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Bioactive Constituents of *Cirsium japonicum* var. *australe*

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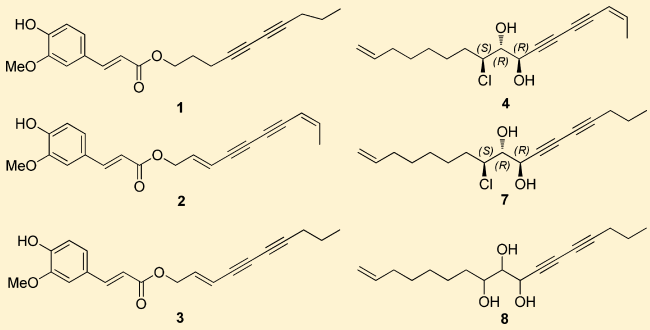
Supporting Information

ABSTRACT: *Cirsium japonicum* var. *australe*, used as a folk medicine in Taiwan, has been employed traditionally in the treatment of diabetes and inflammatory symptoms. Bioactivity-guided fractionation of its ethanolic extract, utilizing centrifugal partition chromatography monitored by DPPH-TLC analysis, led to the isolation of three new acetylenic phenylacrylic acid esters (**1**–**3**) and two new polyacetylenes (**4** and **5**), together with seven known compounds (**6**–**12**). The structures of **1**–**5** were elucidated by spectroscopic methods including 1D and 2D NMR techniques. The absolute configurations of **4** and **7** were determined utilizing Mosher's method and ECD/CD experiments. The DPPH scavenging activity of the constituents isolated from the *C. japonicum* var. *australe* ethanolic extract was evaluated. The potential antidiabetic activity of some of the isolates was evaluated using in vitro cellular glucose uptake and oil red staining assays.



Cirsium japonicum DC. var. *australe* Kitam. (Asteraceae) is known as "Formosan thistle".[1,2] It is native to Taiwan, mainland China, Vietnam, and Australia and is used as a folk medicine for treating diabetes and symptoms associated with inflammation.[1,3] Despite its ethnopharmacological significance, no chemical investigation on *C. japonicum* var. *australe* is available in the scientific literature. However, this plant is frequently mixed up with *Cirsium japonicum* DC. ("Japanese thistle"), which has been utilized as a traditional Chinese medicine for the treatment of hypertension, traumatic hemorrhage, and inflammation.[4] Previous biological studies have indicated that flavones from *C. japonicum* enhanced the differentiation of adipocytes and their glucose uptake activity.[5−7]

The absence of prior phytochemical studies on *C. japonicum* var. *australe* encouraged the present phytochemical investigation to reveal its secondary metabolites and then to determine their biological activities. Bioactivity-guided fractionation of the whole plant ethanolic extract led to the isolation of three new acetylenic phenylacrylic acid esters (**1**–**3**), two new polyacetylenes (**4** and **5**), and seven known compounds (**6**–**12**). Compounds **1**–**3** are esters of a polyacetylene unit and a phenylacrylic acid. The structures of the new compounds were established by means of spectroscopic data interpretation. The absolute configurations of **4** and **7** were determined by CD and by Mosher's experiments. The isolated compounds were evaluated for their antidiabetic and antioxidant activities.

RESULTS AND DISCUSSION

The fresh material of the whole plant of *C. japonicum* var. *australe* was extracted with 90% aqueous EtOH to yield a crude extract, which was then subjected to partition with solvents of different polarities. Among them, the EtOH extract exhibited

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Chart 1

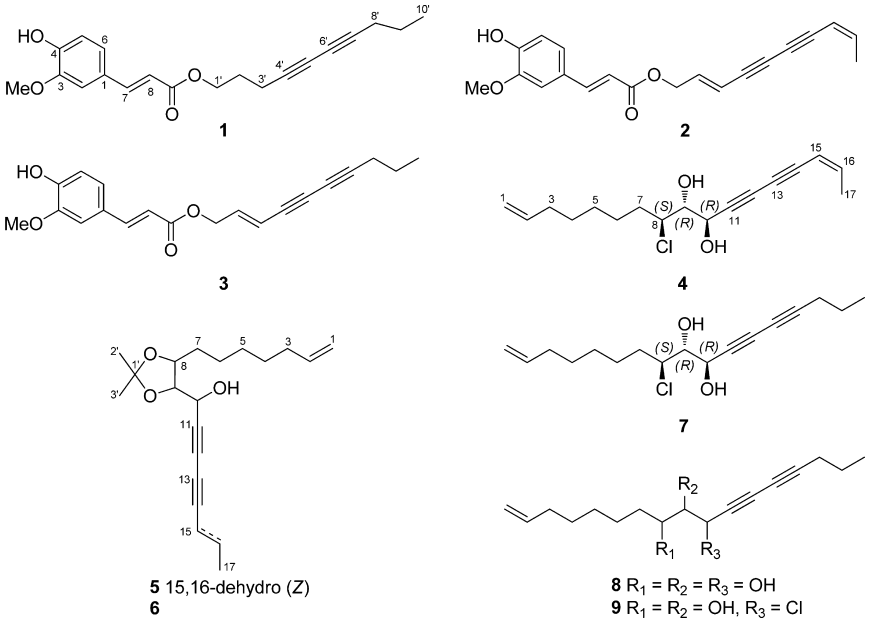

Table 1. ^{1}H NMR and ^{13}C NMR Data for 1−3[a]

	1		2		3	
position	δ_C mult.	δ_H (J in Hz)	δ_C mult.	δ_H (J in Hz)	δ_C mult.	δ_H (J in Hz)
1	127.1, C		127.0, C		126.9, C	
2	109.5, CH	7.03, d (1.8)	109.5, CH	7.03, d (1.7)	109.5, CH	7.02, d (1.3)
3	146.9, C		146.9, C		146.9, C	
4	148.1, C		148.3, C		148.3, C	
5	114.8, CH	6.92, d (8.2)	114.9, CH	6.92, d (8.2)	114.9, CH	6.92, d (8.2)
6	123.3, CH	7.08, dd (8.2, 1.8)	123.4, CH	7.09, dd (8.2, 1.7)	123.4, CH	7.07, dd (8.2, 1.3)
7	145.1, CH	7.61, d (15.9)	145.8, CH	7.64, d (15.9)	145.7, CH	7.63, d (15.9)
8	115.5, CH	6.28, d (15.9)	114.9, CH	6.30, d (15.9)	114.8, CH	6.29, d (15.9)
9	167.3, C		166.7, C		166.7, C	
1′	63.1, CH_2	4.28, t (6.2)	63.7, CH_2	4.77, dd (5.7, 1.6)	63.8, CH_2	4.74, dd (5.8, 1.5)
2′	27.8, CH_2	1.93 m	139.8, CH	6.37, dt (15.9, 5.7)	139.5, CH	6.33, m
3′	16.4, CH_2	2.42, tt (7.0, 1.0)	112.1, CH	5.90, d (15.9)	112.3, CH	5.82, d (15.9)
4′	75.9, C		79.1, C		75.9, C	
5′	66.2, C		75.5, C		72.5, C	
6′	65.4. C		64.9, C		65.2, C	
7′	78.1, C		79.5, C		85.1, C	
8′	21.3, CH_2	2.22, tt (7.0, 1.1)	109.2, CH	5.57, d (10.9)	21.9, CH_2	2.30, t (7.0)
9′	21.9, CH_2	1.53, m	143.4, CH	6.17, dq (10.9, 6.9)	21.7, CH_2	1.57, h (7.3)
10′	13.6, CH_3	0.98, t (7.4)	16.7, CH_3	1.93, dd (6.9, 1.6)	13.6, CH_3	1.00, t (7.4)
O-CH_3	56.1, CH_3	3.93, s	56.1, CH_3	3.94, s	56.1, CH_3	3.92, s

[a]Compound **1** was measured at 600 MHz; compounds **2** and **3** were measured at 400 MHz. For all compounds NMR spectra were obtained in $CDCl_3$; J values (Hz) are given in parentheses. Assignments were made based on the analysis of ^{1}H−^{1}H COSY, HSQC, and HMBC data.

the most potent biological activity. Hence, this extract was selected for further bioactivity-guided isolation.

The fractionation was initiated with polyamide column chromatography using gradient elution with different solvent mixtures possessing a broad polarity range. Elution started with 10% aqueous MeOH, which was increased gradually to 100% MeOH, and then CH_2Cl_2 was added to the solvent system at increasing percentages to reach 100% CH_2Cl_2, resulting in the separation of nine pooled fractions. The active fractions were subjected to different chromatographic techniques such as centrifugal partition chromatography, column chromatography on silica, C_{18}, and Sephadex LH-20 as well as HPLC for final purification. Five new polyacetylenes (**1**−**5**) and seven known compounds, 8-(hept-1-enyl)-1′,1′-dimethyl-8,9-dioxolan-9-yl)-octa-11,13-diyn-1-ol (**6**),[8] ciryneol C (**7**),[9] heptadeca-1-ene-11,13-diyne-8,9,10-triol (**8**),[9] ciryneol H (**9**),[10] ethyl caffeate (**10**),[11] apigenin (**11**),[12] and scopoletin (**12**),[13] were isolated.

Compound **1** was obtained as a pale yellow, amorphous solid. It exhibited a molecular ion peak $[M + Na]^+$ at m/z 349.1413 in the HRESIMS, corresponding to the molecular formula $C_{20}H_{22}O_4$. The IR spectrum showed a hydroxy group (3406 cm^{-1}), an ester carbonyl (1700 cm^{-1}), a conjugated acetylene (2200 cm^{-1}), and an aromatic (1590 and 1509 cm^{-1}) absorption band. The UV absorption maxima at 237, 281, 296,

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and 317 nm suggested the presence of a polyacetylene moiety and a conjugated phenyl group.[14,15]

In the ^{1}H NMR spectrum of **1**, signals for an ABX system (δ_H 6.92, d, J = 8.2 Hz; δ_H 7.03, d, J = 1.8 Hz; δ_H 7.08, dd, J = 8.2, 1.8 Hz) of a phenyl moiety, a *trans*-double bond (δ_H 7.61, d, J = 15.9 Hz; δ_H 6.28, d, J = 15.9 Hz), a methoxy group (δ_H 3.93, 3H, s), a terminal methyl (δ_H 0.98, 3H, t, J = 7.4 Hz), an oxymethylene (δ_H 4.28, 2H, t, J = 6.2 Hz), and four methylenes (δ_H 2.42, 2.22, 1.93 and 1.53) were observed (Table 1). The ^{13}C and DEPT spectra suggested the presence of two methyls, five methylenes, five methines, and eight quaternary carbons including four C−C triple-bond carbons (δ_C 78.1, 75.9, 66.2, and 65.4) (Table 1). On the basis of the information from the HSQC and COSY correlations, a trimethylene unit (δ_H 4.28 for H-1′, δ_H 1.93 for H-2′, and δ_H 2.42 for H-3′) and a propyl group (δ_H 2.22 for H-8′, δ_H 1.50 for H-9′, δ_H 0.98 for H-10′) were suggested (Figure 1). In the HMBC spectrum, the

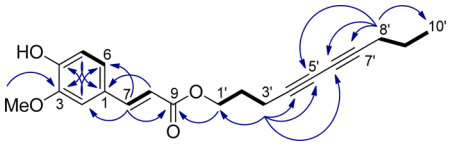

Figure 1. Key COSY (H−H) and HMBC (H→C) correlations of **1**.

correlations of H-8′ (δ_H 2.22) to C-6′ (δ_C 65.4) and C-7′ (δ_C 78.1) and of H-3′ (δ_H 2.42) to C-4′ (δ_C 75.9) and C-5′ (δ_C 66.2) indicated that a diyne unit (C-4′, C-5′, C-6′, and C-7′) may be placed between the trimethylene unit and the propyl group, forming a deca-4,6-diynyl moiety in **1** (Figure 1). Moreover, the HMBC correlations of H-7 (δ_H 7.61) to C-2 (δ_C 109.5), C-6 (δ_C 123.3), and C-9 (δ_C 167.3), H-5 (δ_H 6.92) to C-1 (δ_C 127.1) and C-3 (δ_C 146.9), H-8 (δ_H 6.28) to C-1 (δ_C 127.1), and H-2 (δ_H 7.03) to C-4 (δ_C 148.1) and C-6 (δ_C 123.3) revealed the presence of a 3,4-substituted phenylacrylic ester moiety (Figure 1). The methoxy group (δ_H 3.93) showed an HMBC correlation to C-3 (δ_C 146.9), which along with the NOESY correlation between the methoxy proton and H-2 (δ_H 7.03) suggested the presence of a 4-hydroxy-3-methoxyphenyl acrylic ester moiety. The HMBC correlations between H-1′ (δ_H 4.28) and the ester carbonyl C-9 (δ_C 167.3) confirmed that the deca-4,6-diyne moiety is linked to the 4-hydroxy-3-methoxyphenyl acrylic ester (Figure 1). The structure of **1** was elucidated as (*E*)-deca-4,6-diynyl 3-(4-hydroxy-3-methoxyphenyl)acrylate and was given the trivial name cirsiumyne A.

Compound **2** (cirsiumyne B) was isolated as a pale yellow, amorphous solid, and it was assigned the molecular formula $C_{20}H_{18}O_4$ from the HRESIMS at m/z 345.1102 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_4Na$, 345.1103). The IR spectrum indicated the presence of a hydroxy group (3406 cm^{-1}), a conjugated acetylene (2200 cm^{-1}), an ester carbonyl (1709 cm^{-1}), and aromatic (1590 and 1514 cm^{-1}) units. The UV absorption maxima at 238 and 316 nm suggested the presence of a phenyl moiety possibly conjugated to a carbonyl group. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1** (Table 1). Instead of the four methylenes (δ_H 1.53, 1.93, 2.22, and 2.42) in **1**, the 1D NMR spectra of **2** suggested the presence of a *cis*-disubstituted double bond [δ_H 5.57 (d, J = 10.9 Hz) and δ_H 6.17 (dq, J = 10.9, 6.9 Hz)] and an additional *trans*-disubstituted double bond [δ_H 6.37 (dt, J = 15.9, 5.7 Hz) and δ_H 5.90 (d, J = 15.9 Hz)] (Table 1). The COSY

Table 2. ^{1}H NMR Chemical Shifts (δ) of Compounds 4−7[a]

	4	5	6	7
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	5.00, ddt (17.0, 1.8, 1.7)	4.99, ddt (17.0, 1.7, 1.6)	4.99, ddt (17.0, 1.2, 1.1)	5.00, ddt (17.0, 1.7, 1.6)
	4.94, ddt (10.2, 2.2, 1.7)	4.93, ddt (10.2, 1.7, 1.3)	4.92, ddt (10.3, 2.4, 1.2)	4.94, ddt (10.2, 2.3, 1.6)
2	5.80, ddt (17.0, 10.2, 6.8)	5.80, ddt (17.0, 10.2, 6.8)	5.82, ddt (17.0, 10.1, 6.7)	5.80, ddt (17.0, 10.2, 6.7)
3	2.06, 2H, m	2.05, 2H, m	2.07, 2H, m	2.05, 2H, m
4a	1.40, m	1.42, m	1.42, m	1.42−1.38,[b] m
4b	1.35, m	1.35, m		
5	1.40, 2H, m	1.42, 2H, m	1.42, 2H, m	1.44, 2H, m
6a	1.75−1.90,[b] 2H, m	1.62, m	1.38−1.44,[b] 2H, m	1.38−1.48,[b] 2H, m
6b		1.56, m		
7	1.83, 2H, m	1.63, 2H, m	1.73, 2H, m	1.81, 2H, m
8	4.30, ddd (8.9, 5.0, 4.2)	4.07, td (8.0, 3.8)	4.00, td (8.1, 3.4)	4.28, ddd (10.2, 4.9, 4.0)
9	3.74, dd (5.5, 4.2)	3.81, dd (7.9, 3.8)	3.67, ddd (7.6, 3.4, 5.0)	3.71, dd, br d (6.2, 6.0, 4.0)
10	4.59, d (5.5)	4.61, dd (7.9, 4.3)	4.41, d (5.0)	4.51, d (6.0)
11				
12				
13				
14				
15	5.53, dq (10.9, 1.7)	5.52, dq (10.8. 1.7)	2.28, 2H, td (6.9, 0.7)	2.26, 2H, td (7.0, 0.8)
16	6.20, dq (10.9, 6.9)	6.20, dq (10.8, 6.9)	1.55, 2H, m	1.55, 2H, m
17	1.93, 3H, dd (6.9, 1.7)	1.92, 3H, dd (6.9, 1.7)	1.00, 3H, t (7.4)	0.99, 3H, t (7.4)
1′				
CH_3-1′a		1.43, 3H, s	1.43, 3H, s	
CH_3-1′b		1.42, 3H, s	1.42, 3H, s	

[a]Compounds **4**, **6**, and **7** were measured at 400 MHz; compound **5** was measured at 600 MHz. For all compounds NMR spectra were obtained in $CDCl_3$; J values (Hz) are given in parentheses. Assignments were made based on the analysis of ^{1}H−^{1}H COSY, HSQC, and HMBC data. [b]Multiplicity patterns are unclear due to signal overlapping.

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correlations between the methylene protons adjacent to the allylic and ester functionalities at δ_H 4.77 (H-1′) and the *trans*-disubstituted olefinic proton at δ_H 6.37 (H-2′) and between the terminal methyl proton (δ_H 1.93, dd, J = 6.9, 1.6 Hz, H-10′) and the *cis*-disubstituted olefinic group (δ_H 6.17, dq, J = 10.9, 6.9 Hz, H-9′) indicated the location of the two double bonds. The above data, along with the HMBC correlations of H-2′/C-4′, H-3′/C-5′, and H-10′/C-7′, suggested that **2** contains a diendiynyl moiety between C-1′ and C-10′. Moreover, the 1D and 2D NMR spectra revealed that this compound also possesses the same 4-hydroxy-3-methoxyphenyl acrylic ester moiety as in **1**. The HMBC cross-peak between H-1′ (δ_H 4.77) and the ester carbonyl C-9 (δ_C 166.7) was used to deduce the structure of **2**, and it was identified as (*E*)-((2*E*,8*Z*)-deca-2,8-dien-4,6-diynyl) 3-(4-hydroxy-3-methoxyphenyl)acrylate.

Compound **3** was isolated as a pale yellow, amorphous solid and was assigned the molecular formula $C_{20}H_{20}O_4$, as evidenced by the HRESIMS at m/z 347.1257 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_4Na$, 347.1259), suggesting one additional degree of unsaturation when compared to **1**. The UV absorptions at 269, 286, and 319 nm in combination with an IR band at 2229 cm^{-1} suggested the presence of a polyacetylene functionality and a conjugated phenyl group.[14,15] The IR absorptions at 3511 (hydroxy group), 2229 (conjugated acetylene), 1704 cm^{-1} (ester carbonyl), and 1590/1514 cm^{-1} (aromatic hydrocarbons) of **3** were similar to those of **1** and **2**. The ^1H and ^{13}C NMR spectroscopic data revealed the presence of a similar acrylate moiety to that present in **1** and **2** (Table 1). In addition, the 1D and 2D NMR spectra demonstrated that **3** possesses the same 4-hydroxy-3-methoxyphenyl acrylic ester moiety as in **1**. In comparison with the ^1H NMR data of **1**, the only difference between these two compounds was in the presence of an additional *trans*-olefinic group [δ_H 5.82 (d, J = 15.9 Hz) and δ_H 6.33 (m)] in **3**, in agreement with the observation of the one additional degree of unsaturation. The COSY correlation from the *trans*-olefinic proton (H-2′) to the oxymethylene proton (δ_H 4.74, H-1′), along with the HMBC correlations of H-1′ (δ_H 4.74) to C-3′ (δ_C 146.9), H-3′ (δ_H 5.82) to C-5′ (δ_C 72.5), and H-8′ (δ_H 2.30) to C-6′ (δ_C 65.2) and C-7′ (δ_C 85.1), revealed an endiyne moiety between C-1′ and C-8′. Therefore, compound **3** (cirsiumyne C) was determined as (*E*)-[(*E*)-deca-2-en-4,6-diynyl] 3-(4-hydroxy-3-methoxyphenyl)acrylate.

Compound **4** was isolated as a colorless oil. Its HRESIMS showed a sodiated molecular ion peak at m/z 317.1282 $[M + Na]^+$ (calcd for $C_{17}H_{23}ClO_2Na$, 317.1284), which, together with the ^{37}Cl isotope at m/z 319.1268 $[M + 2 + Na]^+$ (calcd for $C_{17}H_{23}ClO_2Na$, 319.1255), revealed the presence of a chlorine atom. The IR spectrum indicated the presence of a hydroxy group (3397 cm^{-1}), conjugated acetylene (2229 cm^{-1}), and terminal vinyl (1638 and 909 cm^{-1}) absorptions. The UV absorption maxima at 255, 269, and 285 nm suggested that compound **4** contains an ene-diynyl moiety.[14,16] In the ^1H NMR spectrum, a terminal methyl resonance at δ_H 1.93 (dd, J = 6.9, 1.7 Hz, H-17), a terminal vinyl at δ_H 5.00 (ddt, J = 17.0, 1.8, 1.7 Hz, H-1a), 4.94 (ddt, J = 10.2, 2.2, 1.7 Hz, H-1b), and 5.80 (ddt, J = 17.0, 10.2, 6.8 Hz, H-2), and three downfield shifted methine protons (δ_H 4.30, 3.74, and 4.59) were observed. The ^{13}C, DEPT, and HSQC spectra indicated the presence of two C−C triple bonds (δ_C 80.0, 71.9, 76.5, and 77.4), similar to those present in **7**.[9] The ^1H and ^{13}C NMR spectra of **4** were comparable to those of **7** (Tables 2 and 3), a compound previously isolated from *C. japonicum*.[9] Instead of the seven methylenes in **7**, the 1D NMR spectra of **4** revealed the presence of five methylenes (δ_C 33.8, 28.8, 28.6, 26.4, and 34.6) and an additional *cis*-disubstituted double bond [δ_H 5.53 (dq, J = 10.9, 1.7 Hz) and δ_H 6.20 (dq, J = 10.9, 6.9 Hz)]. The chemical shift of C-8 in **4** was shifted upfield to δ_C 64.6, and its proton was shifted downfield to δ_H 4.30, suggesting that the chlorine atom is attached to C-8.[8,9] The COSY correlations of the methine protons revealed the spin system of the 8-chloropropane-9,10-diol (Figure 2). The HMBC correlations of H-9/C-7, C-8, C-10, and C-11 and of H-10/C-11 confirmed that the chlorine atom is connected to C-8 (Figure 2). On the basis of the COSY correlations of H-1a (δ_H 5.00) and H-1b (δ_H 4.94)/H-2 (δ_H 5.80), H-16 (δ_H 6.20)/H-17 (δ_H 1.93), and H-16 (δ_H 6.20)/H-15 (δ_H 5.53), a terminal vinyl and a propenyl group were assigned at both ends of the molecule of **4** (Figure 2). The above data, together with the HMBC correlations of H-3/C-1, C-2, C-4 and C-5, H-7/C-5 and C-9, H-9/C-8 and C-11, H-10/C-11, and H-17/C-13, C-14, C-15 and C-16, suggested that the ene-diyne unit may be placed between C-10 and C-17 (Figure 2). Thus, the planar structure of **4** was assigned as (*Z*)-8-chloroheptadeca-1,15-diene-11,13-diyne-9,10-diol.

On comparing the coupling constants and NOE correlations of **4** and its analogue **7**, it was confirmed that the relative configurations of these compounds are identical. Furthermore,

Table 3. ^{13}C NMR Chemical Shifts (δ) of Compounds 4−7[a]

	4	5	6	7
position	δ_C, mult.	δ_C, mult.	δ_C, mult.	δ_C, mult.
1	114.6, CH_2	114.4, CH_2	114.8, CH_2	114.6, CH_2
2	139.0, CH	139.2, CH	140.1, CH	139.0, CH
3	33.8, CH_2	34.0, CH_2	34.8, CH_2	33.8, CH_2
4	28.8, CH_2	25.4, CH_2	29.9, CH_2	28.5, CH_2
5	28.6, CH_2	33.8, CH_2	30.1, CH_2	28.8, CH_2
6	26.4, CH_2	26.0, CH_2	26.9, CH_2	26.3, CH_2
7	34.6, CH_2	33.8, CH_2	35.1, CH_2	34.6, CH_2
8	64.6, CH	77.4, CH	79.4, CH	64.5, CH
9	75.7, CH	82.7, CH	84.4, CH	75.8, CH
10	64.8, CH	63.4, CH	64.0, CH	64.6, CH
11	80.0, C	87.2, C	75.2, C	72.9, C
12	71.9, C	70.8, C	71.5, C	72.2, C
13	76.5, C	67.7, C	65.5, C	64.4, C
14	77.4, C	72.7, C	82.0, C	82.5, C
15	108.7, CH	108.9, CH	21.7, CH_2	21.4, CH_2
16	144.2, CH	144.0, CH	22.8, CH_2	21.7, CH_2
17	16.8, CH_3	16.6, CH_3	13.7, CH_3	13.6, CH_3
1′		109.0, C	110.3, C	
CH_3-1′a		29.9, CH_3	27.9, CH_3	
CH_3-1′b		27.7, CH_3	27.2, CH_3	

[a]Compounds **4**, **6**, and **7** were measured at 100 MHz; compound **5** was measured at 150 MHz. For all compounds NMR spectra were obtained in $CDCl_3$. Multiplicity was obtained from the DEPT spectrum. Assignments were based on HSQC and HMBC NMR spectra.

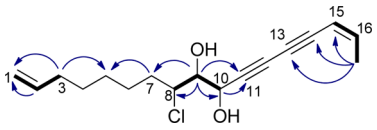

Figure 2. Key COSY (H−H) and HMBC (H→C) correlations of **4**.

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the positive Cotton effect at 220 nm and the negative Cotton effects around 240−270 nm suggested similar absolute configurations for **4** and **7** (Figures S1 and S2, Supporting Information). Unfortunately, the yield of compound **4** was extremely small. Accordingly, **7** was selected for processing with the modified Mosher's method to reveal the absolute configuration of the two analogues.[19,20] Treatment of **7** with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [(*R*)-MTPA-Cl] and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride [(*S*)-MTPA-Cl] in deuterated pyridine afforded the (*S*)- and (*R*)-bis-MTPA ester derivatives (**7s** and **7r**).[19,20] The Δ_{S-R} values were calculated, and the absolute configuration of **7** was found to be 9*R* and 10*R* (Figure S5, Supporting Information).[21,22] In addition, the absolute configuration of **7** was confirmed by electronic circular dichroism (ECD) calculation and circular dichroism (CD) analysis. The experimental CD spectrum of **7** exhibited a positive Cotton effect at 220 nm and two negative Cotton effects around 240−270 nm (Figure S2, Supporting Information), which was in agreement with the calculated ECD spectrum of **7** with an 8*S*, 9*R*, 10*R* configuration (Figures S2 and S4, Supporting Information). Thus, the absolute configuration of **7** was determined as 8*S*, 9*R*, and 10*R*. Therefore, compound **4** (cirsiumyne D) was proposed as (8*S*,9*R*,10*R*,*Z*)-8-chloroheptadeca-1,15-diene-11,13-diyne-9,10-diol.

Compound **5** was obtained as a colorless oil, and it was assigned the molecular formula $C_{20}H_{28}O_3$ from the HRESIMS, $[M + Na]^+$ ion peak at *m*/*z* 339.1933 (calcd for $C_{20}H_{28}O_3Na$, 339.1936), indicating seven degrees of unsaturation. The IR spectrum demonstrated the presence of a hydroxy group (3425 cm^{-1}), a conjugated acetylene (2229 cm^{-1}), and terminal vinyl (1642 and 904 cm^{-1}) functionalities. UV absorptions at 254, 268, and 284 nm were consistent with the presence of an ene-diyne moiety.[16] In the 1D NMR spectra, three methyls [δ_H 1.92 (H-17), δ_H 1.43 (H-2′), and δ_H 1.42 (H-3′)], a *cis*-disubstituted double bond [δ_H 5.52 (dq, *J* = 10.8, 1.7 Hz, H-15) and δ_H 6.20 (dq, *J* = 10.8, 6.9 Hz, H-16)], a terminal double bond [δ_H 4.99 (ddt, *J* = 17.0, 1.7, 1.6 Hz, H-1a), δ_H 4.93 (ddt, *J* = 10.2, 1.7, 1.3 Hz, H-1b), and δ_H 5.80 (ddt, *J* = 17.0, 10.2, 6.8 Hz, H-2)], and three oxymethine carbons (δ_C 77.4 for C-8, δ_C 82.7 for C-9, and δ_C 63.4 for C-10) were observed (Table 3), suggesting a similar skeleton to that of **6**.[8] On comparing their mass and 1D NMR spectra, compound **5** showed one more degree of unsaturation than **6**, which was in agreement with the presence of an additional *cis*-olefinic group (Tables 2 and 3). The COSY correlation between one of the *cis*-olefinic protons at δ_H 6.20 and the terminal methyl at δ_H 1.93 (H-17) revealed the presence of a propenyl group. The HMBC correlations of the methylene proton δ_H 2.05 (H-3) to the terminal vinyl group δ_C 114.4 and δ_C 139.2 (C-1 and C-2) as well as the methyl protons δ_H 1.43 and 1.42 (H-2′ and H-3′) to δ_C 109.0 (C-1′) were consistent with the presence of propene and an acetonide moiety as found in **6**.[8] The EIMS fragment peaks at *m*/*z* 89 and *m*/*z* 65 correlated with the presence of a hepta-5-ene-1,3-diyne fragment (Figures S7 and S8, Supporting Information), which suggested that the diyne unit may be placed at C-11 to C-14 and is connected to the propenyl group (C-15 to C-17). In addition, ion peaks at *m*/*z* 197 and *m*/*z* 119 confirmed that **5** possesses 4-(hept-6-enyl)-2,2-dimethyl-1,3-dioxolane and octa-6-ene-2,4-diyn-1-ol moieties (Figures S7 and S8, Supporting Information).

The relative stereochemistry of **5** could be proposed from the NOESY spectrum and the ^1H NMR coupling constants of H-8, H-9, and H-10 (Table 2). The NOESY correlations of H-9/H-8/H-10/CH_3-1, H-7/CH_3-1/H-10, and H-8/H-9/H-10 showed that H-8 and H-9 have a *syn*/*erythro* relationship (Figure 3). Moreover, the NOESY spectrum showed a

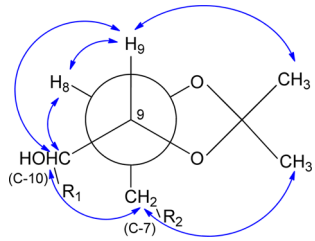

Figure 3. Newman projection of the C-8−C-9 center illustrating the key NOE correlations indicating the stereochemistry of **5** and **6**.

correlation from H-8 to H-10, but no cross-peak between H-10 and CH_3-1, which suggested that H-8 and H-10 are *syn* positioned (Figure 3). Comparing the NOESY spectroscopic data of **5** and **6** confirmed that the relative configurations of these compounds are the same. Moreover, the CD spectrum of **5** showed two positive Cotton effects at 220 and 240 nm and three negative Cotton effects at 246, 260, and 275 nm (Figures S5 and S6, Supporting Information), which were similar to those of **6**, suggesting a similar absolute configuration. Again, the isolated amount of **5** was insufficient to determine its absolute configuration, so its analogue **6** was subjected to the modified Mosher's method.[19,20] Unfortunately, the data were not clear and precluded the determination of the C-10 absolute configuration (the values of $\Delta\delta_{S-R}$ were negative at C-9 and positive at C-8) (Figure S40, Supporting Information). Thus, the structure of **5** (cirsiumyne E) was identified as shown.

The potential antidiabetic effect of the compounds isolated was determined by the percentage of glucose consumption in 3T3-L1 adipocytes.[17] Certain compounds were isolated in minor quantities not sufficient for biological screening, and thus only compounds **8**, **10**, and **11** were selected for the evaluation of their in vitro hypoglycemic activity. The crude extract and its EtOH layer promoted glucose uptake activity in 3T3-L1 adipocytes (Figure 4). Previously, the antidiabetic activity of *Cirsium* was attributed to its flavonoid content.[5−7] However, only two pure flavones from *C. japonicum*, pectolinarin and 5,7-dihydroxy-4′,6-dimethoxyflavone, were investigated for their potential antidiabetic activity, and both showed PPARγ activation.[6] Polyacetylenes from this genus have never been evaluated for their antidiabetic activity. According to the results obtained, heptadeca-1-ene-11,13-diyne-8,9,10-triol (**8**) and ethyl caffeate (**10**) showed significant in vitro hypoglycemic activity, while apigenin (**11**) decreased the glucose uptake of 3T3-L1 adipocytes (Figure 4). The 3T3-L1 adipocytes were stained with Oil Red O after coculturing with the test samples for 7 days. Compound **8** increased glucose consumption in adipocytes without any promotion of lipid accumulation (Figure 5). Furthermore, the major compound (**10**) showed significant DPPH and $ABTS^+$ scavenging activities with EC_{50} values of 0.18 and 0.19 μg/mL, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with JASCO DIP-370 and P-1020 digital polarimeters. UV

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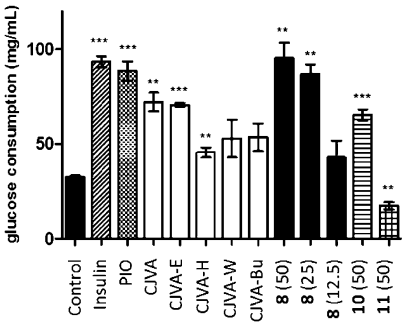

Figure 4. Effects of *C. japonicum* var. *australe* extracts and pure compounds on the glucose consumption of 3T3-L1 adipocytes. 3T3-L1 cells were treated with CJVA (*C. japonicum* var. *australe* ethanolic extract, 50 μg/mL), CJVA-E (ethanolic layer from CJVA, 50 μg/mL), CJVA-H (*n*-hexane layer from CJVA, 50 μg/mL), CJVA-W (water layer from CJVA, 50 μg/mL), CJVA-Bu (butanol layer from CJVA, 50 μg/mL), heptadeca-1-ene-11,13-diyne-8,9,10-triol (**8**) (50, 25, 12.5 μg/mL), ethyl caffeate (**10**) (50 μg/mL), and apigenin (**11**) 50 μg/mL. DMSO (0.1%, v/v) was used as the control. Insulin (3.2×10^{-7} M) and pioglitazone (PIO, 50 μg/mL) were selected as the positive controls. The data are expressed as mean ± SEM of four independent experiments. **: $p < 0.01$, ***: $p < 0.001$ compared to the control. The glucose consumption data showed no significant difference between **8** (25 and 50 μg/mL) and insulin. This means that **8** showed equal hypoglycemic activity to insulin.

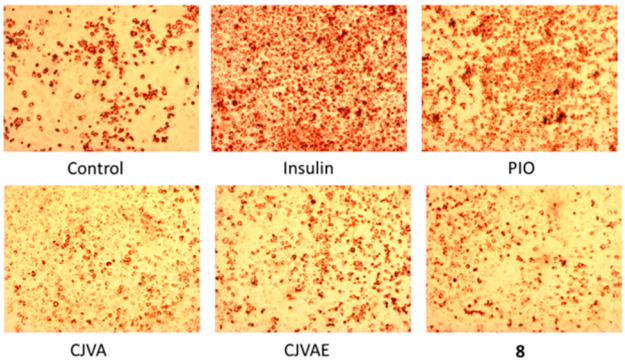

Figure 5. Effect of the extracts and pure compound **8** from *C. japonicum* var. *australe* on lipid accumulation in 3T3-L1 adipocytes. 3T3-L1 cells were treated with CJVA (20 μg/mL), CJVAE (20 μg/mL), and compound **8** (20 μg/mL) for 7 days. Insulin (3.2×10^{-7} M) and PIO (pioglitazone, 50 μg/mL) were taken as positive controls. Cells were fixed and stained with Oil Red O dye. Images of the representative cells were scanned and captured with a microscope (40× magnification).

spectra were obtained using JASCO UV-530 ultraviolet spectrophotometers. IR spectra were obtained on a PerkinElmer FTIR-spectrometer and Spectrum 2000 spectrophotometer. The CD spectra were obtained using a JASCO J-810 spectrophotometer. NMR (400 and 600 MHz) spectra were obtained on a Varian Unity 400 MHz FT-NMR, a JEOL JNM ECS 400 MHz FT-NMR, and a Varian Unity 600 MHz FT-NMR. ESIMS data were collected on a VG Biotech Quattro 5022 mass spectrometer. HRESIMS data were obtained on a Bruker APEX II spectrometer (FT-ICR/MS, FTMS) (Bruker Daltonics Inc., Billerica, MA, USA). MN polyamide SC-9 gel was purchased from ICN Biomedicals GmbH (Eschwege, Germany). Analytical and preparative TLC was performed on silica gel 60 F_{254} plates (Merck KGaA, Darmstadt, Germany), and spots were visualized by UV and by spraying with vanillin sulfuric acid reagent followed by heating for 5 min. Rotational planar chromatography (RPC) was performed on a Chromatotron apparatus (Harrison Research, Palo Alto, CA, USA). Centrifugal partition chromatography (CPC) was performed on an SCPC-250 instrument from Armen Instrument (Armen, Saint-Ave, France) with a 250 mL column volume. For the preparative reversed-phase HPLC, a Phenomenex Gemini-NX C_{18} (110A, 5 μm, 250 × 10.0 mm; Phenomenex Inc., Torrance, CA, USA), a Zorbax Eclipse XDB-C_8 (5 μm, 250 × 9.4 mm; Agilent Technologies Inc., Santa Clara, CA, USA), or a Merck Hibar Purospher STAR C_{18} (5 μm, 250 × 10.0 mm) semipreparative column (Merck KGaA, Darmstadt, Germany) was used, and HPLC equipment consisted of two JASCO PU-2080 HPLC pumps connected to a JASCO MD-2010 Plus multiwavelength detector (JASCO Inc., Tokyo, Japan). The instrumentation for normal-phase HPLC (NP-HPLC) consisted of dual Shimadzu LC-10AT pumps and a Shimadzu SPD-10A UV−vis detector (Shimadzu Inc., Kyoto, Japan), and a Phenomenex Luna CN (5 μm, 250 × 10.0 mm) semipreparative column (Phenomenex Inc., Torrance, CA, USA) was used.

Plant Material. The fresh plant of *C. japonicum* var. *australe* was collected from Puli, Nantou County, Taiwan, in November 2011. The material was identified by Dr. Ming-Hong Yen, and a voucher specimen (voucher number: Cirsium 01-1) was deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The fresh whole plants of *C. japonicum* var. *australe* (12.0 kg) were extracted with 90% aqueous EtOH (20 L × 4) at room temperature and then concentrated at 37 °C under reduced pressure. The crude extract (320.0 g) was partitioned with 10% aqueous MeOH (2 L × 3) and EtOAc (2 L × 3) to yield an aqueous layer (233.8 g), an insoluble portion (8.0 g), and an EtOAc layer (77.4 g). The EtOAc layer was then partitioned between *n*-hexane (2 L) and 90% aqueous EtOH (2 L × 3) to provide an *n*-hexane layer (38.2 g) and an EtOH layer (30.9 g). Among the layers obtained, the EtOH layer exhibited the most potent potential hypoglycemic, DPPH scavenging, and xanthine oxidase inhibitory activities and was selected for further bioactivity-guided fractionation.

The EtOH layer (29.8 g) was subjected to polyamide column chromatography (20.0 × 25.5 cm) under a gradient elution of 10% aqueous MeOH to pure MeOH and then increasing ratios of CH_2Cl_2 in MeOH to 100% CH_2Cl_2 to yield nine fractions (Fr. 1−9). The DPPH scavenging and xanthine oxidase inhibitory assays revealed the most potent fractions to be Fr. 5 (2.8 g) and Fr. 6 (6.7 g). Accordingly, Fr. 5 was eluted with CH_2Cl_2−MeOH (50:1 → 10:1) from silica gel to obtain 10 subfractions (Fr. 5-1−5-10).

Fraction 5-4 (125.0 mg) was subjected to CPC using a solvent system of *n*-hexane−EtOAc−MeOH−water (4:1:4:1) in the ascending mode to give 12 subfractions (Fr. 5-4-1−5-4-12). Among them, Fr. 5-4-4 (2.7 mg) was purified utilizing normal-phase HPLC (CN) (*n*-hexane−CH_2Cl_2−MeOH, 100:10:1; flow rate 2.2 mL/min, UV 254 nm, 5 μm, 250 × 10.0 mm column) to obtain compounds **6** (1.2 mg) and **5** (1.0 mg). Fraction 5-4-5 (1.3 mg) was purified with normal-phase HPLC (CN) (flow rate 2.0 mL/min, UV 254 nm, 5 μm, 250 × 10.0 mm column) in *n*-hexane−CH_2Cl_2−MeOH (130:10:1) to obtain compound **6** (0.5 mg).

Fraction 5-5 (138.9 mg) was subjected to RPC eluting with *n*-hexane−EtOAc−EtOH (200:10:0.5 → 1:2:0.1), and Fr. 5-5-4 (13.7 mg) was purified by reversed-phase HPLC (C_{18}) (3 mL/min, PDA, 250 × 10.0 mm column) eluting with 66% aqueous ACN to give an additional amount of compound **6** (5.5 mg). Fraction 5-5-18 (30.1 mg) was purified by reversed-phase HPLC (C_8) (3 mL/min, PDA, 250 × 9.4 mm column) eluting with 43% aqueous MeOH to obtain compound **10** (8.5 mg).

Fraction 5-7 was purified by CPC in *n*-hexane−EtOAc (1:1, 1 L) and MeOH−H_2O (1:1, 250 mL) to give compound **8** (94.0 mg) and subfractions Fr. 5-7-1−5-7-14. Then, the pooled subfractions Fr. 5-7-7 and 5-7-8 (56.7 mg) were eluted from a Sephadex LH-20 column by 98% aqueous MeOH to obtain compound **10** (45.1 mg).

Fraction 6 (2.8 g) was chromatographed on a silica gel open column with a gradient solvent system, from pure *n*-hexane to CH_2Cl_2−MeOH (50:3), to yield subfraction Fr. 6-6 (548.5 mg) eluted with *n*-hexane−CH_2Cl_2 (1:6). This fraction was loaded on a Sephadex LH-20 column and eluted with CH_2Cl_2−MeOH (3:1), to yield eight subfractions (Fr. 6-6-1−6-6-8). Among them, Fr. 6-6-4 (245.8 mg)

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was purified using silica gel and eluted with a gradient of *n*-hexane−CH_2Cl_2 (2:3) to *n*-hexane−CH_2Cl_2−MeOH (10:30:1) to obtain subfractions Fr. 6-6-4-5 (39.5 mg) and Fr. 6-6-4-6 (6.14 mg). Then, normal-phase HPLC (CN) (2.2 mL/min, UV 254 nm, 5 μm, 250 × 10.0 mm column) was utilized by eluting with *n*-hexane−CH_2Cl_2−MeOH (40:10:1) to give pure compounds **7** (5.1 mg) and **4** (0.5 mg) from subfraction 6-6-4-5. In addition, normal-phase HPLC eluting with *n*-hexane−CH_2Cl_2−MeOH (100:10:1) gave the pure compounds **9** (1.2 mg) and **7** (1.0 mg) from fraction 6-6-4-6.

Fraction 6-6-6 (30.7 mg) was purified by normal-phase HPLC (CN) (2.2 mL/min, UV 254 nm, 5 μm, 250 × 10.0 mm column), eluting with *n*-hexane−CH_2Cl_2−MeOH (240:20:1), to give pure compound **3** (1.9 mg). The residue (12.2 mg) was further purified by reversed-phase HPLC (C_{18}) (2 mL/min, PDA, 5 μm, 250 × 10.0 mm column) and eluted with 72% aqueous MeOH to obtain compounds **1** (0.6 mg), **2** (2.7 mg), and **3** (2.1 mg).

Fraction 3 (1.3 g) was chromatographed by CPC in *n*-hexane−EtOAc−MeOH−H_2O (2:8:2:8, 1 L), and its subfraction Fr. 3-10 (45.6 mg) obtained from this separation was purified by reversed-phase HPLC (C_{18}) (2 mL/min, RI detector, 5 μm, 250 × 10.0 mm column), eluting with 40% aqueous MeOH, to obtain compound **12** (2.3 mg).

Fraction 8 (1.0 g) was loaded on a C_{18} silica gel open column eluting with 10% aqueous MeOH−100% MeOH. A subfraction obtained, Fr. 8-2 (186.2 mg), was purified by RPC, eluting with a gradient system of CH_2Cl_2−MeOH (50:1 → 6:1), to give compound **11** (2.3 mg).

*Cirsiumyne A (**1**):* pale yellow, amorphous solid; UV (CH_2Cl_2) λ_{max} (log ε) 237 (4.07), 281 (2.91), 296 (4.60), 317 (4.60) nm; IR (neat) ν_{max} 3406, 2918, 2200, 1700, 1590, 1509, 1152, 1028, 976 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 1; HRESIMS *m*/*z* 349.1413 $[M + Na]^+$ (calcd for $C_{20}H_{22}O_4Na$, 349.1416).

*Cirsiumyne B (**2**):* pale yellow, amorphous solid; UV (CH_2Cl_2) λ_{max} (log ε) 238 (4.57), 280 (2.61), 296 (3.80), 316 (3.94) nm; IR (neat) ν_{max} 3406, 2928, 2200, 1709, 1590, 1514, 1152, 1028, 976 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; HRESIMS *m*/*z* 345.1102 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_4Na$, 345.1103).

*Cirsiumyne C (**3**):* pale yellow, amorphous solid; UV (CH_2Cl_2) λ_{max} (log ε) 241 (3.01), 254 (2.63), 269 (3.25), 286 (3.76), 319 (3.11) nm; IR (neat) ν_{max} 3511, 3416, 2956, 2229, 1704, 1628, 1590, 1514, 1266, 1152, 1028, 976 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table 1; HRESIMS *m*/*z* 347.1257 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_4Na$, 347.1259).

*Cirsiumyne D (**4**):* colorless oil; $[\alpha]^{25}_D$ −18.5 (*c* 0.1, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 242 (2.10) 255 (3.43), 269 (4.47), 285(3.51) nm; IR (neat) ν_{max} 3626, 3397, 2919, 2851, 2229, 1638, 1457, 1433, 1300, 1033, 995, 909, 719 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 2 and 3; HRESIMS *m*/*z* 317.1282 $[M + Na]^+$, 319.1268 $[M + 2 + Na]^+$ (calcd for $C_{17}H_{23}ClO_2Na$, 317.1284 and 319.1255).

*Cirsiumyne E (**5**):* colorless oil; $[\alpha]^{25}_D$ −30.0 (*c* 0.05, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} (log ε) 242 (1.01), 254 (1.47), 268 (1.90), 284 (1.62) nm; IR (neat) ν_{max} 3425, 2918, 2851, 2315, 2354, 2229, 1728, 1642, 1452, 1371, 1095, 1042, 904, 871 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Tables 2 and 3; HRESIMS *m*/*z* 339.1933 $[M + Na]^+$ (calcd for $C_{20}H_{28}O_3Na$, 339.1936).

Preparation of (*R*)- and (*S*)-MTPA Esters (6r, 6s, 7r, 7s). Compounds **6** and **7** (1.0 mg) were stored in separate NMR tubes and dried under vacuum.[19,20] Deuterated pyridine (0.6 mL) and (*R*)-MTPA-Cl (10 μL) were added to the NMR tubes under a N_2 gas stream. The reaction NMR tubes were permitted to stand at room temperature and monitored by 400 MHz NMR every hour. After 5 h, the reaction was found to be completed, and the ^1H NMR data was obtained (400 MHz, in pyridine-d_5). (*S*)-MTPA esters of **6** (**6s**) and **7** (**7s**) were obtained, and the ^1H NMR data in $CDCl_3$ (400 MHz) were analyzed. Similar to **6s** and **7s**, (*S*)-MTPA-Cl (10 μL) and deuterated pyridine (0.6 mL) were reacted at room temperature for 5 h, to afford the (*R*)-MTPA ester derivatives (**6r** and **7r**), in separate experiments. The ^1H NMR spectra were measured with 400 MHz NMR in $CDCl_3$.[19,20] **(*R*)-MTPA ester 6**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.43−7.37 (5H, m, aromatic H), 5.81 (1H, ddt, *J* = 17.2, 10.0, 6.7 Hz, H-2), 5.37 (1H, d, *J* = 7.1 Hz, H-10), 5.00, (1H, ddt, *J* = 17.2, 2.0, 1.7 Hz, H-1a), 4.93 (1H, ddt, *J* = 10.0, 2.0, 1.7 Hz, H-1b), 3.97 (1H, m, H-8), 3.93 (1H, m, H-9), 3.58 (3H, OCH_3), 2.27 (2H, t, *J* = 7.1 Hz, H-15), 2.03 (2H, q, *J* = 6.7 Hz, H-3), 1.69 (2H, m, H-7), 1.57 (2H, dq, *J* = 7.1, 7.4 Hz, H-16), 1.42−1.26 (6H, m, H-4, 5, 6), 1.40 (3H, s, H-2′), 1.34 (3H, s, H-3′), 0.99 (3H, t, *J* = 7.4 Hz, H-17). **(*S*)-MTPA ester 6**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.40−7.38 (5H, m, aromatic H), 5.78 (1H, ddt, *J* = 17.2, 10.0, 6.9 Hz, H-2), 5.72 (1H, d, *J* = 3.6 Hz, H-10), 4.99 (1H, m, H-1a), 4.94 (1H, m, H-1b), 4.99 (1H, m, H-8), 3.83 (1H, dd, *J* = 3.8, 7.1 Hz, H-9), 3.52 (3H, OCH_3), 2.26 (2H, t, *J* = 7.0 Hz, H-15), 1.99 (2H, q, *J* = 7.0 Hz, H-3), 1.73 (2H, m, H-7), 1.57 (2H, dq, *J* = 7.0, 7.4 Hz, H-16), 1.37−1.28 (6H, m, H-4, 5, 6), 1.33, (3H, s, H-2′), 1.25, (3H, s, H-3′), 0.99 (3H, t, *J* = 7.4 Hz, H-17). **(*R*)-Bis-MTPA ester 7**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.44−7.34 (10H, m, aromatic H), 5.80 (2H, m, H-2), 5.75 (1H, d, *J* = 6.8 Hz, H-10), 5.57 (1H, dd, *J* = 6.8, 1.5 Hz, H-9), 5.01 (1H, m, H-1a), 4.94 (1H, m, H-1b), 4.00 (1H, m, H-8), 3.50 (OCH_3), 3.55 (OCH_3), 2.27 (2H, m, H-15), 1.99 (2H, m, H-3), 1.86 (2H, m, H-7), 1.57 (2H, m, H-16), 1.45−1.15 (6H, m, H-4, 5, 6), 0.99 (3H, t, *J* = 7.4 Hz, H-17). **(*S*)-Bis-MTPA ester 7**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.42−7.34 (10H, m, aromatic H), 5.88 (1H d, *J* = 8.0 Hz, H-10), 5.78 (2H, m, H-2), 5.50 (1H, dd, *J* = 8.0, 3.0 Hz, H-9), 5.00 (1H, m, H-1a), 4.94 (1H, m, H-1b), 3.56 (1H, m, H-8), 3.50 (3H, OCH_3), 3.55 (3H, OCH_3), 2.27 (2H, m, H-15), 2.01 (2H, dd, *J* = 14.6, 6.8 Hz, H-3), 1.79 (1H, m, H-7), 1.57 (2H, m, H-16), 1.49−1.05 (6H, m, H-4, 5, 6), 0.98 (3H, t, *J* = 7.4 Hz, H-17).

ECD Calculation. Both 8*R*,9*R*,10*R* and 8*S*,9*R*,10*R* conformational analysis of compounds **4** and **7** were conducted via Monte Carlo searching with the MMFF94 molecular mechanics force field using Molecular Operating Environment (MOE) software (Chemical Computing Group, Montreal, Canada).[23] The conformers were optimized using DFT at the CAM-B3LYP/6-31G(d) level in the gas phase with GAUSSIAN 09.[23] The CAM-B3LYP/6-31G(d) harmonic vibrational frequencies were computed to confirm their stability and to provide their relative thermal free energy, which are used to evaluate their equilibrium populations. The energies, rotational strengths, and oscillator strengths of the 20 weakest electronic excitations of the conformers were computed utilizing the TDDFT methodology at the CAM-B3LYP/6-31G(d)/dichloromethane level in the gas phase. Furthermore, the ECD spectra were then simulated using GaussSum2.2.5 with a bandwidth σ of 0.20 eV. The corresponding theoretical ECD spectra of (8*R*,9*R*,10*R*)-**4** and (8*R*,9*R*,10*R*)-**7** were depicted by inverting those of (8*S*,9*R*,10*R*)-**4** and (8*S*,9*R*,10*R*)-**7**. The ECD spectra of the two stereostructures were compared with the CD spectrum of **4** and **7** to determine their absolute configuration.

2-Deoxyglucose Uptake Assay. 3T3-L1 preadipocytes were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's minimal essential medium (DMEM) supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, 1% nonessential amino acid, and 10% calf serum (Hyclone, Logan, UT, USA) in 5% CO_2 at 37 °C.[14] When the cell density reached 100% confluence, 3T3-L1 preadipocytes were induced to differentiate by treating the culture with 450 mg/dL glucose, 0.32 μM insulin, 0.5 mM 3-isobutyl-1-methylxanthine, and 1 μM dexamethasone.[17] The induction medium was removed, and the cells were maintained in the culture medium with 450 mg/dL glucose in the presence or absence of test samples and insulin after 2 days. Fresh medium and test samples were treated every day, and 24 h glucose consumption was measured by a Roche Cobas Integra 400 chemistry analyzer (Roche Diagnostics, Taipei, Taiwan).[17] The samples were dissolved in DMSO, to which the medium was added to obtain the final concentration of 0.1% (v/v) of DMSO. Pioglitazone (50 mg/mL) was used as a positive control, and DMSO was added to the blank control group.

Oil Red O Staining. Fixed cells were washed with PBS buffer and placed in 60% 2-propanol in PBS.[18] Then, they were stained in freshly diluted Oil Red O (Wako Pure Chemical, Tokyo, Japan) solution [0.3% stock in 2-propanol−H_2O (3:2)] for 15 min at 37 °C. DMSO (0.1%, v/v) was used as the blank. Insulin (3.2×10^{-7} M) and PIO (pioglitazone, 50 μg/mL) were taken as positive controls. The stained

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lipid droplets were scanned and captured with a microscope (40× magnification).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR spectra of compounds **1**−**5**; GC-MS spectrum of compound **5**; CD spectra of compounds **4**−**7**; ECD spectra of compounds **4** and **7** are available free of charge via the Internet at http://pubs.acs.org.

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#W.-C. Lai and Y.-C. Wu contributed equally to this study.

Notes

The authors declare no competing financial interest.

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