, J=8.1 Hz, H-5′), two *meta*-coupled aromatic protons at δ 6.31 (1H, d, J=1.8 Hz, H-5) and δ 6.24 (1H, d, J=1.8 Hz, H-7), two methylene protons at δ 3.03 (1H, dd, J=2.7, 16.5 Hz, H-4a) and δ 3.32 (1H, dd, J=12.3, 16.5 Hz, H-4b), one methine proton at δ 5.54 (1H, dd, J=2.7, 12.3 Hz, H-3), an *O*-methyl group at δ 3.79 (3H, s, 3′-OMe), as well as three phenolic hydroxy protons at δ 9.21 (1H, s, 4′-OH), δ 10.78 (1H, s, 6-OH) and δ 11.11 (1H, s, 8-OH). The ^{13}C–^{1}H long range correlations were observed at H-5/C-4 and H-7/C-1 in the HMBC spectrum, which also supported the existence of the dihydroisocoumarin skeleton of **3**. The attachment site of the B-ring to δ-lactone was determined on the basis of HMBC correlations between H-3 and C-2′, C-6′. Furthermore, one hydroxy and one *O*-methyl groups were assigned to the B-ring according to the ^{13}C–^{1}H long range correlations founded at δ 9.21 (4′-OH)/C-3′, C-4′, C-5′, as well as δ 3.79 (3′-OMe)/C-3′ in the HMBC spectrum (Fig. 1). Compound **3** was optically inactive, and the CD spectrum showed no significant Cotton effect, demonstrating that it was likely racemic. Furthermore, compound **3** was characterized as a pair of enantiomers by on-line chiral high-performance liquid chromatography-circular dichroism (HPLC-CD) analysis.[14] The two enantiomers (**3a**, **3b**) corresponding to the two peaks in a ratio of 49 : 51 by the Chiral CD-Ph S5 column analysis were separated (Chart 2A). Full CD spectra were taken directly on-line for these two peaks, giving a couple of mirror imaged CD curves (Chart 2B). For the pair of enantiomers (**3a**, **3b**) could be converted to each other in the aquenous solution after separation, we could not obtain the enantiomers respectively. Hence, the structure of **3** was elucidated as (3*RS*)-3,4-dihydro-6,8-dihydroxy-3-(4′-hydroxy-3′-methoxyphenyl) isochromen-1-one, named (±)-matteucen B.

Compound **4** was obtained as a white amorphous powder, $[\alpha]_D^{25}$ 0.5 (c= 0.5, $CHCl_3$). Its HR-ESI-MS showed a quasi-molecular ion peak at m/z 273.0763 $[M+H]^+$, in agreement with the molecular formula $C_{15}H_{12}O_5$. The ^{1}H-NMR spectrum (Table 2) displayed resonances for five aromatic protons at δ 7.34 (2H, d, J=7.2 Hz, H-2′, 6′), δ 7.30 (2H, t, J=7.2 Hz, H-3′, 5′) and δ 7.24 (1H, t, J=7.2 Hz, H-4′), appearing as a monosubstituted benzene ring, and two phenolic hydroxy protons at δ 10.30 (2H, s, 5-OH, 7-OH). The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **4** showed the presence of five aromatic quaternary carbons (two oxygenated), seven aromatic methine, two oxygenated methine, and one carbonyl carbon (δ 167.7, C-1) characteristic of lactone.[12] Analysis of the COSY spectrum led to the HO–CH–CH structural fragment in the structure by the correlations between δ 4.94 (H-8) and δ 5.44 (H-3), δ 5.71 (8-OH). In the HMBC spectrum, the ^{13}C–^{1}H long range correlations were observed at H-3/C-1, C-3a, C-7a, H-4/C-3 and H-6/C-1 respectively, which resulted in the presence of benzofuranone skeleton. Additionally, the monosubstituted benzene ring located on the C-8 was derived from the HMBC correlations between H-8 and C-1′, C-2′, C-6′ (Fig. 2). The relative configurations at C-3 and C-8 were deduced from the NMR data in comparison to those reported.[15,16] The optical purity of **4** was determined by HPLC analysis of the corresponding (*R*)-α-methoxy-α-(trifluoromethyl) phenlacetate [(*R*)-MTPA ester, **6**], which was prepared by methylation with trimethylsilyl–diazomethane (TMS–CH_2N_2), followed by esterification with (*R*)-α-methoxy-α-(trifluoromethyl) phenlacetyl chloride [(*R*)-MTPA chloride] in the presence of pyridine.[17] The HPLC chromatogram of **6** showed two peaks in a ratio of 40 : 60, demonstrating that **4** was a mixture of two enantiomers.[16] On the basis of the above evidence, the structure of **4** was determined as (3*RS*,8*SR*)-5,7-dihydroxy-3-(hydroxy(phenyl)methyl)-isobenzofuran-1(3*H*)-one, which was named as (±)-matteucen C.



4 and **5**

Fig. 2. Key HMBC (→) Correlations of **4** and **5**

Compound **5** was a white amorphous powder. It had a molecular formula $C_{15}H_{12}O_5$ as determined by HR-ESI-MS. The ^{1}H- and ^{13}C-NMR data of **5** (Table 2) were fairly similar to those of **4**, with the exception of the coupling constant between C-3 and C-8. Thus, compound **5** was assumed to be an epimer of **4**.[15,16] The optical rotation for **5** was −1°, as well as the CD spectrum showed no Cotton effect, indicating that **5** was also likely racemic. With the same method as **4**, the optical purity of **5** was determined by HPLC analysis of the corresponding (*R*)-MTPA ester (**7**), which was also showed two peaks in a ratio of 57 : 43 in the HPLC chromatogram.[16,17] From the above evidence, the structure of **5** was determined as (3*RS*,8*RS*)-5,7-dihydroxy-3-(hydroxy-(phenyl)methyl)isobenzofuran-1(3*H*)-one, and named (±)-matteucen D.

Experimental

General Optical rotations were measured with a JASCO P-1020 digital polarimeter (l=1 cm). UV spectra were measured on a JASCO V-550 UV/VIS spectrometer. IR spectra (KBr) were obtained with a Bruker IFS-55 spectrometer. 1D- and 2D-NMR spectra were acquired in DMSO-d_6 on Bruker Avance-300 and Bruker Avance-600 NMR spectrometers with TMS as internal standard. ESI-MS data were recorded on a Bruker Esquire 2000 mass spectrometer. HR-ESI-time of flight (TOF)-MS spectra were acquired using an Agilent 6210 LC/MSD TOF mass spectrometers. CD spectra were measured with a JASCO J810-150S spectrometer. The HPLC-CD coupling system consisted of a JASCO pump PU 2080, a CD 2095 detector and Borwin chromatographic software (JASCO, Tokyo, Japan). The analytical HPLC were performed on an Agilent 1200 equipped with DAD detector using a reversed-phase C18 column (5 μm, 4.60×250 mm; Phenomenex Gemini). Preparative HPLC was carried out on a Shimazu LC-6AD with UV SPD-20A detector using a reversed-phase C18 column (10 μm, 20×250 mm; YMC-Pack ODS-A), and a Chiral CD-Ph S5 column (5 μm, 4.6×250 mm; Shiseido), respectively. Column chromatography (CC) was performed using silica gel (200—300 mesh, Qingdao), macroporous adsorptive resins (Diaion HP20, Mitsubishi Chemical), Sephadex LH-20 (Amersham Biosciences) and ODS (60—80 μm, YMC).

Plant Material The rhizomes of *M. orientalis* were collected from Jiangxi province, China, in September 2007, and authenticated by Research Professor Ceming Tan, at Jiujiang Forest Herbarium, Jiangxi, China. A

voucher specimen was deposited at College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China.

Extraction and Isolation The dry rhizomes (10 kg) of *M. orientalis* were refluxed with 60% EtOH (v/v) three times (2 h each). The combined extracts were concentrated in vacuo to afford a yellow residue (1.3 kg), which was dissolved in H_2O and subjected to column chromatography over Diaion HP20 macroporous adsorptive resins eluted with EtOH/H_2O gradiently. The 50% EtOH (v/v) eluate (100 g) was subjected to column chromatography on silica gel (200—300 mesh, 700 g) eluted with $CHCl_3$/MeOH (100 : 0→0 : 100) to afford 9 fractions. Fraction 2 (3.0 g) was passed over a Sephadex LH-20 column with $CHCl_3$/MeOH (1 : 1) as eluent and then applied to ODS MPLC eluted with MeOH/H_2O (30 : 70→60 : 40). Subfraction 2-6 (MeOH–H_2O, 50 : 50, eluant) was further purified by preparative HPLC (MeOH–H_2O, 45 : 55) to afford compound **3** (21.7 mg). Fraction 3 (3.2 g) was chromatographed over a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1) and ODS MPLC (MeOH–H_2O, 20 : 80→60 : 40). Subfraction 3-2 was purified by preparative HPLC (MeOH–H_2O, 50 : 50) to yield the racemic mixture (11.7 mg), which was identified by CD, MS and NMR spectra. Compounds **1** (5 mg) and **2** (4.7 mg) were obtained from the racemic mixture by preparative HPLC (MeOH–H_2O, 40 : 60; flow rate, 0.6 ml/min; detection, UV at 220 nm) with a Chiral CD-Ph S5 column. Fraction 4 (2.0 g) was passed on a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1). Subfrcation 4-2 was further separated by column chromatography over ODS eluted with MeOH/H_2O gradiently. Compounds **4** (10 mg) and **5** (12 mg) was obtained from the successive purification over Sephadex LH-20 and preparative HPLC (MeOH–H_2O, 35 : 65) from subfraction 4-2-3 (MeOH–H_2O, 20 : 80, eluant).

Preparation of MTPA Esters of 4 and 5 A mixture of **4** (2 mg, 0.74×10^{-5} mol) and TMS–CH_2N_2 (2 M solution in *n*-hexane, 5 ml) (0.5 ml) in MeOH (0.5 ml) was stirred at room temperature (25 °C) overnight and the solvent was evaporated under reduced pressure to furnish the dimethyl ether (2.2 mg). A solution of dimethyl ether in pyridine (0.5 ml) was added (*R*)-MTPA chloride (6×10^{-5} mol), and the whole mixture was stirred at room temperature (25 °C) for 12 h. Evaporation of the solvent from the reaction mixture under reduced pressure afford a residue. The resulting residue was purified by preparative TLC [$CHCl_3$–EtOAc (85 : 15)] to give a mixture of **6a** and **6b** (1.1 mg). The MTPA ester (a mixture of **7a** and **7b**) was also prepared from **5** (2 mg) by the same procedure as described above.

(−)-Matteucen A (**1**): White powder. $[\alpha]_D^{25}$ −38.2 (c=0.5, MeOH). UV λ_{max} (MeOH) nm (log ε): 211 (4.19), 271 (3.75), 304 (3.54). CD ($c=1\times10^{-3}$, MeOH) $\Delta\varepsilon$ (nm): +10.74 (217), −3.47 (233), +1.53 (253). IR (KBr) cm^{-1}: 3430, 1669, 1629, 1163, 800, 696. ESI-MS *m*/*z*: 271 $[M-H]^-$, 543 $[2M-H]^-$. HR-ESI-MS *m*/*z*: 295.0572 $[M+Na]^+$ (Cacld for $C_{15}H_{12}O_5Na$: 295.0577). ^{1}H- and ^{13}C-NMR data, see Table 1.

(+)-Matteucen A (**2**): White powder. $[\alpha]_D^{25}$ 37.9 (c=0.5, MeOH). UV λ_{max} (MeOH) nm (log ε): 211 (4.32), 271 (3.89), 305 (3.71). CD ($c=1\times10^{-3}$, MeOH) $\Delta\varepsilon$ (nm): −7.78 (217), +5.56 (234), −0.78 (253). IR (KBr) cm^{-1}: 3430, 1668, 1634, 1164, 800, 697. ESI-MS *m*/*z*: 271 $[M-H]^-$, 543 $[2M-H]^-$. HR-ESI-MS *m*/*z*: 295.0574 $[M+Na]^+$ (Cacld for $C_{15}H_{12}O_5Na$: 295.0577). ^{1}H- and ^{13}C-NMR data, see Table 1.

(±)-Matteucen B (**3**): Brown powder. $[\alpha]_D^{25}$ −0.5 (c=0.5, MeOH). UV λ_{max} (MeOH) nm (log ε): 212 (4.16), 270 (3.77), 300 (3.48). IR (KBr) cm^{-1}: 3423, 3023, 1669, 1628, 1163, 798, 722. ESI-MS *m*/*z*: 325 $[M+Na]^+$, 303 $[M+H]^+$, 301 $[M-H]^-$. HR-ESI-MS *m*/*z*: 325.0682 $[M+Na]^+$ (Cacld for $C_{16}H_{14}O_6Na$: 325.0683). ^{1}H- and ^{13}C-NMR data, see Table 1.

(±)-Matteucen C (**4**): White powder. $[\alpha]_D^{25}$ 0.5 (c=0.5, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 218 (4.39), 258 (3.98), 293 (3.52). IR (KBr) cm^{-1} 3364, 1724, 1681, 1616, 1169, 710, 691 . ESI-MS *m*/*z*: 271 $[M-H]^-$, 273 $[M+H]^-$. HR-ESI-MS *m*/*z*: 273.0763 $[M+H]^+$ (Cacld for $C_{15}H_{13}O_5$: 273.0757). ^{1}H- and ^{13}C-NMR, see Table 2.

(±)-Matteucen D (**5**): White powder. $[\alpha]_D^{25}$ −1 (c=0.5, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 222 (4.18), 257 (3.98), 292 (3.68). IR (KBr) cm^{-1} 3367, 1724, 1681, 1617, 1170, 710, 691. ESI-MS *m*/*z*: 271 $[M-H]^-$, 543 $[2M-H]^-$. HR-ESI-MS *m*/*z*: 295.0572 $[M+Na]^+$ (Cacld for $C_{15}H_{12}O_5Na$: 295.0577). ^{1}H- and ^{13}C-NMR, see Table 2.

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References

1) Flora of China Editorial Committee, "Flora Reipublicate Popularis Sinicae," Vol. 4, Science Press, Beijing, 2006, pp. 159—164.
2) Jiangsu Institute of Botany, "Xinhua Herbal Schem," Shanghai Scientific and Technologic Press, Shanghai, 1990, pp. 684—685.
3) Mohri K., Takemoto T., Kondo Y., *Yakugaku Zasshi*, **102**, 310—312 (1982).
4) Jiang J. Q., Zhou R. H., Meng Z. M., Li N., *Zhongguo Yaoke Daxue Xuebao*, **25**, 199—201 (1994).
5) Basnet P., Kadota S., Shimizu M., Xu H.-X., Namba T., *Chem. Pharm. Bull.*, **41**, 1790—1795 (1993).
6) Kadota S., Basnet P., Hase K., Namba T., *Chem. Pharm. Bull.*, **42**, 1712—1714 (1994).
7) Basnet P., Kadota S., Hase K., Namba T., *Chem. Pharm. Bull.*, **43**, 1558—1564 (1995).
8) Yoshikawa M., Uchida E., Chatani N., Kobayashi H., Naitoh Y., Okuno Y., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **40**, 3352—3354 (1992).
9) Stephen J. H., Andrew P., Rodolfo M., *Org. Lett.*, **10**, 2813—2816 (2008).
10) Hashimoto T., Tori M., Asakawa W., *Phytochemistry*, **26**, 3323—3330 (1987).
11) Krohn K., Bahramsari, R., Flörke U., Ludewig K., Kliche-Spory C., Michel A., Aust H. J., Draeger S., Schulz B., Antus S., *Phytochemistry*, **45**, 313—320 (1997).
12) Paraschos S., Magiatis P., Kalpoutzakis E., Harvala C., Skaltsounis A. L., *J. Nat. Prod.*, **64**, 1585—1587 (2001).
13) Hosoi S., Kamiya M., Ohta T., *Org. Lett.*, **3**, 3659—3662 (2001).
14) Ren D. M., Guo H. F., Wang S. Q, Lou H. X., *J. Chromatogr. A*, **1161**, 334—337 (2007)
15) Sarı A., Zidorn C., Ellmerer E. P., Özgökçe F., Ongania K.-H., Stuppner H., *Helv. Chim. Acta*, **90**, 311—317 (2007).
16) Yoshikawa M., Shimada H., Yagi, N., Murakami N., Shimoda H., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **44**, 1890—1898 (1996).
17) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc.*, **113**, 4092—4096 (1991).