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Determination of the Absolute Configuration of Khellactone Esters from *Peucedanum japonicum* Roots

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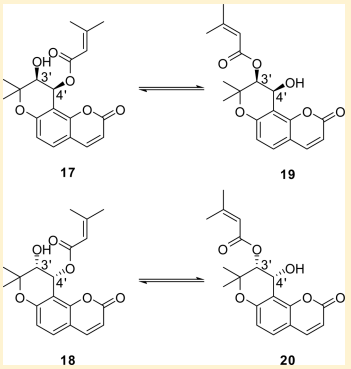
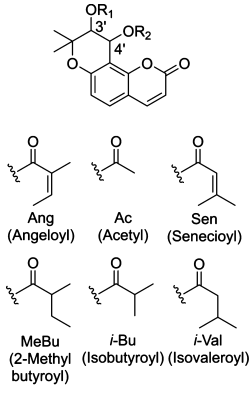
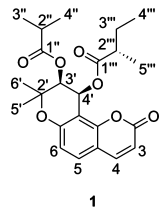
Supporting Information

ABSTRACT: Sixteen new angular dihydropyranocoumarins (**1**−**16**) and 24 known compounds were isolated from the roots of *Peucedanum japonicum* Thunb. The absolute configuration of diacylkhellactone was established by partial hydrolysis, the Mosher method, and X-ray crystallography. In addition, ECD spectroscopy was used to assign the absolute configurations of several of the angular dihydropyranocoumarins. Enantiomers were detected by RP-HPLC using MTPA esters while acyl migration of the substituents was observed in *cis*-monoacylkhellactones.



Peucedanum japonicum Thunberg, a member of the Umbelliferae family, is widely distributed in southern and eastern Asia. Its roots have been used as a folk medicine for cold and neuralgic diseases in Korea and Taiwan.[1,2] Phytochemical studies have revealed that the roots of this plant contain coumarins,[3−7] chromones, polyacetylenes,[8] inositols,[9] and steroid glycosides.[10] A literature survey showed that coumarins,[11,12] flavonoids, phenylpropanoid glycosides,[13] phenol derivatives, C_{13} norisoprenoid glycosides, nucleosides, nucleobases, amino acids, and benzofuran glycosides have been isolated from the leaves of *P. japonicum*. In this study, 16 new angular dihydropyranocoumarins (**1**−**16**) and 24 known angular dihydropyranocoumarins (**17**−**40**) were isolated from the *n*-hexane and $CHCl_3$ fractions of the MeOH extract of the roots of *P. japonicum*. In the case of the diacylkhellactones, partial hydrolysis combined with MTPA derivatization and X-ray crystallography were applied to determine the configurations at C-3′ and C-4′. In addition, ECD spectroscopy combined with TDDFT calculations was used to define the absolute configurations. Compounds **2**, **7**, **9**, **12**, and **14** were assigned (3′*S*,4′*S*) absolute configurations, while the enantiomers of these compounds have been previously reported.[14−17]





	R_1	R_2	3′	4′
2	Ac	Sen	*S*	*S*
3	MeBu	*i*-Bu	*S*	*S*
4	MeBu	Sen	*S*	*S*
5	Sen	MeBu	*S*	*S*
6	*i*-Bu	*i*-Val	*S*	*S*
7	MeBu	Ang	*S*	*S*
8	Bu	MeBu	*S*	*S*
9	*i*-Val	Ang	*S*	*S*
10	Ac	3-hydroxy-*i*-Val	*S*	*S*
11	Ac	3-hydroxy-MeBu	*S*	*S*
12	Ac	MeBu	*S*	*S*
13	MeBu	H	*S*	*S*
14	H	MeBu	*S*	*S*
15	MeBu	Me	*S*	*S*
16	H	Sen	*S*	*R*
17	H	Sen	*S*	*S*
18	H	Sen	*R*	*R*
19	Sen	H	*S*	*S*
20	Sen	H	*R*	*R*

RESULTS AND DISCUSSION

The air-dried roots of *P. japonicum* (30.0 kg) were extracted with 100% MeOH under ultrasonication. After removing the solvent under reduced pressure, the MeOH extract was partitioned using *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-hexane and $CHCl_3$ fractions were subjected to repeated chromatography to afford 16 new angular dihydropyranocoumarins (**1**−**16**), including five new enantiomers 3′-*O*-acetyl-4′-*O*-senecioylkhellactone (isosamidin) (**2**),[14] 4′-*O*-angeloyl-3′-*O*-(2-methylbutyroyl)khellactone (**7**),[15] 3′-*O*-isovaleroyl-4′-*O*-angeloylkhellactone (**9**),[16] 3′-*O*-acetyl-4′-*O*-(2-methylbutyroyl)-khellactone (hyuganin C) (**12**),[17] and 4′-*O*-(2-methylbutyroyl)khellactone (**14**),[14] and 24 known compounds.

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The known compounds were identified as (3′*S*,4′*S*)-peujaponisinol B (**17**),[5] 4′-*O*-senecioylkhellactone [(3′*R*,4′*R*)-peujaponisinol B] (**18**),[18,19] (3′*S*,4′*S*)-peujaponisinol A (**19**),[5] 3′-*O*-senecioylkhellactone [(3′*R*,4′*R*)-peujaponisinol A] (**20**),[18,19] 4′-*O*-angeloyl-3′-*O*-senecioylkhellactone (**21**),[20] 3′,4′-di-*O*-senecioylkhellactone (**22**),[21] peujaponisin (**23**),[6] qianhucoumarin B (**24**),[22,23] 3′-*O*-acetyl-4′-*O*-angeloylkhellactone (pteryxin) (**25**),[24] hyuganin D (**26**),[20] corymbocoumarin (**27**),[25] (−)-*cis*-khellactone (**28**),[20] qianhucoumarin C (**29**),[23] 3′-hydroxy-4′-angeloyloxy-3′,4′-dihydroseselin (**30**),[25] praeruptorin F (**31**),[26] praeruptorin B (**32**),[27] 3′-angeloyloxy-4′-hydroxy-3′,4′-dihydroseselin (**33**),[25] 3′-*O*-angeloyl-4′-*O*-senecioylkhellactone (calipteryxin) (**34**),[27] 3′-*O*-isovaleroyl-4′-*O*-senecioylkhellactone (**35**),[21] 3′,4′-di-*O*-isovaleroylkhellactone (**36**),[27] praeruptorin H (**37**),[26] *O*-senecioyllomatin (**38**),[28,29] *O*-isovaleroyllomatin (**39**),[28,29] and qianhucoumarin D (**40**)[30] (Figure S1, Supporting Information).

Absolute Configuration Determination of the Diacylkhellactones by Partial Hydrolysis Combined with the Mosher Method and X-ray Crystallography. Compound **1** was isolated as colorless needles. The molecular formula was determined as $C_{23}H_{28}O_7$ based on the *m*/*z* 417.1912 ion $[M + H]^+$ in HRCIMS. In the ^{1}H NMR spectrum, **1** showed signals attributable to an α-pyrone moiety at δ_H 6.20 (1H, d, J = 9.5 Hz, H-3) and 7.57 (1H, d, J = 9.5 Hz, H-4), and an *o*-disubstituted benzene moiety at δ_H 7.33 (1H, d, J = 8.6 Hz, H-5) and 6.78 (1H, d, J = 8.6 Hz, H-6), typical patterns of a disubstituted coumarin. A pair of methine doublets [δ_H 5.30 (1H, d, J = 4.8 Hz, H-3′) and 6.53 (1H, d, J = 4.8 Hz, H-4′)] and geminal dimethyls [δ_H 1.38 and 1.43 (each 3H, s, H_3-5′, H_3-6′)] revealed the presence of an angular dihydropyran unit (Table 1). The signals at δ_H 2.55 (1H, sep, J = 7.0 Hz, H-2″), 1.18, and 1.17 (each 3H, d, J = 7.0 Hz, H_3-3″, H_3-4″) were due to an isobutyroyl group with 2-methylbutyroyl signals resonating at δ_H 2.37 (1H, sext, J = 7.0 Hz, H-2‴), 1.71, 1.44 (each 1H, m, H-3‴), 0.91 (3H, t, J = 7.4 Hz, H_3-4‴), and 1.19 (3H, d, J = 7.0 Hz, H_3-5‴). The isobutyroyl (C-1″) and 2-methylbutyroyl (C-1‴) groups were correlated to H-3′ (δ_H 5.30) and H-4′ (δ_H 6.53), respectively, as indicated by the MS fragmentation (base peak, M^+ − OMeBu) and confirmed by HMBC data. The relative configurations of the C-3′ and C-4′ stereogenic carbons were considered to be *cis* based on the coupling constant (4.8 Hz) and NOESY correlation between H-3′ and H-4′.[27,31]

The 2D structure of the angular dihydropyranocoumarin moiety was readily defined by 1D and 2D NMR data. The Mosher's method was selected to define the absolute configurations at C-3′ and C-4′. In the case of the diacyldihydropyranocoumarins, partial alkaline hydrolysis was required to release a hydroxy group prior to the MTPA (α-methoxy-α-trifluoromethylphenylacetyl) esterification.

Upon alkaline hydrolysis, **1** gave a mixture of three products (**1a**−**c**), which were isolated by HPLC (Figure 1). Compounds **1a** and **1b** showed epimerization at C-4′ as previously reported.[27] The partially hydrolyzed product (**1a**) was found to be *trans*-configured based on the $J_{3',4'}$ value of 3.9 Hz (Table S1, Supporting Information).[27,31] The fully hydrolyzed products were identified as (+)-*trans*-khellactone (**1b**) and (−)-*cis*-khellactone (**1c**) by NMR data analysis and specific rotations (Table S1, Supporting Information).[5] The absolute configuration of **1a** was defined using Mosher's reagents to yield the diastereomeric (*S*)- and (*R*)-MTPA esters (**1aa** and **1ab**). In the (*S*)-MTPA ester of **1a**, the protons in the C-3′, C-4′, C-5′, C-6′, and C-6 portion were shielded, and those in the C-3 and C-4 portion were deshielded (vice versa in the (*R*)-MTPA ester) (Figure 2). Thus, the absolute configuration of compound **1** was assigned as (3′*S*,4′*S*). The (3′*S*,4′*S*) absolute

Table 1. ^{1}H and ^{13}C Spectroscopic Data of Compounds 1−2 and 16[a]

	1		2		16	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
2	159.7		159.8		160.1	
3	113.3	6.20, d (9.5)	113.3	6.19, d (9.5)	113.1	6.21, d (9.4)
4	143.1	7.57, d (9.5)	143.1	7.56, d (9,5)	143.3	7.58, d (9.4)
5	129.2	7.33, d (8.6)	129.1	7.32, d (8.6)	129.1	7.32, d (8.6)
6	114.4	6.78, d (8.6)	114.4	6.77, d (8.6)	114.5	6.79, d (8.6)
7	156.6		156.7		157.0	
8	107.5		107.5		107.6	
9	154.0		154.0		154.2	
10	112.4		112.5		112.6	
2′	77.4		77.4		79.3	
3′	70.3	5.30, d (4.8)	70.6	5.29, d (4.8)	73.7	3.93, br s
4′	60.4	6.53, d (4.8)	59.6	6.56, d (4.8)	67.8	6.08, d (3.4)
5′	25.5	1.38, s	25.3	1.40, s	24.9	1.46, s
6′	22.0	1.43, s	22.3	1.44, s	21.0	1.37, s
			3′-ester			
1	175.7		169.9			
2	33.9	2.55, sep (7.0)	20.7	2.07, s		
3	19.1	1.18, d (7.0)				
4	18.5	1.17, d (7.0)				
			4′-ester			
1	175.4		165.2		167.3	
2	41.3	2.37, sxt (7.0)	115.0	5.62, s	115.0	5.68, s
3	26.5	1.71, m 1.44, m	158.2		159.6	
4	11.6	0.91, t (7.4)	20.4	2.21, s	20.6	2.21, s
5	16.4	1.19, d (7.0)	27.5	1.87, s	27.6	1.89, s

[a]Data were obtained at 400 MHz in $CDCl_3$.

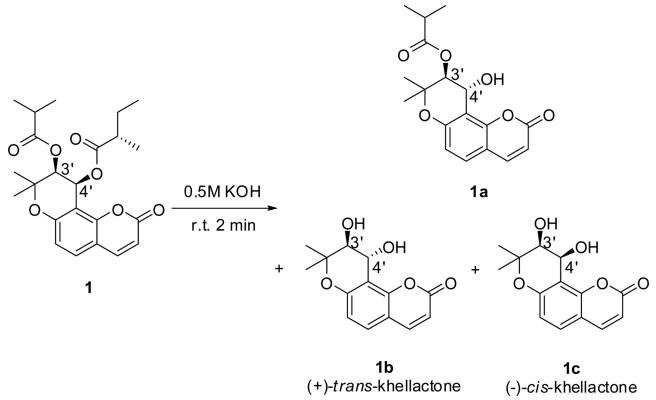

Figure 1. Partial and total alkaline hydrolysis of **1**.

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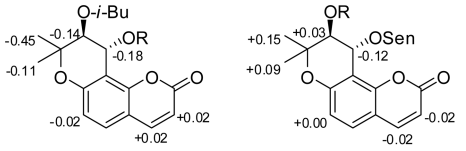

1aa R=(*S*)-MTPA ester
1ab R=(*R*)-MTPA ester

16a R=(*S*)-MTPA ester
16b R=(*R*)-MTPA ester

Figure 2. $\Delta\delta$ ($\delta_S - \delta_R$) values obtained from the MTPA esters of compound **16** and partial hydrolysis product **1a**.

configuration was confirmed by single-crystal X-ray diffraction analysis (Figure 3).

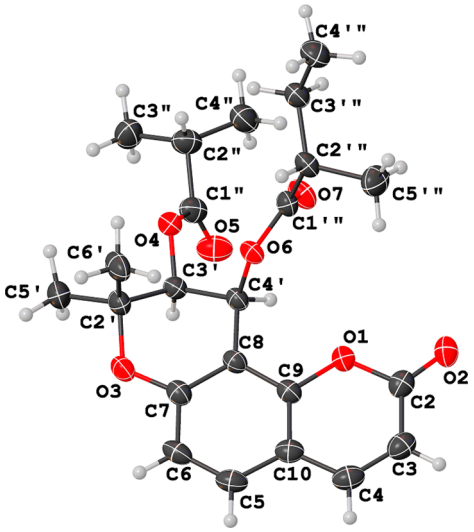

Figure 3. X-ray crystallographic structure of **1** (ORTEP drawing).

Compound **2** was obtained as colorless needles. The molecular formula was identified as $C_{19}H_{20}O_6$ by HRESIMS data. The ^{1}H NMR spectrum of **2** was similar to that of **1** except for the substituents at C-3′ and C-4′ (Table 1). The linkage between the khellactone, acetyl (C-1″), and senecioyl (C-1‴) moieties was confirmed by the HMBC cross-peaks from H-3′ (δ_H 5.29) to C-1″ (δ_C 169.9) and from H-4′ (δ_H 6.56) to C-1‴ (δ_C 165.2). The 3′,4′-*cis* relative configuration was identified based on the $^3J_{3',4'}$ value of 4.8 Hz and NOESY interactions.

The Mosher method could not be applied to compound **2**, because the ester functionalities at both C-3′ and C-4′ were hydrolyzed under alkaline conditions. The (3′*S*,4′*S*) absolute configuration of compound **2** was defined by single-crystal X-ray diffraction analysis (Figure 4).

Assignment of the Absolute Configuration of the Khellactone Esters by ECD Spectroscopy. The structures of compounds **3**−**15** were similar to **1** except for the 3′ and 4′ substituents that were confirmed by ^{1}H and ^{13}C NMR data (Tables S2−S5, Supporting Information). The locations of the C-3′ and C-4′ substituents were determined by the HMBC interactions from H-3′ to C-1″ and from H-4′ to C-1‴. The 3′,4′-*cis* relative configuration was confirmed by the NOESY interactions and *J* values (4.6−5.0 Hz).

The configuration of **1** was assigned as (3′*S*,4′*S*) based on the MTPA experiment and X-ray diffraction analysis, while the (3′*S*,4′*S*) configuration of **2** was defined via X-ray data only. In addition, the calculated ECD spectra of **1** and **2** were in good

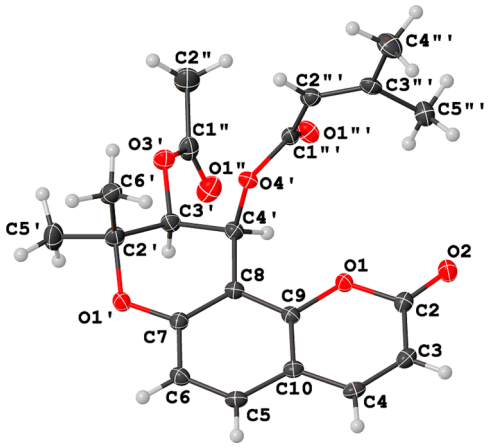

Figure 4. X-ray crystallographic structure of **2** (ORTEP drawing).

agreement with the experimental ECD spectra (Figures S3 and S5, Supporting Information).

Compounds **3**−**15** displayed similar patterns to compounds **1** and **2** in their experimental ECD spectra (Figure S2, Supporting Information). Therefore, the absolute configurations of compounds **3**−**15** were defined as (3′*S*,4′*S*). If the khellactone ester showed negative ECD Cotton effects at ca. 220 and 320 nm, a NOESY correlation between H-3′ and H-4′, and a $^3J_{3',4'}$ value of 4.6−5.0 Hz, the absolute configuration could be identified as (3′*S*,4′*S*), regardless of the nature of the C-3′ and C-4′ substituents.

Structure Determination of *trans*-Monoacylkhellactone. Compound **16** (t_R = 37.8 min) was isolated along with **E1** (enantiomeric mixture of **17** and **18**, t_R = 36.6 min) and **E2** (enantiomeric mixture of **19** and **20**, t_R = 39.6 min) by RP-HPLC [CH_3CN−H_2O (55:45, 5 mL/min)] from fraction H39. The molecular composition of compound **16**, $C_{19}H_{20}O_6$, was determined by HRCIMS data (*m*/*z* 345.1335 [M + H]$^+$). The ^{1}H and ^{13}C NMR spectra of **16** were similar to those of its diastereomer, *cis*-4′-*O*-senecioylkhellactone (peujaponisinol B, **17**)[5] except for the protons and carbons of the dihydropyran ring, especially at C-4′ [**16**: δ_H 6.08 (1H, d, *J* = 3.4 Hz, H-4′), δ_C 67.8 (C-4′); **17**: δ_H 6.43 (1H, d, *J* = 4.8 Hz, H-4′), δ_C 63.0 (C-4′)] (Tables 1 and S3 and S5, Supporting Information). The 3′,4′-*trans* relative configurations were characterized based on the *J* value (3.4 Hz) and the absence of a NOESY correlation between H-3′ and H-4′.

The 3′,4′-*trans* relative configuration of **16** was determined via ^{1}H NMR data. The absolute configuration was determined by the Mosher method using the free 3′-OH group. The ^{1}H NMR chemical shift differences of the MTPA esters (**16a** and **16b**) [H-3 (−0.02 ppm), H-4 (−0.02 ppm), H-4′ (−0.12 ppm), H-6 (+0.00 ppm), H-5′ (+0.09 ppm), H-6′ (+0.15 ppm), and H-3′ (+0.03 ppm)] indicated a (3′*S*) configuration (Figure 2). Therefore, the C-4′ absolute configuration had to be *R* based on the 3′,4′-*trans* relative configuration. Thus, the structure of compound **16** was assigned as (3′*S*,4′*R*)-4′-*O*-senecioylkhellactone. Interestingly, a structure identical to compound **16** was reported but without substantial spectroscopic evidence (Table S6, Supporting Information).[32]

Separation of the Senecioylkhellactone Enantiomers. Before the presence of enantiomers was recognized, **E1** (enantiomeric mixture of compounds **17** and **18**) was esterified by the Mosher method to determine its absolute configuration. After the MTPA reaction of **E1** with (*R*)-(−)-MTPA-Cl, major

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and minor peaks (**17a** and **18a**, respectively) were isolated by HPLC (Figure S10, Supporting Information). MTPA esters with (*S*)-(+)-MTPA-Cl gave **17b** and **18b** as the major and minor constituents, respectively. Based on the Mosher method, the absolute configurations of the major (**17**) and minor (**18**) compounds were assigned as (3′*S*,4′*S*) and (3′*R*,4′*R*), respectively (Figure S11, Supporting Information).

E2, the enantiomeric mixture of compounds **19** and **20**, was also derivatized by Mosher reagent (Figure S12, Supporting Information). The absolute configurations of the major (**19**) and minor (**20**) compounds were determined as (3′*S*,4′*S*) and (3′*R*,4′*R*), respectively (Figure S13, Supporting Information).

Because enantiomeric mixtures **E1** and **E2** were discovered during the separation of the MTPA reaction product, separation of the enantiomer was performed on a CHIRALPAK IC column (4.6 mm × 250 mm, 5 μm). (3′*S*,4′*S*)-Peujaponisinol B (**17**) and (3′*R*,4′*R*)-peujaponisinol B (**18**) (ratio = 2.44:1) were isolated from **E1** (Figure S14, Supporting Information), and (3′*S*,4′*S*)-peujaponisinol A (**19**) and (3′*R*,4′*R*)-peujaponisinol A (**20**) (ratio = 2.70:1) were isolated from **E2** (Figure S15, Supporting Information). The absolute configurations of the enantiomers were confirmed by their opposite specific rotations, mirror-image related ECD patterns, and similar NMR spectra (Tables S3 and S5, Supporting Information). It is difficult to find appropriate columns that have good resolution to resolve enantiomers (Figures S11 and S12, Supporting Information). Therefore, HPLC analysis after MTPA derivatization is a feasible alternative to detect the presence of enantiomers. In addition, the ratios of the major and minor MTPA reaction products and enantioseparation products were similar.

ECD Spectroscopy of the Senecioylkhellactone Enantiomers. The monoacylkhellactones **16**–**18** have the same 2D structures. The ECD curves of **16** and **18** showed mirror-image related Cotton effects compared to that of **17**. The calculated ECD spectra of **16**–**18** (Figure 5) showed

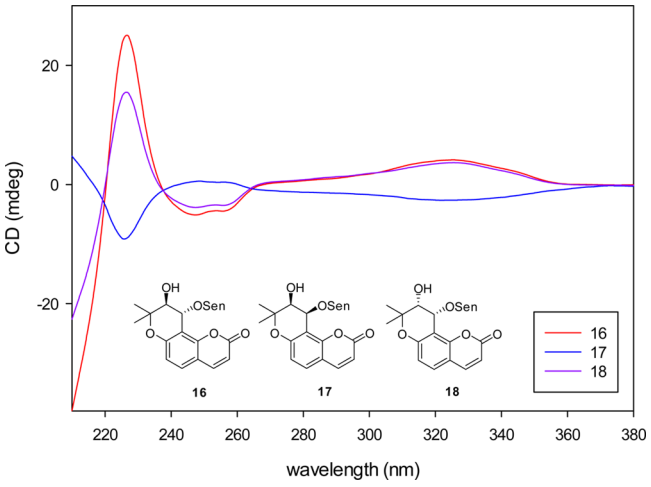

Figure 5. Experimental ECD spectra of **16**–**18** in MeOH.

similar patterns to the experimental ECD spectra (Figures S18, S20, and S22, Supporting Information). The (4′*R*)-khellactone ester without a 3′-*O*-acyl substituent displayed a mirror-image related ECD pattern with the (3′*S*,4′*S*)-*cis*-khellactone ester.

Acyl Migration of the *cis*-Monoacylkhellactones. Compounds **17**–**20** were separated from fraction H39. After their isolation, interchange of the substituents in solution was observed between **17** and **19** and between **18** and **20** due to transesterification involving the hydroxy and senecioyl moieties (Figures S16 and S17, Supporting Information). Equilibrium was established through mutual acyl migration between C-3′ and C-4′ approximately 4 days after purification. The migration of the C-3′ acyl moiety to C-4′ was slightly dominant compared to the reverse migration. Positional isomers, such as compounds **13** and **14**, **17** and **19**, **18** and **20**, **24** and **29**, and **30** and **33**, were isolated in this experiment.

MS Fragmentation of the Khellactone Esters. For compound **1**, the location of the isobutyroyl (C-3′) and 2-methylbutyroyl (C-4′) groups was deduced by the diagnostic fragment at *m*/*z* 315 (base peak, M^+ − OMeBu) in the CI mass spectrum. In addition, the fragment ion of **2** was detected at *m*/*z* 287 (M^+ − OSen), and that of **16** (4′-*O*-senecioylkhellactone) was detected at *m*/*z* 245 (base peak, M^+ − OSen) (Table S7, Supporting Information).

The MS fragment peaks of khellactone esters without a C-4′ substituent were detected as major peaks, which were more dominant than those from esters without a 3′-ester moiety. The ion resulting from fragmentation of the 3′-substituent was small or undetectable, depending on the ionization method. The determination of the position of substituents by MS fragmentation analysis corresponded with the HMBC analysis. Based on these results, the positions of the C-3′ and C-4′ substituents could be determined by MS fragmentation.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 polarimeter at 20 °C using a sodium lamp (589 nm). UV spectra were recorded using a PerkinElmer Lambda 25 UV/vis spectrophotometer. ECD and UV spectra were measured on an Applied Photophysics Chirascan-plus CD spectrometer. IR spectra were acquired using a JASCO FT/IR-4200 spectrophotometer. The ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC, and NOESY spectra were obtained in $CDCl_3$ on Bruker AVANCE digital 400 or 500 spectrometers, a JEOL JNM-ECA 600 spectrometer, or a Bruker AVANCE III HD 800 spectrometer with a 5 mm CPTCI cryoprobe. Solvent peaks were used as internal standards. High-resolution and low-resolution CIMS were conducted on a JEOL JMS 700 spectrometer. High-resolution and low-resolution ESIMS data were obtained using an AB SCIEX Q-TOF 5600 mass spectrometer or an Agilent Technologies 6130 Quadrupole LC/MS spectrometer with an Agilent Technologies 1260 Infinity LC system and an INNO C_{18} column (4.6 × 150 mm, S-5 μm, 12 nm). Column chromatography (CC) was performed with Merck silica gel 60 (40–63 μm, 230–400 mesh). TLC was conducted using Merck silica gel 60 F_{254}-precoated TLC plates and Merck RP-18 F_{254s}-precoated TLC plates. Spots were visualized by spraying with anisaldehyde–H_2SO_4 followed by heating at 120 °C. The MPLC system was equipped with Combi Flash Companion (Teledyne Isco Inc., Lincoln, NE, USA) and a Reveleris C_{18} Reversed-Phase 120 g Cartridge (Grace & Co., Columbia, MD, USA). Fisher HPLC-grade solvents were used for the MeOH–H_2O solution. The HPLC system was composed of a Gilson 321 pump, Gilson UV/vis 151 detector (detection at 254 or 327 nm), and a Hydrosphere C_{18} column (20 × 250 mm, S-5 μm, 12 nm, YMC Co., Ltd., Kyoto, Japan), INNO C_{18} column (20 × 250 mm, S-5 μm, 12 nm, Young Jin Biochrom Co., Ltd., Seoul, South Korea), YMC J'sphere ODS H80 column (10 × 250 mm, S-4 μm, 8 nm), INNO C_{18} column (10 × 250 mm, S-5 μm, 12 nm), or CHIRALPAK IC column (4.6 × 250 mm, 5 μm, Daicel Corporation, Osaka, Japan). Fisher HPLC-grade solvents were used for the CH_3CN–H_2O and MeOH system. Pyridine-d_5 (Cambridge Isotope Laboratories, Inc.), (*R*)-(−)- and (*S*)-(+)-MTPA-Cl, and DMAP (4-dimethylaminopyridine) (Sigma-Aldrich Co., St. Louis, MO, USA) were used to prepare the Mosher esters.

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Plant Material. *P. japonicum* roots were collected at Taean-gun, Chungcheongnam-do, Korea, in November 2012. Plant specimens were deposited at the herbarium of Seoul National University in Korea (ref: SNUPH 2012−02/KOR) after identification by Prof. Je Hyun Lee, College of Oriental Medicine, Dongguk University.

Extraction and Isolation. Dried roots of *P. japonicum* (30.0 kg) were extracted with MeOH (3 × 45.5 L, 200 min each) at room temperature in an ultrasonicator and filtered. After condensing the filtrate under reduced pressure, the MeOH extract (2.7 kg, yield: 8.9%) was partitioned into *n*-hexane (454.2 g, yield: 16.9%), $CHCl_3$ (87.9 g, yield: 3.3%), EtOAc (19.9 g, yield: 0.7%), *n*-BuOH (97.5 g, yield: 3.6%), and residual aqueous fractions (1.6 kg, yield: 59.6%).

A part of the *n*-hexane fraction (406.0 g) was subjected to silica column chromatography (CC, 34 × 15.3 cm) using gradient mixtures of *n*-hexane-EtOAc (95:5 → 0:100) to afford 51 fractions (H1−H51). Fraction H32 (5.3 g) was separated using RP-MPLC (MeOH−H_2O, 75:25, 20 mL/min) to yield 16 subfractions, H32−1 to H-32−16. H32−4 was subjected to RP-HPLC (CH_3CN−H_2O, 75:25, 5 mL/min) to obtain compound **15** (0.8 mg, t_R = 26.9 min). H32−5 was divided by RP-HPLC (CH_3CN−H_2O, 75:25, 5 mL/min) to obtain seven subfractions (H32−5−1 to H32−5−7). H32−5−5 was applied to chiral-selective HPLC (1 mL/min) using MeOH to furnish **1** (15.1 mg, t_R = 6.3 min), **3** (24.0 mg, t_R = 6.0 min), **6** (4.7 mg, t_R = 5.5 min), and a subfraction (H32-5-5-4). H32-5-5-4 was chromatographed using chiral-selective HPLC (1 mL/min) with MeOH to give **8** (0.6 mg, t_R = 6.6 min). H32-6 was separated by RP-HPLC (CH_3CN−H_2O, 75:25, 5 mL/min) to give compound **9** (2.2 mg, t_R = 41.6 min). Fraction H34 (45.2 g) was chromatographed using RP-MPLC (MeOH-H_2O, 70:30, 40 mL/min) to give 13 subfractions, H34−1 to H-34−13. H34−7 was separated by RP-HPLC (CH_3CN−H_2O, 75:25, 7 mL/min) to acquire compound 7 (11.1 mg, t_R = 37.2 min) and subfractions (H34-7-3, 4).

Compound **5** (2.1 mg, t_R = 45.8 min) was obtained from H34-7-4 by RP-HPLC (7 mL/min), using a mixture of CH_3CN−H_2O (70:30). Subfraction H34-7-3 was subjected to HPLC (1 mL/min) using a chiral-selective column with MeOH to provide compound **4** (3.5 mg, t_R = 5.5 min). Fraction H35 (14.7 g) was applied to silica CC and eluted using a mixture of *n*-hexane−EtOAc−MeOH (60:4:1) followed by RP-HPLC (CH_3CN−H_2O, 80:20, 5 mL/min) to obtain subfractions (H35-11-1, **2**). Compound **2** (2.8 mg, t_R = 27.4 min) was isolated from H35-11-1 by RP-HPLC using a mixture of CH_3CN−H_2O (65:35, 7 mL/min), and **12** (109.6 mg, t_R = 24.5 min) was isolated from H35-11-2 by eluting with CH_3CN−H_2O (80:20, 5 mL/min). Fraction H37 (5.8 g) was subjected to RP-MPLC using a MeOH−H_2O (55:45, 15 mL/min) elution system followed by RP-HPLC (CH_3CN−H_2O, 55:45, 5 mL/min) to yield subfractions H37-9-1 and H37-9-4. Chiral-selective HPLC was performed with MeOH to obtain compounds **13** (6.7 mg, t_R = 5.4 min) and **14** (21.7 mg, t_R = 4.7 min) from H37-9-4. Fraction H39 (1.7 g) was purified by RP-HPLC using a CH_3CN−H_2O (55:45, 5 mL/min) elution system to give compounds **16** (2.5 mg, t_R = 37.8 min) and **40** (11.4 mg, t_R = 34.6 min) and the enantiomeric mixtures **E1** (8.1 mg, t_R = 36.6 min) and **E2** (7.2 mg, t_R = 39.6 min). The CHIRALPAK IC column (MeOH, 1 mL/min) was used to yield compounds **17** (1.0 mg, t_R = 4.5 min) and **18** (0.5 mg, t_R = 5.9 min) from **E1** and compounds **19** (0.8 mg, t_R = 6.3 min) and **20** (0.5 mg, t_R = 5.3 min) from **E2**. Fractions H41−43 (3.7 g) were applied to MPLC over ODS and eluted with MeOH−H_2O (55:45, 10 mL/min) to give ten subfractions H41-43-1 to H41-43-10. H41-43-7 was chromatographed by RP-HPLC (5 mL/min) using an isocratic solvent system of CH_3CN−H_2O (45:55) to afford **10** (3.6 mg, t_R = 50.9 min).

The $CHCl_3$ fraction was divided by CC (64 × 15.3 cm) over silica gel with mixtures of *n*-hexane−EtOAc (85:15 → 0:100) as a gradient solvent system to produce 36 fractions (C1−C36). Fraction C20−22 (4.8 g) was subjected to MPLC (20 mL/min) over ODS using MeOH−H_2O (50:50) as the eluent to give 16 subfractions (C20-22-1 to C20-22-16). C20-22-12 was separated by RP-HPLC (CH_3CN−H_2O, 45:55, 5 mL/min) to give seven subfractions (C20-22-12-1 to C20-22-12-7). C20-22-12-7 was purified by semipreparative RP-HPLC (CH_3CN−H_2O, 45:55, 2 mL/min) to obtain compound **11** (0.8 mg, t_R = 31.5 min).

(3′S,4′S)-3′-O-Isobutyroyl-4′-O-(2S-methylbutyroyl)khellactone ***(1)***. Colorless needles; $[\alpha]^{20}_D$ + 1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.69), 255 (3.10), 321 (3.64) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (−3.73), 244 (+0.92), 256 (+0.73), 323 (−0.87) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; CIMS *m*/*z* 417 $[M + H]^+$; HRCIMS *m*/*z* 417.1912 $[M + H]^+$ (calcd for $C_{23}H_{29}O_7$, 417.1913).

(3′S,4′S)-3′-O-Acetyl-4′-O-senecioylkhellactone ***(2)***. Colorless needles; mp 104−106 °C; $[\alpha]^{20}_D$ − 6 (*c* 1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.28), 255 (3.50), 323 (3.87) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (−17.00), 245 (+2.86), 255 (+2.46), 319 (−2.44) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m*/*z* 409.1258 $[M + Na]^+$ (calcd for $C_{21}H_{22}O_7Na$, 409.1258).

(3′S,4′S)-4′-O-Isobutyroyl-3′-O-(2-methylbutyroyl)khellactone ***(3)***. Colorless needles; $[\alpha]^{20}_D$ + 52 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.36), 256 (3.84), 323 (4.23) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 225 (−19.43), 245 (+3.31), 256 (+2.63), 323 (−4.16) nm; IR ν_{max} 2974, 2938, 2875, 1745, 1608, 1491, 1220, 1147, 772 cm^{-1}; ^{1}H and ^{13}C NMR data, see Supporting Information; CIMS *m*/*z* 417 $[M + H]^+$; HRCIMS *m*/*z*:417.1910 ($[M + H]^+$, calcd for $C_{23}H_{29}O_7$:417.1913).

(3′S,4′S)-3′-O-(2-Methylbutyroyl)-4′-O-senecioylkhellactone ***(4)***. White amorphous powder; $[\alpha]^{20}_D$ −20 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.50), 256 (3.85), 323 (4.17) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 225 (−29.78), 246 (+4.94), 256 (+4.21), 326 (−5.38) nm; IR ν_{max} 2976, 2938, 2877, 1741, 1608, 1491, 1221, 1144, 1074, 772 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Supporting Information; CIMS *m*/*z*:429 $[M + H]^+$; HRCIMS *m*/*z*:429.1909 $[M + H]^+$ (calcd for $C_{24}H_{29}O_7$, 429.1913).

(3′S,4′S)-4′-O-(2-Methylbutyroyl)-3′-O-senecioylkhellactone ***(5)***. White amorphous powder; $[\alpha]^{20}_D$ + 6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.55), 256 (3.86), 323 (4.23) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 225 (−17.32), 246 (+3.05), 256 (+2.14), 323 (−4.93) nm; IR ν_{max} 2976, 2934, 1747, 1608, 1220, 1146, 772 cm^{-1}; ^{1}H and ^{13}C NMR data, see Supporting Information; CIMS *m*/*z* 429 $[M + H]^+$; HRCIMS *m*/*z* 429.1908 $[M + H]^+$ (calcd for $C_{24}H_{29}O_7$, 429.1913).

(3′S,4′S)-3′-O-Isobutyroyl-4′-O-isovaleroylkhellactone ***(6)***. White amorphous powder; $[\alpha]^{20}_D$ −9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.09), 255 (3.49), 323 (4.05) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (−8.79), 245 (+2.53), 255 (+1.94), 323 (−1.80) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; CIMS *m*/*z* 417 $[M + H]^+$; HRCIMS *m*/*z* 417.1917 $[M + H]^+$ (calcd for $C_{23}H_{29}O_7$, 417.1913).

(3′S,4′S)-4′-O-Angeloyl-3′-O-(2-methylbutyroyl)khellactone ***(7)***. White amorphous powder; $[\alpha]^{20}_D$ −59 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.33), 255 (3.62), 323 (4.12) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (−7.15), 246 (+1.36), 254 (+1.24), 318 (−1.25) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m*/*z* 451.1729 $[M + Na]^+$ (calcd for $C_{24}H_{28}O_7Na$, 451.1727).

(3′S,4′S)-3′-O-Butyroyl-4′-O-(2-methylbutyroyl)khellactone ***(8)***. Colorless needles; $[\alpha]^{20}_D$ + 7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.78), 255 (3.23), 323 (3.75) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (−4.58), 243 (+1.03), 257 (+0.90), 326 (−1.04) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m*/*z* 439.1719 $[M + Na]^+$ (calcd for $C_{23}H_{28}O_7Na$, 439.1727).

(3′S,4′S)-4′-O-Angeloyl-3′-O-isovaleroylkhellactone ***(9)***. White amorphous powder; $[\alpha]^{20}_D$ −40 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.32), 255 (3.62), 323 (4.01) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 225 (−19.19), 246 (+2.61), 256 (+2.66), 326 (−2.57) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m*/*z* 451.1724 $[M + Na]^+$ (calcd for $C_{24}H_{28}O_7Na$, 451.1727).

(3′S,4′S)-3′-O-Acetyl-4′-O-(3-hydroxyisovaleroyl)khellactone ***(10)***. White amorphous powder; $[\alpha]^{20}_D$ + 23 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 219 (4.27), 256 (3.78), 322 (4.19) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (−7.73), 245 (+2.32), 255 (+1.72),

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322 (−1.43) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; CIMS *m/z* 405 $[M + H]^+$; HRCIMS *m/z* 405.1548 $[M + H]^+$ (calcd for $C_{21}H_{25}O_8$, 405.1549).

(3′S,4′S)-3′-O-Acetyl-4′-O-(3-hydroxy-2-methylbutyroyl)-khellactone (11). White amorphous powder; $[\alpha]^{20}_D$ − 3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.06), 257 (3.56), 323 (4.00) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (−8.32), 244 (+1.98), 255 (+1.52), 321 (−1.44) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; CIMS *m/z* 404 $[M]^+$; HRCIMS *m/z* 404.1466 $[M]^+$ (calcd for $C_{21}H_{24}O_8$, 404.1471).

(3′S,4′S)-3′-O-Acetyl-4′-O-(2-methylbutyroyl)khellactone (12). White amorphous powder; $[\alpha]^{20}_D$ + 5 (*c* 2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (3.37), 255 (2.71), 325 (3.23) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (−11.8), 244 (+3.16), 256 (+2.24), 324 (−3.03) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m/z* 411.1400 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_7Na$, 411.1414).

(3′S,4′S)-3′-O-(2-Methylbutyroyl)khellactone (13). White amorphous powder; $[\alpha]^{20}_D$ − 3 (*c* 1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.18), 256 (3.56), 326 (4.20) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (−6.05), 235 (+1.58), 258 (+0.67), 328 (−2.16) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m/z* 369.1309 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_6Na$, 369.1309).

(3′S,4′S)-4′-O-(2-Methylbutyroyl)khellactone (14). White amorphous powder; $[\alpha]^{20}_D$ − 73 (*c* 1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.22), 257 (3.65), 323 (4.25) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 225 (−4.15), 243 (+0.68), 257 (+0.53), 318 (−2.01) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m/z* 369.1312 $[M + Na]^+$ (calcd for $C_{19}H_{22}O_6Na$, 369.1309).

(3′S,4′S)-4′-O-Methyl-3′-O-(2-methylbutyroyl)khellactone (15). White amorphous powder; $[\alpha]^{20}_D$ + 4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.66), 255 (3.11), 323 (3.39) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 223 (−1.16), 248 (+0.15), 259 (+0.13), 329 (−0.33) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m/z* 383.1452 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_6Na$, 383.1465).

(3′S,4′R)-4′-O-Senecioylkhellactone (16). White amorphous powder; $[\alpha]^{20}_D$ + 81 (*c* 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 219 (4.33), 257 (3.69), 324 (3.99) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (+18.91), 247 (−3.39), 256 (−2.86), 324 (+2.57) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; CIMS *m/z* 345 $[M + H]^+$; HRCIMS *m/z* 345.1335 $[M + H]^+$ (calcd for $C_{19}H_{21}O_6$, 345.1338).

(3′S,4′S)-4′-O-Senecioylkhellactone (17). White amorphous powder; $[\alpha]^{20}_D$ − 52 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.31), 257 (3.53), 326 (3.99) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (−6.38), 247 (+0.44), 255 (+0.33), 324 (−1.75) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; ESIMS *m/z* 367 $[M + Na]^+$.

(3′R,4′R)-4′-O-Senecioylkhellactone (18). White amorphous powder; $[\alpha]^{20}_D$ + 39 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.14), 257 (3.38), 326 (3.83) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (+11.74), 248 (−2.48), 257 (−2.29), 326 (+2.31) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; HRESIMS *m/z* 367.1144 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_6Na$, 367.1152).

(3′S,4′S)-3′-O-Senecioylkhellactone (19). White amorphous powder; $[\alpha]^{20}_D$ + 24 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.32), 256 (3.45), 326 (4.00) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (−4.31), 246 (+1.25), 257 (+1.63), 323 (−1.25) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; ESIMS *m/z* 367 $[M + Na]^+$.

(3′R,4′R)-3′-O-Senecioylkhellactone (20). White amorphous powder; $[\alpha]^{20}_D$ − 2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.13), 255 (3.28), 326 (3.81) nm; ECD (*c* 50 μM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (+0.78), 233 (−1.06), 257 (−0.36), 333 (+0.53) nm; ^{1}H and ^{13}C NMR data, see Supporting Information; ESIMS *m/z* 367 $[M + Na]^+$.

Partial and Total Alkaline Hydrolysis of 1. A solution of **1** (3.2 mg, 7.7 μmol) in 1,4-dioxane (0.5 mL) containing 0.5 M KOH (0.5 mL) was stirred at room temperature for 2 min. The progress of the reaction was monitored by NP-TLC using a mixture of *n*-hexane and EtOAc (2:1). The reaction mixture was acidified with 5% H_2SO_4 (~120 μL), extracted with $CHCl_3$ (1 mL), and evaporated. Reaction products **1a** (t_R = 11.0 min, 0.2 mg, 0.7 μmol, yield: 9.4%), **1b** (t_R = 7.1 min, 0.2 mg, 0.7 μmol, yield: 8.9%), and **1c** (t_R = 7.6 min, 1.0 mg, 2.9 μmol, yield: 37.2%) were purified by RP-HPLC using a mixture of CH_3CN−H_2O (75:25, 2 mL/min, 327 nm). The structures of **1a**, **1b**, and **1c** were confirmed by ^{1}H NMR ($CDCl_3$, 600 MHz) spectroscopy, see Supporting Information. In addition, (*S*)-(+)-2-methylbutyric acid was isolated (t_R = 8.6 min, 210 nm) using HPLC (CH_3CN−H_2O, 5:95 → 95:5, 1 mL/min) with a BDS Hypersil C_{18} column (4.6 × 150 mm, S-5 μm, Thermo Scientific, Waltham, MA, USA).

Preparation of the MTPA Esters of 1a. Compound **1a** (0.5 mg, 1.4 μmol) was resuspended in pyridine-d_5 (800 μL), and (*R*)-(−)-MTPA-Cl (10 μL, 0.05 mmol) and DMAP were added under a stream of nitrogen gas. The reaction mixture was incubated at room temperature overnight, dried, and dissolved in $CHCl_3$ (50 μL). Silica gel TLC using *n*-hexane and EtOAc (2:1) was conducted to monitor progress of the reaction. Using an INNO C_{18} column (10 × 250 mm, S-5 μm, 12 nm), the resulting (*S*)-MTPA ester **1aa** (t_R = 13.9 min, 0.4 mg, 0.7 μmol, yield: 52.7%) was isolated (2 mL/min) with CH_3CN−H_2O (90:10). In the same manner, (*S*)-(+)-MTPA-Cl (10 μL, 0.05 mmol) was added to **1a** (0.5 mg, 1.5 μmol) to obtain the (*R*)-MTPA ester **1ab** (t_R = 13.1 min, 0.3 mg, 0.5 μmol, yield: 37.1%).

Preparation of the MTPA Esters of 16. Compound **16** (0.4 mg, 1.2 μmol) was dissolved in deuterated pyridine (600 μL), and mixed with (*R*)-(−)-MTPA-Cl (10 μL, 0.05 mmol) under a stream of nitrogen gas. The resulting mixture was kept at room temperature overnight, evaporated, dissolved in $CHCl_3$ (50 μL), and subjected to silica capillary CC (7 × 0.5 cm) using a mixture of *n*-hexane-EtOAc (5:1). Fifty drops were collected per vial, and the (*S*)-MTPA ester (0.2 mg, 0.3 μmol, yield: 27.0%) was obtained from the 15th−21st vials. The progress of the reaction was monitored by NP-TLC using a mixture of *n*-hexane and EtOAc (3:1). To obtain the (*R*)-MTPA ester (0.1 mg, 0.2 μmol, yield: 11.7%), (*S*)-(+)-MTPA-Cl (10 μL, 0.05 mmol) was added to **16** (0.5 mg, 0.002 mmol), and subsequent processes were performed in the same way as above.

X-ray Crystallographic Analysis of 1 and 2. The absolute configurations of **1** and **2** were determined using data collected on a SuperNova, Dual, Cu at zero, AtlasS2 diffractometer with Cu Kα radiation (λ = 1.54184). With Olex2, the structure was solved by direct methods using the ShelXT structure solution program and was refined by least-squares minimization using the ShelXL refinement package. Crystallographic data for **1** and **2** have been deposited at the Cambridge Crystallographic Data Centre (**1**: CCDC 1488681, **2**: CCDC 1474283). Copies of the data can be obtained free of charge by application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk).

Crystal Data of 1. Colorless crystal, $C_{23}H_{28}O_7$, M = 416.45, monoclinic, crystal size 0.3 × 0.101 × 0.031 mm^3, space group $P2_1$ (no. 4), *a* = 24.3633(4) Å, *b* = 9.12727(13) Å, *c* = 31.0898(6) Å, β = 109.128(2)°, *V* = 6531.8(2) Å^3, *Z* = 12, *T* = 100(80) K, μ (Cu Kα) = 0.774 mm^{-1}, D_{calc} = 1.270 g/cm^3, Reflections collected: 39478 (7.274° ≤ 2Θ ≤ 153.672°). Independent reflections: 20639 (R_{int} = 0.0243, R_{sigma} = 0.0335). The goodness of fit on F^2 = 1.031. The final R_1 values were 0.0417 ($I > 2\sigma(I)$) and 0.0464 (all data). The final wR_2 values were 0.1069 ($I > 2\sigma(I)$) and 0.1107 (all data). Flack parameter = 0.08(6).

Crystal Data of 2. Colorless crystal, $C_{21}H_{22}O_7$, M = 386.38, monoclinic, crystal size 0.2 × 0.059 × 0.024 mm^3, space group $P2_1$ (no. 4), *a* = 9.68785(12) Å, *b* = 6.62844(9) Å, *c* = 15.2024(2) Å, β = 103.8888(13)°, *V* = 947.69(2) Å^3, *Z* = 2, *T* = 100.0(2) K, μ (Cu Kα) = 0.851 mm^{-1}, D_{calc} = 1.354 g/cm^3, Reflections collected: 19091 (9.404° ≤ 2Θ ≤ 152.988°). Independent reflections: 3883 (R_{int} = 0.0347, R_{sigma} = 0.0220). The goodness of fit on F^2 = 1.062. The final R_1 values were 0.0276 ($I > 2\sigma(I)$) and 0.0287 (all data). The final wR_2 values were 0.0705 ($I > 2\sigma(I)$) and 0.0715 (all data). Flack parameter = −0.03(6).

ECD Calculations. Conformational searches were performed using the MMFF94s force field in Conflex 7 with a search limit of 10.0 kcal/mol. Conformers used for geometry optimization were selected by a lower energy level up to 90% population. Turbomole was used for the ground-state geometry optimization with the def-SV(P) basis set for all

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atoms, B3LYP functional, and density functional theory (DFT). The calculated ECD data were obtained using TDDFT at the B3LYP/def-SV(P) level with the COSMO model in MeOH. The ECD spectra were simulated using Gaussian functions overlapping each transition, where σ is the width of the band at $1/e$ height and ΔE_i and R_i are the excitation energies and rotatory strengths for transition i, respectively. The value of σ was 0.10 eV and R was R_{len} in this work.

$$\Delta\epsilon(E) = \frac{1}{2.297 \times 10^{-39}} \frac{1}{\sqrt{2\pi}\sigma} \sum_{i}^{A} \Delta E_i R_i \, e^{[-(E-\Delta E_i)^2/(2\sigma)^2]}$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00947.

X-ray crystallographic data for **1** (CIF)
X-ray crystallographic data for **2** (CIF)
Data including ^{1}H (**1**−**20**, and **1a**−**1c**), ^{13}C (**1**−**20**), and 2D (**1**−**16**) NMR, experimental ECD (**1**−**27**, and **29**−**40**), conformational analysis (**1**−**2**, and **16**−**18**), calculated ECD (**1**−**2**, and **16**−**18**), UV (**1**−**2**, and **16**−**18**), MS (**1**−**20**), ^{1}H NMR of MTPA esters (**1a**, and **16**−**20**), experimental ECD of MTPA esters (**17**−**20**), HPLC chromatograms of MTPA esters (**17**−**20**), enantioseparation (**17**−**20**), acyl migration (**17**−**20**), isolation of compounds **21**−**39**, preparation of MTPA esters of **17**−**20**, comparison of 3′α-hydroxy-4′β-senecioyloxy-3′,4′-dihydroseselin and **16**, structures of compounds **21**−**40**, and HPLC chromatograms of **E1** and **E2** on CHIRALPAK IA-IF (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Gan, W. S. *Manual of Medicinal Plants in Taiwan*; National Research Institute of Chinese Medicine: Taichung, Taiwan, 1965; Vol. 3, p 675.
(2) Bae, K. H. *Medicinal Plants of Korea*; Kyo-Hak Publishing Corporation: Seoul, Korea, 2001; p 379.
(3) Chen, I.-S.; Chang, C.-T.; Sheen, W.-S.; Teng, C.-M.; Tsai, I.-L.; Duh, C.-Y.; Ko, F.-N. *Phytochemistry* **1996**, *41*, 525−530.
(4) Ikeshiro, Y.; Mase, I.; Tomita, Y. *Phytochemistry* **1994**, *35*, 1339−1341.
(5) Ikeshiro, Y.; Mase, I.; Tomita, Y. *Phytochemistry* **1993**, *33*, 1543−1545.
(6) Ikeshiro, Y.; Mase, I.; Tomita, Y. *Phytochemistry* **1992**, *31*, 4303−4306.
(7) Duh, C. Y.; Wang, S. K.; Wu, Y. C. *Phytochemistry* **1992**, *31*, 1829−1830.
(8) Lee, G.; Park, H.-G.; Choi, M.-L.; Kim, Y. H.; Park, Y. B.; Song, K.-S.; Cheong, C.; Bae, Y.-S. *J. Microbiol. Biotechnol.* **2000**, *10*, 394−398.
(9) Lee, S. O.; Choi, S. Z.; Lee, J. H.; Chung, S. H.; Park, S. H.; Kang, H. C.; Yang, E. Y.; Cho, H. J.; Lee, K. R. *Arch. Pharmacal Res.* **2004**, *27*, 1207−1210.
(10) Shin, K. H.; Kang, S. S.; Chi, H. J. *Saengyak Hakhoechi* **1992**, *23*, 20−23.
(11) Jang, K.-C.; Kim, S.-C.; Song, E.-Y.; Um, Y.-C.; Kim, S. C.; Lee, Y.-J. *Acta Hortic.* **2008**, *765*, 49−53.
(12) Hisamoto, M.; Kikuzaki, H.; Ohigashi, H.; Nakatani, N. *J. Agric. Food Chem.* **2003**, *51*, 5255−5261.
(13) Hisamoto, M.; Kikuzaki, H.; Nakatani, N. *J. Agric. Food Chem.* **2004**, *52*, 445−450.
(14) Chen, Y.-C.; Chen, P.-Y.; Wu, C.-C.; Tsai, I.-L.; Chen, I.-S. *Yaowu Shipin Fenxi* **2008**, *16*, 15−25.
(15) Xiong, Y.-Y.; Wu, F.-H.; Wang, J.-S.; Li, J.; Kong, L.-Y. *J. Ethnopharmacol.* **2012**, *141*, 314−321.
(16) Nielsen, B. E.; Larsen, P. K.; Lemmich, J. *Acta Chem. Scand.* **1971**, *25*, 529−533.
(17) Matsuda, H.; Murakami, T.; Nishida, N.; Kageura, T.; Yoshikawa, M. *Chem. Pharm. Bull.* **2000**, *48*, 1429−1435.
(18) Swager, T. M.; Cardellina, J. H. *Phytochemistry* **1985**, *24*, 805−813.
(19) Gonzalez, A. G.; Barroso, J. T.; Lopez-Dorta, H.; Luis, J. R.; Rodriguez-Luis, F. *Phytochemistry* **1979**, *18*, 1021−1023.
(20) Wang, X.-Y.; Li, J.-F.; Jian, Y.-M.; Wu, Z.; Fang, M.-J.; Qiu, Y.-K. *J. Chromatogr. A* **2015**, *1387*, 60−68.
(21) Lee, J. W.; Lee, C.; Jin, Q.; Yeon, E. T.; Lee, D.; Kim, S.-Y.; Han, S. B.; Hong, J. T.; Lee, M. K.; Hwang, B. Y. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2717−2719.
(22) Kong, L.-Y.; Min, Z.-D.; Li, Y.; Pei, Y.-H. *Phytochemistry* **1996**, *42*, 1689−1691.
(23) Kong, L. Y.; Pei, Y. H.; Li, X.; Wang, S. X.; Hou, B. L.; Zhu, T. R. *Yaoxue Xuebao* **1993**, *28*, 772−776.
(24) Song, Y.-L.; Jing, W.-H.; Tu, P.-F.; Wang, Y.-T. *Nat. Prod. Res.* **2014**, *28*, 545−550.
(25) Tosun, A.; Ozkal, N.; Baba, M.; Okuyama, T. *Turk. J. Chem.* **2005**, *29*, 327−334.
(26) Lv, H.; Luo, J.; Wang, X.; Kong, L. *Chromatographia* **2013**, *76*, 141−148.
(27) Song, Y.-L.; Zhang, Q.-W.; Li, Y.-P.; Ru, Y.; Wang, Y.-T. *Molecules* **2012**, *17*, 4236−4251.
(28) Buendia-Trujillo, A. I.; Torrres-Valencia, J. M.; Joseph-Nathan, P.; Burgueno-Tapia, E. *Tetrahedron: Asymmetry* **2014**, *25*, 1418−1423.
(29) Bohlmann, F.; Rode, K. M. *Chem. Ber.* **1968**, *101*, 2741−2746.
(30) Valencia-Islas, N.; Abbas, H.; Bye, R.; Toscano, R.; Mata, R. *J. Nat. Prod.* **2002**, *65*, 828−834.
(31) Macias, F. A.; Massanet, G. M.; Rodriguez-Luis, F.; Salva, J.; Fronczek, F. R. *Magn. Reson. Chem.* **1989**, *27*, 653−658.
(32) Mikailova, N. K.; Serkerov, S. V. *Chem. Nat. Compd.* **2015**, *51*, 826−828.

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