JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Diarylheptanoids from Rhizomes of *Alpinia officinarum* Inhibit Aggregation of α-Synuclein

Guangmiao Fu,[†,‡] Wei Zhang,[†,§] Dongsheng Du,[‡] Yu Pong Ng,[‡] Fanny C. F. Ip,[‡,∥,⊥] Rongbiao Tong,[*,§] and Nancy Y. Ip[*,‡,∥,⊥]

[‡]Division of Life Science, [§]Department of Chemistry, and [∥]State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong, People's Republic of China

[⊥]Guangdong Provincial Key Laboratory of Brain Science, Disease and Drug Development, HKUST Shenzhen Research Institute, Shenzhen, Guangdong 518057, People's Republic of China

Supporting Information

ABSTRACT: Two new diarylheptanoids, alpinin A (**1**) and alpinin B (**2**), together with 18 known diarylheptanoids (**3**–**20**), were isolated from the rhizomes of *Alpinia officinarum*. Their structures were elucidated by comprehensive spectroscopic analysis, including high-resolution mass spectrometry, infrared spectroscopy, and one- and two-dimensional nuclear magnetic resonance spectroscopy. Structurally, alpinin A is a new member of the small family of oxa-bridged diarylheptanoids and contains the characteristic 2,6-*cis*-configured tetrahydropyran motif (C_1–C_5 oxa bridge). The absolute configuration of alpinin A was confirmed by asymmetric total synthesis of the enantiomer (*ent*-**1**), corroborating the assignment of the molecular structure. The absolute configuration of alpinin B was determined on the basis of the analysis of the circular dichroism exciton chirality spectrum. We evaluated the inhibitory activity of all isolated diarylheptanoids against α-synuclein aggregation at 10 μM. Alpinins A and B significantly inhibited α-synuclein aggregation by 66 and 67%, respectively.

KEYWORDS: *Alpinia officinarum, diarylheptanoids, alpinin A, alpinin B, inhibit α-synuclein aggregation*

INTRODUCTION

Parkinson's disease is the second most common progressive neurodegenerative disorder. The characteristic phenotypes of the disease are majorly the resting tremor and postural instability with cognitive and emotional disorders. Although the etiology and pathogenesis of Parkinson's disease are not fully understood, recent evidence suggests that environmental and genetic factors might account for the progression of disease.[1] α-Synuclein, a protein expressed predominantly in neurons, especially at synaptic terminals, is the major component of Lewy bodies in Parkinson's disease patients[2,3] and is therefore implicated in the pathogenesis of the disease. α-Synuclein protein readily adopts various conformations;[2,3] it has a strong tendency to self-aggregate into oligomers, followed by fibrils that are deposited as Lewy bodies and other similar pathologies. Therefore, small organic molecules that can inhibit α-synuclein aggregation might provide a potentially effective treatment of Parkinson's disease. Herein, we report the isolation and/or synthesis of new natural diarylheptanoid products and the preliminary evaluation of their inhibition activity against α-synuclein aggregation.

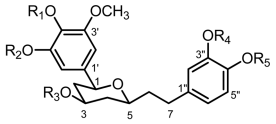
Alpinia officinarum (Hance) is a perennial herb from the Zingiberaceae family that is ubiquitous in tropical and subtropical Asian regions, especially southern China. The rhizome of *A. officinarum* is used as a traditional Chinese medicine for strengthening the circulatory system and treating stomach ache, cold, and swelling symptoms.[4] Besides its medicinal use, *A. officinarum* is also a valuable dietary material. Phytochemical studies have revealed that diarylheptanoids,[5−7] flavonoids,[8] and volatile oils are the major characteristic compounds of *A. officinarum*. It is noteworthy that many diarylheptanoid natural products present in *Alpinia* species possess cytotoxic, antioxidant, anti-inflammatory, antiplatelet, and antiproliferative activities.[9−12] We were intrigued by the finding that the total extract of *A. officinarum* inhibited the aggregation of α-synuclein and exhibits a neuroprotective effect. In this paper, we describe the isolation and structural elucidation of 2 new diarylheptanoids and 18 known diarylheptanoids (Figure 1). We also investigated their inhibitory effects on α-synuclein aggregation and accomplished the first asymmetric total synthesis of the enantiomer of compound **1**.

MATERIALS AND METHODS

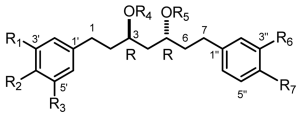
General Experimental Procedures. Solvents [analytical reagent (AR), Merck] for isolation were used as received unless stated otherwise. Silica gel (200−300 mesh, Qingdao Marine Chemical Incorporation), MCI GEL CHP20P (63−150 μm, Supelco), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. Fractions were monitored by thin-layer chromatography (TLC) on precoated silica gel 60 GF_{254} aluminum plates (Merck), and the signals were observed on heated silica gel plates after spraying 10% H_2SO_4 in EtOH. Preparative high-performance liquid chromatography (HPLC) was performed using a Waters 2996 photodiode array detector (220 nm) equipped with a preparative optimum bed density (OBD) column (150 × 19 mm, 5.0 μm, flow rate of 15.0 mL/min, Waters Xbridge). Optical rotation was obtained by a Jasco P-2000 polarimeter. Spectral data on ultraviolet (UV),

Received: May 10, 2017
Revised: July 10, 2017
Accepted: July 14, 2017
Published: July 14, 2017

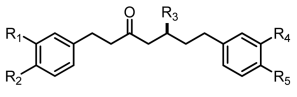
DOI: 10.1021/acs.jafc.7b02021
J. Agric. Food Chem. 2017, 65, 6608−6614



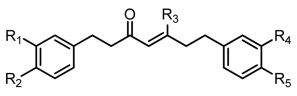
No.	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	H
1a	CH_3	CH_3	H	CH_3	CH_3



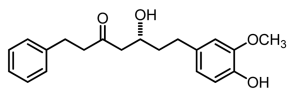
No.	R_1	R_2	R_3	R_4	R_5	R_6	R_7
2	OCH_3	OH	OH	H	H	OH	OH
2a	OCH_3	OCH_3	OCH_3	H	H	OCH_3	OCH_3
2b	OCH_3	OCH_3	OCH_3	*p*-Br-Bz	*p*-Br-Bz	OCH_3	OCH_3
3	OCH_3	OH	H	H	H	OH	OH
4	OCH_3	OH	H	H	H	H	OH
5	OH	OH	H	H	H	OH	OH
6	OCH_3	OH	OH	H	H	H	OH
7	OH	OH	H	H	H	H	OH
8	H	OH	H	H	H	H	OH



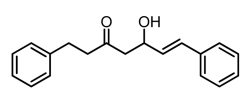
No.	R_1	R_2	R_3	R_4	R_5
9	OCH_3	OH	OH	OH	OH
10	H	OH	OH	H	OH
12	H	H	OH	H	OH
13	H	H	OH	H	H
14	H	H	OCH_3	H	H



No.	R_1	R_2	R_3	R_4	R_5
15	OCH_3	OH	H	OH	OH
16	OH	OH	H	H	OH
17	H	H	H	H	OH
18	H	H	H	H	H
20	H	H	OH	H	H



11



19

Figure 1. Chemical structure of natural diarylheptanoids **1**−**20** and their derivatives **1a**, **2a**, and **2b**.

circular dichroism (CD), infrared (IR), and nuclear magnetic resonance (NMR) were gained from a PerkinElmer Lambda 900 UV/vis/NIR spectrophotometer, a Chirascan Applied Photophysics spectropolarimeter, a PerkinElmer 577 spectrometer (KBr discs), and a Bruker AM-400 spectrometer, respectively. High-resolution mass spectrometry (HRMS) was recorded using a Waters Xevo G2-XS QTOF in an electrospray ionization (ESI) mode.

Plant Material. The dried rhizomes of *A. officinarum* (Hance) were procured in August 2009 from Zhanjiang, Guangdong, China, authenticated by one of the authors (Guangmiao Fu), and preserved as a voucher specimen (TCM-40) in the room CYT5014 at HKUST, Hong Kong, China.

Extraction and Isolation. A schematic flowchart of the details of extraction and isolation is provided in the Supporting Information. The dried, ground rhizomes (8.0 kg) of *A. officinarum* were extracted with 70% EtOH (40.0 L × 3, 2 h each), and the pooled extract was then concentrated *in vacuo* to give a brown paste (700.0 g), which was successively partitioned between chloroform (1.0 L × 3) and *n*-BuOH (1.0 L × 3) with water (1.0 L) to yield fractions of chloroform (120.0 g), *n*-BuOH (150.0 g), and water (420.0 g), respectively.

The *n*-BuOH fraction (80.0 g) was fractionated by silica gel column chromatography using a stepwise gradient elution of CH_2Cl_2/MeOH (100:0, 50:1, 20:1, 15:1, 10:1, 5:1, and 1:1, v/v) to yield seven fractions designated fractions 1−7. Purification of fraction 2 (9.7 g) was performed with a MCI GEL CHP20P column elution with a gradient of MeOH/H_2O (2:8, 4:6, 5:5, 6:4, 8:2, and 9:1, v/v), Sephadex LH-20 column elution with a mixture of $CHCl_3$/MeOH (1:1, v/v), and finally preparative HPLC using ACN/H_2O/TFA (20:80:0.025, v/v/v, in 20 min). This provided the new, analytically pure compounds **1** (39 mg, alpinin A) and **2** (28 mg, alpinin B) as well as the previously known diarylheptanoid **3** (28 mg). The separation of fraction 3 (6.8 g) with Sephadex LH-20 elution with $CHCl_3$/CH_3OH (1:1, v/v) and preparative HPLC elution with ACN/H_2O/TFA (10:90:0.025, v/v/v, in 40 min) yielded three known diarylheptanoid compounds: **6** (15 mg), **7** (13 mg), and **8** (20 mg). Fraction 4 (10.6 g) was chromatographed over silica gel with gradient-eluting solvents $CHCl_3$/MeOH (10:0, 9:1, 8:2, 7:3, 6:4, and 1:1, v/v) to give three subfractions designated fractions 4-1−4-3, which were categorized according to their polarity estimated by TLC. Compound **11** (17 mg) was obtained from fraction 4-1 by Sephadex LH-20 column chromatography elution with $CHCl_3$/MeOH (1:1, v/v), and compound **9** (8 mg) was found in fraction 4-2 through silica gel column chromatography elution with a gradient of $CHCl_3$/MeOH (20:1, 10:1, and 5:1, v/v).

The chloroform fraction (68.0 g) was separated by a silica gel column chromatography using gradient elution with petroleum ether (60−80 °C)/EtOAc (10:0, 9:1, 8:2; 7:3, 6:4, 5:5, and 0:10, v/v) affording six subfractions designated fractions A−F, which were categorized according to their polarity estimated by TLC. Separation of fraction B (9.6 g) on a silica gel column chromatography elution with a gradient of petroleum ether (60−80 °C)/EtOAc (30:1, 20:1, 10:1, 5:1, and 1:1, v/v) yielded two pure compounds **17** (35 mg), and **19** (28 mg) as well as a mixture of compounds **18** and **20**, which was efficiently separated by Sephadex LH-20 column chromatography elution with $CHCl_3$/MeOH (1:1, v/v) (**18**, 38 mg; **20**, 25 mg). Fraction C (7.5 g) was further fractionated on a silica gel column eluted with petroleum ether (60−80 °C)/EtOAc (15:1, v/v) to give compound **12** (20 mg) and three subfractions designated fractions C-1−C-3. Compounds **13** (22 mg) and **14** (8 mg) from fraction C-2 and compound **15** (16 mg) from fraction C-3 were then obtained through Sephadex LH-20 column chromatography elution with $CHCl_3$/MeOH (1:1, v/v). Similarly, compounds **10** (11 mg) and **16** (25 mg) were isolated through sequential separation of fraction D (5.5 g) by Sephadex LH-20 (eluted with 1:1, v/v, $CHCl_3$/MeOH) and silica gel column chromatography [eluted with 20:1−10:1, v/v, petroleum ether (60−80 °C)/EtOAc]. Fraction E (8.8 g) was further fractionated by Sephadex LH-20 (eluted with 1:1, v/v, $CHCl_3$/MeOH) and preparative HPLC (eluted with 10:90:0.025, v/v/v, ACN/H_2O/TFA, in 40 min) to give compounds **4** (17 mg) and **5** (9 mg).

Methylation of Compound 1. K_2CO_3 (41.0 mg) was added to an acetone solution of compound **1** (13.1 mg in 2 mL) at room temperature and stirred under a nitrogen atmosphere for 10 min. Iodomethane (0.6 mL) was then added and mixed for 1.5 h at room temperature, followed by heating under reflux at 60 °C overnight (∼16 h). Upon cooling to room temperature, the reaction mix was worked up and organic residue was purified by column chromatography on silica gel (40:1, v/v, CH_2Cl_2/MeOH) to give the methylated analogue of alpinin A (**1a**, 12.0 mg).

Methylation of Compound 2. Following the same procedure as described for synthesis of compound **1a** from alpinin A, the methylated analogue (**2a**, 11.8 mg) of alpinin B was obtained from 12.8 mg of alpinin B (**2**).

Bis-*p*-bromobenzoate (2b) of Compound 2a. Et_3N (32 μL), 4-dimethylaminopyridine (DMAP, 15 mg), and *p*-bromobenzoyl chloride (40 mg) were added sequentially to a solution of compound **2a** (10 mg) in anhydrous CH_2Cl_2 (0.6 mL). The reaction mixture was heated to 60 °C and stirred for 9 h. Upon cooling to room

DOI: 10.1021/acs.jafc.7b02021
J. Agric. Food Chem. 2017, 65, 6608−6614

Table 1. NMR Data for Compounds 1, 2, 1a, and 2a [400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in CD_3OD]

	1		1a	2		2a
position	δ_H (J in Hz)	δ_C type	δ_C	δ_H (J in Hz)	δ_C type	δ_C
1	4.18, brd (11.1)	78.9 CH	79.0	2.59, m	32.9 CH_2	33.4
2	2.08, m	39.1 CH_2	39.0	1.68, m	41.4 CH_2	41.2
	1.41, dd (5.80, 11.7)					
3	3.78, m	76.3 CH	76.3	3.84, m	68.7 CH	68.6
4	1.95, m	43.6 CH_2	44.0	1.54, m	45.6 CH_2	45.7
	1.21, m					
5	3.43, m	69.0 CH	68.9	3.84, m	68.7 CH	68.5
6	1.84, m	41.8 CH_2	41.9	1.68, m	41.3 CH_2	41.1
	1.75, m					
7	2.60, m	32.0 CH_2	32.4	2.52, m	32.4 CH_2	32.6
1′		134.5 C	136.4		134.5 C	136.6
2′	6.51, s	102.8 CH	104.4	6.32, s	104.8 CH	106.7
3′		149.4 C	154.4		149.5 C	154.3
4′		134.5 C	140.1		132.9 C	139.9
5′		146.3 C	154.4		146.3 C	154.3
6′	6.52, s	108.0 CH	104.4	6.33, s	109.8 CH	106.7
1″		135.0 C	138.2		135.3 C	136.7
2″	6.64, d (2.0)	116.3 CH	113.6	6.63, d (2.0)	116.5 CH	113.5
3″		146.1 C	150.3		146.0 C	150.3
4″		144.2 C	148.6		144.1 C	148.5
5″	6.62, d (8.0)	116.6 CH	113.2	6.66, d (8.0)	116.2 CH	113.1
6″	6.48, dd (2.0, 8.0)	120.8 CH	121.6	6.51 dd (2.0, 8.0)	120.6 CH	121.6
3′-OCH_3	3.81, s	56.6 CH_3	56.6	3.81, s	56.5 CH_3	56.6
4′-OCH_3			61.2			61.1
5′-OCH_3			56.6			56.6
3″-OCH_3			56.5			56.5
4″-OCH_3			56.4			56.4

temperature, the reaction was quenched by H_2O (20 mL). The mixture was then extracted with EtOAc (10 mL × 3), and the combined organic fractions were concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography (9.5:0.5, v/v, CH_2Cl_2/MeOH) to afford compound **2b** (6 mg).

*Alpinin A (**1**):* yellowish oil; $[\alpha]_D^{25}$, +51.1° (*c* 0.42, MeOH); UV (MeOH, nm) λ_{max} (log ε), 281 (1.05); IR (KBr, cm^{-1}) ν_{max}, 3358, 2941, 2856, 2361, 2032, 1674, 1607, 1527, 1455, 1340, 1284, 1203, 1090, 990, 958, 800; see Table 1 for the ^{1}H and ^{13}C NMR data; and HRMS(ESI), *m*/*z* 399.1447 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_7Na$, 399.1420), *m*/*z* 377.1607 $[M + H]^+$ (calcd for $C_{20}H_{25}O_7$, 377.1600).

*(1R,3S,5R)-1,5-Epoxy-3-hydroxy-1-(3,4,5-trimethoxyphenyl)-7-(3,4-dimethoxyphenyl)heptane (**1a**):* amorphous powder; $[\alpha]_D^{25}$, −3.2° (*c* 0.10, MeOH); UV (MeOH, nm) λ_{max} (log ε), 230 (2.54), 280 (2.02); IR (KBr, cm^{-1}) ν_{max}, 3418, 3073, 2999, 2933, 2853, 2837, 1591, 1516, 1464, 1453, 1418, 1260, 1235, 1154, 1139, 1070, 1027, 807; ^{1}H NMR (CD_3OD, 400 MHz), δ 4.31 (1H, brd, J = 11.1 Hz, H-1), 1.95, 1.15 (each 1H, m, H-2), 3.77 (1H, m, H-3), 2.01, 1.33 (each 1H, m, H-4), 3.35 (1H, m, H-5), 1.90, 1.80 (each 1H, m, H-6), 2.70 (2H, m, H-7), 6.70 (2H, s, H-2′ and H-6′), 6.84 (1H, d, J = 2.0 Hz, H-2″), 6.79 (1H, d, J = 2.0 and 8.0 Hz, H-5″), 6.70 (1H, d, J = 8.0 Hz, H-6″), 3.84 (6H, s, H-3′ and H-5′), 3.78 (3H, s, H-4′), 3.75 (6H, s, H-3″ and H-4″); see Table 1 for the ^{13}C NMR data (CD_3OD, 100 MHz); and HRMS(ESI), *m*/*z* 455.2047 $[M + Na]^+$ (calcd for $C_{24}H_{32}O_7Na$, 455.2046).

*Alpinin B (**2**):* yellowish oil; $[\alpha]_D^{25}$, +14.6° (*c* 0.22, MeOH); UV (MeOH, nm) λ_{max} (log ε), 281 (2.11); IR (KBr, cm^{-1}) ν_{max}, 3375, 2942, 2856, 2361, 2034, 1609, 1528, 1455, 1342, 1285, 1203, 1090, 991, 960, 800; see Table 1 for the ^{1}H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data; and HRMS(ESI), *m*/*z* 401.1584 $[M + Na]^+$ (calcd for $C_{20}H_{26}O_7Na$, 401.1576), *m*/*z* 379.1752 $[M + H]^+$ (calcd for $C_{20}H_{27}O_7$, 379.1757).

*(3R,5R)-3,5-Dihydroxy-1-(3,4,5-trimethoxyphenyl)-7-(3,4-dimethoxyphenyl)heptane (**2a**):* amorphous powder; $[\alpha]_D^{25}$, +6.5° (*c* 0.10, MeOH); UV (MeOH, nm) λ_{max} (log ε), 230 (2.66), 280 (2.25); IR (KBr, cm^{-1}) ν_{max}, 3418, 3000, 2934, 2853, 1591, 1516, 1465, 1453, 1419, 1261, 1235, 1155, 1140, 1070, 1028, 807, 764; ^{1}H NMR (CD_3OD, 400 MHz), δ 2.69 (2H, m, H-1), 1.75 (2H, m, H-2), 3.70 (1H, m, H-3), 1.55 (2H, m, H-4), 3.70 (1H, m, H-5), 1.75 (2H, m, H-6), 2.63 (2H, m, H-7), 6.50 (2H, s, H-2′, H-6′), 6.82 (1H, d, J = 2.0 Hz, H-2″), 6.79 (1H, d, J = 2.0 and 8.0 Hz, H-5″), 6.70 (1H, d, J = 8.0 Hz, H-6″), 3.80 (6H, s, H-3′, H-5′), 3.78 (3H, s, H-4′), 3.74 (6H, s, H-3″, H-4″); see Table 1 for the ^{13}C NMR (CD_3OD, 100 MHz) data; and HRMS(ESI), *m*/*z* 457.2198 $[M + Na]^+$ (calcd for $C_{24}H_{34}O_7Na$, 457.2202).

Total Synthesis of Compound *ent*-1. *(S)-1-(3′,4′-Dibenzyloxyphenyl)hex-5-ene-3-ol (**23**).*[13] Compound **22** (336 mg, 1.7 mmol) was added to a solution of (1*R*)-(−)-10-camphorsulfonic acid (CSA, 13 mg, 0.06 mmol) and aldehyde **21** (200 mg, 0.6 mmol) in CH_2Cl_2 (0.096 mL) under a nitrogen atmosphere. After stirring at room temperature for 5 days, the reaction mixture was diluted with 20 mL of CH_2Cl_2, quenched with 10 mL of saturated $NaHCO_3$ solution, and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resultant residue was purified by silica gel chromatography (5:1 hexane/EtOAc) to give compound **23** as a white solid (130 mg, 58%). $[\alpha]_D^{25}$: −13.5° (*c* 1.0, MeOH). Enantiomeric excess (ee) = 92.5%. $t_R(R)$ = 20.8 min. $t_R(S)$ = 18.7 min. Hexane/i-PrOH = 90:10. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.63 (s, 1H,OH), 1.68−1.74 (m, 2H), 2.15 (dt, J = 14.8 and 8.0 Hz, 1H), 2.28 (dt, J = 14.0 and 5.6 Hz, 1H), 2.60 (dt, J = 16.4 and 8.4 Hz, 1H), 2.70 (dt, J = 14.0 and 7.6 Hz, 1H), 3.55−3.63 (m, 1H), 5.10−5.20 (m, 6H), 5.73−5.90 (m, 1H), 6.72 (dd, J = 8.4 and 2.0 Hz, 1H), 6.81 (d, J = 1.6 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 7.27−7.49 (m, 10H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 31.5, 38.4, 42.1, 69.7, 71.3, 71.6, 115.4, 115.7, 118.3, 121.2, 127.36, 127.39, 127.74, 127.77, 128.5, 134.7, 135.5, 137.5, 137.6, 147.2, 148.9.

*(1S,3R,5S)-1,5-Epoxy-3-acetoxy-1-(3,4-diacetoxy-5-methoxyphenyl)-7-(3,4-dibenzyloxyphenyl)heptane (**25**).* BF_3−OEt_2 (0.077

DOI: 10.1021/acs.jafc.7b02021
J. Agric. Food Chem. 2017, 65, 6608−6614

mL, 0.62 mmol) was added to a stirred solution of aldehyde **24** (78 mg, 0.31 mmol), homoallylic alcohol **23** (120 mg, 0.31 mmol), TMSOAc (204 mg, 1.55 mmol), and AcOH (0.12 mL, 2.17 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was warmed to room temperature and was subsequently stirred for 2 h. When the reaction was completed, the mixture was diluted with CH_2Cl_2 and quenched by saturated aqueous $NaHCO_3$ solution (3 mL). While the separated aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL), the combined organic extracts were washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (5:1 hexane/EtOAc) to afford two colorless oils, compound **25** (80 mg, 38%) and its 3-fluoro derivative (62 mg, 31%). $[\alpha]_D^{25}$: −15.8° (*c* 1.0, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2954, 2919, 2849, 1769, 1728, 1604, 1505, 1455, 1424, 1367, 1324, 1240, 1240, 1207, 1180, 1136, 1087, 1012, 891, 850, 750, 696. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.38 (q, *J* = 11.6 Hz, 1H), 1.50 (q, *J* = 11.6 Hz, 1H), 1.66−1.79 (m, 1H), 1.85−1.95 (m, 1H), 1.95−2.02 (m, 1H), 2.04 (s, 3H), 2.20−2.27 (m, 1H), 2.28 (s, 3H), 2.30 (s, 3H), 2.56−2.76 (m, 2H), 3.37−3.49 (m, 1H), 3.83 (s, 3H), 4.32 (d, *J* = 11.2 Hz, 1H), 4.91−5.03 (m, 1H), 5.13 (d, *J* = 3.6 Hz, H), 6.65−6.75 (m, 1H), 6.75−6.85 (m, 2H), 6.85−6.90 (m, 2H), 7.22−7.39 (m, 6H), 7.39−7.50 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.3, 20.6, 21.3, 31.1, 37.0, 37.5, 39.1, 56.3, 70.4, 71.3, 71.6, 74.5, 76.3, 107.3, 112.4, 115.4, 115.7, 121.2, 127.3, 127.4, 127.7, 128.4, 131.0, 135.3, 137.4, 137.6, 140.6, 143.2, 147.2, 148.9, 152.2, 167.9, 168.3, 170.5. HRMS(ESI): *m*/*z* 682.2781 $[M]^+$ (calcd for $C_{40}H_{42}O_{10}$, 682.2778).

(1S,3R,5S)-1,5-Epoxy-3-hydroxy-1-(3,4-dihydroxy-5-methoxyphenyl)-7-(3,4-dihydroxyphenyl)heptane (ent-1). Concentrated HCl (0.2 mL) was added to a solution of compound **25** (80 mg, 0.12 mmol) in MeOH (2 mL) at room temperature, followed by stir mixing at room temperature for 12 h. The solvent was removed under vacuum; the crude product was then redissolved in MeOH (2 mL), and 10% Pd/C (10 mg) was carefully added. The mixture was then degassed and filled with nitrogen, degassed again, and refilled with hydrogen (1 atm). The reaction was stirred at room temperature for another 12 h. When the starting material was completely consumed, the reaction mixture was filtered through Celite and the filtrate was concentrated under vacuum. The resultant residue was purified by silica column chromatography (1:1 hexane/EtOAc) to afford *ent*-alpinin A (*ent*-**1**) as a white solid (32 mg, 73%). $[\alpha]_D^{25}$: −40.5° (*c* 1.0, MeOH). IR (KBr, cm^{-1}) ν_{max}: 3364, 2922, 2852, 1632, 1526, 1462, 1365, 1288, 1237, 1203, 1077, 785, 651. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.23 (q, *J* = 11.6 Hz, 1H), 1.43 (q, *J* = 11.6 Hz, 1H), 1.65−1.80 (m, 1H), 1.80−1.92 (m, 1H), 1.93−2.02 (m, 1H), 2.04−2.14 (m, 1H), 2.48−2.70 (m, 2H), 3.40−3.50 (m, 1H), 3.77−3.85 (m, 1H), 3.86 (s, 3H), 4.22 (d, *J* = 11.2 Hz, 1H), 4.63 (br, 1H), 6.50−6.60 (m, 3H), 6.60−6.75 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 30.6, 37.7, 40.4, 42.2, 55.3, 67.6, 74.9, 77.6, 101.5, 106.6, 114.9, 115.2, 119.3, 133.1, 133.2, 133.6, 142.8, 144.7, 144.9, 148.1.

Activity Bioassay. A filter trap assay was performed, and aggregated α-synuclein was detected using western blot analysis as described previously, with slight modification.[14] Recombinant human α-synuclein (GenWay Biotech, San Diego, CA, U.S.A.) was diluted in 1× Tris-buffered saline (20 mM Tris and 500 mM NaCl at pH 7.5) and incubated with extracts or compounds at room temperature for 7 days. After incubation, the mixtures were loaded onto a Bio-Dot SF microfiltration apparatus (Bio-Rad, Hercules, CA, U.S.A.) sandwiched with a 0.45 μm nitrocellulose membrane (Bio-Rad). After filtration and washing twice with Tris-buffered saline, the amount of trapped α-synuclein was determined by western blot analysis using an anti-α-synuclein antibody (1:2000, BD Bioscience, San Jose, CA, U.S.A.) and an anti-mouse secondary antibody (1:1000, Cell Signaling Technology, Danvers, MA, U.S.A.) and visualized by an enhanced chemiluminescent (ECL) detection kit (GE Healthcare, Buckinghamshire, U.K.). The band intensity was quantified using ImageJ software (https://imagej.nih.gov/ij/features.html). Dimethyl sulfoxide (DMSO) and Congo red served as the solvent and positive control, respectively. We diluted the DMSO stock solution (0.02%) with Tris-buffered saline to obtain the working concentrations for the assay. Data are expressed as the mean ± standard error of the mean (SEM) of three individual experiments (n = 3 per treatment group).

RESULTS AND DISCUSSION

The molecular formula of alpinin A (**1**, yellowish oil) is determined as $C_{20}H_{24}O_7$ based on the positive-ion HRMS(ESI) spectrum, with pseudo-molecular ion peaks at *m*/*z* 399.1447 for $[M + Na]^+$ and *m*/*z* 377.1607 for $[M + H]^+$. The IR spectrum of compound **1** displayed absorptions for hydroxyl (broad, 3359 cm^{-1}) and substituted benzene groups (1674, 1608, and 1527 cm^{-1}). In the ^{1}H NMR spectrum (Table 1), five low-field resonances (δ_H 6.48−6.62 ppm) demonstrated the presence 1,3,4,5-tetrasubstituted benzene [δ_H 6.51 (1H, s, H-2′) and 6.52 (1H, s, H-6′)] and 1,3,4-trisubstituted benzene [δ_H 6.62 (1H, d, *J* = 8.1 Hz, H-5″), 6.64 (1H, d, *J* = 1.8 Hz, H-2″), and 6.48 (1H, dd, *J* = 8.1 and 1.8 Hz, H-6″)], and four signals (δ_H 4.18, 3.78, 3.43, and 3.81 ppm) suggested that there were four hydrogens on the carbons with oxygen substitution. The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra (Table 1) revealed that this molecule consisted of 20 carbons, 12 of which were aromatic (sp^2 hybridized, δ_C 103−150 ppm) and 8 of which were aliphatic (sp^3 hybridized, δ_C 32−79 ppm). This is concordant with two aromatic rings [1,3,4,5-tetrasubstituted benzene, δ_C 134.5 (C-1′), 102.8 (C-2′), 149.4 (C-3′), 134.5 (C-4′), 146.3 (C-5′), 108.0 (C-6′) ppm; and 1,3,4-trisubstituted benzene, δ_C 135.0 (C-1″), 116.3 (C-2″), 146.1 (C-3″), 144.2 (C-4″), 116.6 (C-5″), 120.7 (C-6″) ppm] and a heptane moiety with three oxygenated carbons [δ_C 78.9 (C-1), 76.3 (C-3 with a hydroxyl group), and 69.0 (C-5) ppm] plus a methoxy group (δ_C 56.7 ppm). Analysis of the DEPT and ^{13}C NMR spectra suggested one methoxy, four methylenes, eight methines, including three oxymethines, and seven quaternary carbons, which are consistent with cyclic ether substituted with two aromatic rings, as represented in compound **1**. The cyclic ether ring was proposed to be a tetrahydropyran (THP) on the basis of comprehensive analysis of ^{1}H−^{1}H correlation spectroscopy (COSY), heteronuclear single-quantum correlation (HSQC), heteronuclear multiple-bond correlation (HMBC), and nuclear Overhauser effect spectroscopy (NOESY) [nuclear Overhauser effect (NOE) correlation between H-5 and H-1], which also allowed us to confirm one methoxy group at the aromatic ring (see the Supporting Information). The spectral analysis results consistently indicated that alpinin A (**1**) is a THP-containing diarylheptanoid. A literature search also indicated that our proposed structure of compound **1** is similar to two known THP-containing diarylheptanoids 5-[4-hydroxy-6-(4-hydroxyphenethyl)-tetrahydro-2*H*-pyran-2-yl]-3-methoxybenzene-1,2-diol isolated from the rhizomes of *Zingiber officinale*[15] and 1,5-epoxy-3-hydroxy-1-(3,4-dihydroxy-5-methoxyphenyl)-7-(3,4-dihydroxyphenyl)heptane.[16] A comparison of the NMR data of these three diarylheptanoids revealed obvious differences at either the aromatic or THP region, which indicate that compound **1** is a new member of THP-containing diarylheptanoids; its relative structure of compound **1** was assigned as shown in Figure 1. However, its absolute configuration could not be established unambiguously with Mosher's ester method, because contrary shielding effects were observed for the protons on the same side. Therefore, to fully establish the relative and absolute configurations of alpinin A and provide sustainable expedited access to the THP-containing diarylheptanoids, we initiated the asymmetric total synthesis of alpinin A (Scheme 1).

DOI: 10.1021/acs.jafc.7b02021
J. Agric. Food Chem. 2017, 65, 6608−6614

Scheme 1. Enantioselective Synthesis of the Proposed Structure of Compound 1

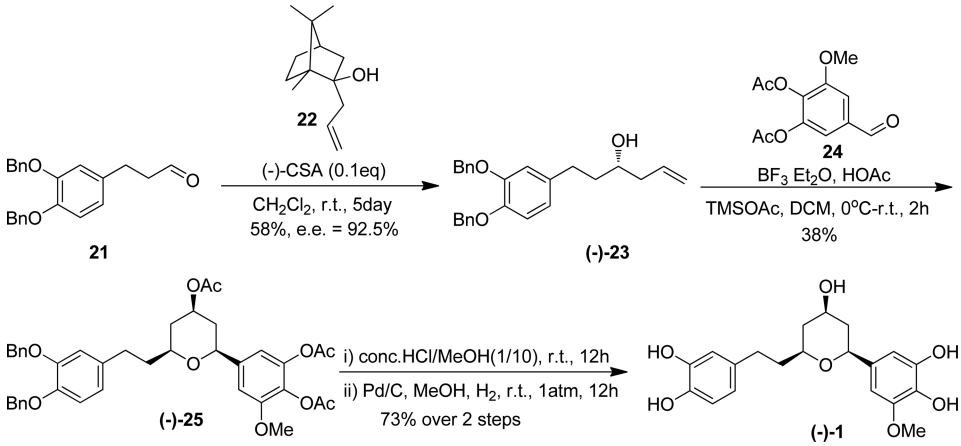


Table 2. Effects of the Isolated Compounds in Inhibiting α-Synuclein Aggregation

compound	inhibition (%)	compound	inhibition (%)	compound	inhibition (%)
1	66.1 ± 4.4	**8**	32.2 ± 11.7	**16**	0.0
2	67.3 ± 6.2	**9**	0.0	**17**	0.0
3	72.4 ± 4.4	**10**	10.6 ± 3.8	**18**	40.4 ± 17.2
4	16.6 ± 10.6	**12**	40.3 ± 14.9	**20**	30.3 ± 5.5
5	65.9 ± 9.8	**13**	20.1 ± 14.6	DMSO	0.0
6	76.0 ± 3.5	**14**	0.0	Congo red[a]	64.5 ± 7.6
7	61.3 ± 6.3	**15**	59.1 ± 11.2		

[a]Congo red and the isolated compounds at the concentration of 10 μM (inhibition percentage compared to the control DMSO).

The synthesis of *ent*-**1** commenced with asymmetric allylation of aldehyde **21**[13] using Loh's allyl transfer method,[17] which provided the homoallylic alcohol **23** with a yield of 58% and 92.5% ee. The Prins cyclization of homoallylic alcohol **23** and the known aldehyde **24**[18] afforded THP **25** with a yield of 38%, along with a 3-fluoro derivative (31%).[18] The two-step global deprotection (deacetylation with HCl/MeOH and Pd/C-catalyzed benzylation) was carried out to furnish alpinin A with a 73% overall yield for two steps. The NMR and MS spectroscopic data of our synthetic material were identical to those derived from natural alpinin A isolated from *A. officinarum*, which confirmed the molecular structure of our proposed compound **1**. However, the opposite sign of the specific rotation of the synthetic and natural materials [synthetic, $[\alpha]_D^{25}$ −40.0° (*c* 1.0, MeOH); natural, $[\alpha]_D^{25}$ +51.1° (*c* 0.42, MeOH)] indicated that synthetic alpinin A (*ent*-**1**) was the enantiomer of compound **1**, which, in turn, confirmed the absolute configuration of the natural (+)-alpinin A. The total synthesis was not only a conclusive and powerful way to confirm the relative and absolute configurations of alpinin A but also provided efficient (only four steps) and expedited access to alpinin A and related THP-containing diarylheptanoids.

The molecular formula of alpinin B (**2**, yellowish oil) is determined as $C_{20}H_{26}O_7$ based on the HRMS(ESI) spectrum, with pseudo-molecular ion peaks *m*/*z* at 379.1751 for $[M + H]^+$ and *m*/*z* at 401.1558 for $[M + Na]^+$. The carbon number of this formula was verified by the ^{13}C NMR spectrum. Similar to the IR spectrum of compound **1**, the IR spectrum of compound **2** also indicated the presence of hydroxyl (3376 cm^{-1}) and substituted benzene (1609 and 1527 cm^{-1}) groups, which was consistent with a maximum absorption in the UV spectrum (281.4 nm). Careful analysis of its ^1H and ^{13}C NMR data indicated that the compound has the same 1,3,4,5-tetrasubstituted and 1,3,4-trisubstituted aromatic rings as compound **1**. Additionally, seven carbons of the heptane-3,5-diol moiety were also observed at δ_C 32.9 (C-1), 41.4 (C-2), 68.7 (C-3), 45.6 (C-4), 68.7 (C-5), 41.3 (C-6), and 32.4 (C-7) in the ^{13}C NMR spectrum and were further confirmed by the HSQC and $^1H-^1H$ COSY spectral data. Furthermore, the sp^3-hybridized aliphatic hydrogen and carbon resonances in the NMR spectra were nearly identical to those of the previously reported linear diarylheptanoid derived from the rhizomes of *Tacca chantrieri*.[19] However, these two compounds differ mainly with respect to the chemical shifts derived from their aromatic rings. Compound **2** resonates at δ_C 104.8 (C-2′), 149.5 (C-3′), 132.9 (C-4′), 146.3 (C-5′), and 109.8 (C-6′) and δ_H 6.32 and 6.33 (each 1H, s, H-2′ and H-6′), while the known diarylheptanoid has the corresponding signals at δ_C 116.5 (C-2′), 146.0 (C-3′), 144.1 (C-4′), 116.2 (C-5′), and 120.6 (C-6′) and δ_H 6.65 (1H, d, *J* = 8.1 Hz, H-5′), 6.62 (1H, d, *J* = 2.0 Hz, H-2′), and 6.50 (1H, dd, *J* = 8.1, 2.0 Hz, H-6′).[19] This indicates that C-3′ was substituted by a methoxy group, whose assignment was unequivocally substantiated by long-range correlation (HMBC) analysis: protons of the methoxy group at δ_H 3.81 were correlated with carbon C-3′ at δ_C 149.5. The HMBCs between δ_H 6.32 (H-2′) and δ_C 32.9 (C-1) and between δ_H 6.63 (H-2″) and δ_H 6.51 (H-6″) and δ_C 32.4 (C-7), indicated that the two benzene rings were located at two ends of the heptane chain. On the basis of the above evidence, the planar structure of compound **2** was proposed to be a linear diarylheptanoid (Figure 1) and named alpinin B.

To determine the absolute configuration of alpinin B (**2**), the two hydroxyl groups were derivatized to *p*-bromobenzoate for the CD spectrum (using the CD exciton chirality method).[16] First, the phenolic hydroxyl groups of 4′-OH, 5′-OH, 3″-OH, and 4″-OH were alkylated by iodomethane to provide the

DOI: 10.1021/acs.jafc.7b02021
J. Agric. Food Chem. 2017, 65, 6608−6614

corresponding tetramethyl ether (**2a**), and then the compound was reacted with *p*-bromobenzoyl chloride to give the dibenzoate **2b**. The CD spectrum of compound **2b** exhibited positive (238.5 nm, $\Delta\varepsilon$ +15.1) and negative (253.0 nm, $\Delta\varepsilon$ −13.4) Cotton effects, which confirmed the absolute configuration as 3*R* and 5*R*.[19,20] Therefore, the absolute configuration of compound **2** was proposed to be (3*R*,5*R*)-alpinin B.

The structures of compounds **3**−**20** were elucidated on the basis of careful comparison of the NMR data to those in the literature, and their molecular structures are shown as compounds **3**−**5**,[19] **6**,[21] **7** and **8**,[19] **9**,[22] **10**,[23] **11**,[24] **12**,[5] **13**,[25] **14**,[26] **15**,[27] **16**,[28] **17** and **18**,[24] **19**,[29] and **20**[26] in Figure 1.

The inhibitory effects of compounds **1**−**10**, **12**−**18**, and **20** on α-synuclein aggregation were evaluated (Table 2). A filter trap assay was performed, and aggregated α-synuclein was detected by western blot analysis. Compounds **1**−**3** and **5**−**7**, which featured the presence of 3,4-dihydroxy substitution on the benzene ring, exhibited the most potent inhibitory activity against α-synuclein aggregation, which suggests that the presence of the 3,4-dihydroxy group on the benzene ring is crucial to their inhibitory activity. In addition, in comparison of the inhibitory activity of compounds **3**−**4** to that of compounds **6**−**8**, the more phenolic hydroxyl groups on benzene exhibited a greater activity, which suggests that the inhibition of α-synuclein aggregation is strongly correlated with the number of hydroxyl groups on the aromatic ring. Furthermore, compound **1a**, a tetramethylated analogue of compound **1**, showed no inhibitory activity, further supporting the importance of hydroxyl groups in the observed inhibition. Finally, the results show that the methoxy substitution on C-3 of the benzene ring is not essential for inhibitory activity, because no significant activity difference was observed for compounds **4** and **8** as well as compounds **2** and **5**.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b02021.

HR−ESI−MS, UV, IR, ^{1}H and ^{13}C NMR, DEPT 135, ^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY spectra of compounds **1**, **1a**, **2**, **2a**, **2b**, **23**, **25**, and *ent*-**1** and flowchart for compound isolation (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Telephone: 852-2358-7304. Fax: 852-2358-1552. E-mail: boip@ust.hk.

*Telephone: 852-2358-7357. Fax: 852-2358-1594. E-mail: rtong@ust.hk.

ORCID

Rongbiao Tong: 0000-0002-2740-5222

Nancy Y. Ip: 0000-0002-2763-8907

Author Contributions

†Guangmiao Fu and Wei Zhang contributed equally to this work.

Funding

This study was supported in part by the National Basic Research Program of China (2013CB530900), the Hong Kong Research Grants Council Theme-Based Research Scheme (T13-607/12R), the General Research Fund (16305314), the Innovation and Technology Fund for State Key Laboratory (ITCPT/17-9), the Shenzhen Peacock Plan, the Shenzhen Knowledge Innovation Program (JCYJ20151030140325152, JCYJ20151030154629774, and GJHS20140425165746765), and the S. H. Ho Foundation.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Ascherio, A.; Schwarzschild, M. A. The epidemiology of Parkinson's disease: Risk factors and prevention. *Lancet Neurol.* **2016**, *15*, 1257−1272.

(2) Ghosh, D.; Mehra, S.; Sahay, S.; Singh, P. K.; Maji, S. K. α-Synuclein aggregation and its modulation. *Int. J. Biol. Macromol.* **2017**, *100*, 37−54.

(3) Benskey, M. J.; Perez, R. G.; Manfredsson, F. P. The contribution of α synuclein to neuronal survival and function—Implications for Parkinson's disease. *J. Neurochem.* **2016**, *137*, 331−359.

(4) State Pharmacopoeia Commission of the People's Republic of China. *Pharmacopoeia of the People's Republic of China*; China Medical Science and Technology Press: Beijing, China, 2010, Vol. *1*, pp 270−271.

(5) Shin, D.; Kinoshita, K.; Koyama, K.; Takahashi, K. Antiemetic principles of *Alpinia officinarum*. *J. Nat. Prod.* **2002**, *65*, 1315−1318.

(6) Sun, Y.; Matsubara, H.; Kitanaka, S.; Yasukawa, K. Diarylheptanoids from the rhizomes of *Alpinia officinarum*. *Helv. Chim. Acta* **2008**, *91*, 118−123.

(7) Zhao, L.; Liang, J. Y.; Zhang, J. Y.; Chen, Y. A novel diarylheptanoid bearing flavonol moiety from the rhizomes of *Alpinia officinarum* Hance. *Chin. Chem. Lett.* **2010**, *21*, 194−196.

(8) An, N.; Yang, S. L.; Zou, Z. M.; Xu, L. Z. Flavonoids of *Alpinia officinarum*. *Chin. Tradit. Herb. Drugs* **2006**, *37*, 663−664.

(9) Lv, H. N.; She, G. M. Naturally occurring diarylheptanoids. *Nat. Prod. Commun.* **2010**, *5*, 1687−708.

(10) Lv, H. N.; She, G. M. Naturally occurring diarylheptanoids—A supplementary version. *Rec. Nat. Prod.* **2012**, *6*, 321−333.

(11) Zhu, J. P.; Islas-Gonzalez, G.; Bois-Choussy, M. Recent progress in isolation, bioactivity evaluation and total synthesis of diarylheptanoids. *Org. Prep. Proced. Int.* **2000**, *32*, 505−546.

(12) Ruedi, P.; Juch, M. Chemistry and biological activities of long-chain alkyloxy-catechols of the [n]-gingerol-type. *Curr. Org. Chem.* **1999**, *3*, 623−646.

(13) Voigt, T.; Gerding-Reimers, C.; Ngoc Tran, T. T.; Bergmann, S.; Lachance, H.; Schölermann, B.; Brockmeyer, A.; Janning, P.; Ziegler, S.; Waldmann, H. A natural product inspired tetrahydropyran collection yields mitosis modulators that synergistically target CSE1L and tubulin. *Angew. Chem., Int. Ed.* **2013**, *52*, 410−414.

(14) Wan, O. W.; Chung, K. K. The role of α-synuclein oligomerization and aggregation in cellular and animal models of Parkinson's disease. *PLoS One* **2012**, *7*, e38545.

(15) Tao, Q. F.; Xu, Y.; Lam, R. Y.Y.; Schneider, B.; Dou, H.; Leung, P. S.; Shi, S. Y.; Zhou, C. X.; Yang, L. X.; Zhang, R. P.; Xiao, Y. C.; Wu, X. M.; Stöckigt, J.; Zeng, S.; Cheng, C. H. K.; Zhao, Y. Diarylheptanoids and a monoterpenoid from the rhizomes of *Zingiber officinale*: Antioxidant and cytoprotective properties. *J. Nat. Prod.* **2008**, *71*, 12−17.

(16) He, W. S.; Wei, X. Y.; Li, L.; Li, Y.; Guo, S. Y.; Guo, B. J. A new antioxidant cyclic diarylheptanoid from the rhizomes of *Zingiber officinale*. *J. Integr. Plant Biol.* **2001**, *43*, 757−759.

(17) Lee, C.; Loh, T. A highly enantioselective allyl-transfer through suppression of epimerization. *Tetrahedron Lett.* **2004**, *45*, 5819−5822.

(18) Parker, G. D.; Seden, P. T.; Willis, C. L. Synthesis of a novel diarylheptanoid isolated from *Zingiber officinale*. *Tetrahedron Lett.* **2009**, *50*, 3686−3689.

(19) Yokosuka, A.; Mimaki, Y.; Sakagami, H.; Sashida, Y. New diarylheptanoids and diarylheptanoid glucosides from the rhizomes of *Tacca chantrieri* and their cytotoxic activity. *J. Nat. Prod.* **2002**, *65*, 283−289.

DOI: 10.1021/acs.jafc.7b02021
J. Agric. Food Chem. 2017, 65, 6608−6614

(20) Harada, N.; Saito, A.; Ono, H.; Gawronski, J.; Gawronska, K.; Sugioka, T.; Uda, H.; Kuriki, T. A CD method for determination of the absolute stereochemistry of acyclic glycols. 1. Application of the CD exciton chirality method to acyclic 1,3-dibenzoate systems. *J. Am. Chem. Soc.* **1991**, *113*, 3842−3850.
(21) Qiu, F.; Li, J.; Kang, N.; Chen, L. X. Diarylheptanoid compounds in *Curcuma zedoary* and medicinal application thereof. CN Patent 101979366 A, Feb 23, 2011.
(22) Ma, J. P.; Jin, X. L.; Yang, L.; Liu, Z. L. Diarylheptanoids from the rhizomes of *Zingiber officinale*. *Phytochemistry* **2004**, *65*, 1137−1143.
(23) Ohta, S.; Koyama, M.; Aoki, T.; Suga, T. Absolute configuration of platyphylloside and [-)-centrolobol. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 2423−2424.
(24) Liu, Z. H.; Sang, S. M.; Hartman, T. G.; Ho, C. T.; Rosen, R. T. Determination of diarylheptanoids from *Alpinia officinarum* (Lesser Galangal) by HPLC with photodiode array and electrochemical detection. *Phytochem. Anal.* **2005**, *16*, 252−256.
(25) Kiuchi, F.; Shibuya, M.; Sankawa, U. Inhibitors of prostaglandin biosynthesis from *Alpinia officinarum*. *Chem. Pharm. Bull.* **1982**, *30*, 2279−2282.
(26) Pulkkinen, J. T.; Honkakoski, P.; Perakyla, M.; Berczi, I.; Laatikainen, R. Synthesis and evaluation of estrogen agonism of diaryl 4,5-dihydroisoxazoles, 3-hydroxyketones, 3-methoxyketones, and 1,3-diketones: A compound set forming a 4D molecular library. *J. Med. Chem.* **2008**, *51*, 3562−3571.
(27) Kikuzaki, H.; Kobayashi, M.; Nakatani, N. Diarylheptanoids from rhizomes of *Zingiber officinale*. *Phytochemistry* **1991**, *30*, 3647−3651.
(28) Tung, N.; Kim, S.; Ra, J.; Zhao, Y.-Z.; Sohn, D.; Kim, Y. Antioxidative and hepatoprotective diarylheptanoids from the bark of *Alnus japonica*. *Planta Med.* **2010**, *76*, 626−629.
(29) Jurgens, T. M.; Frazier, E. G.; Schaeffer, J. M.; Jones, T. E.; Zink, D. L.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J. Novel nematocidal agents from *Curcuma comosa*. *J. Nat. Prod.* **1994**, *57*, 230−235.

DOI: 10.1021/acs.jafc.7b02021
J. Agric. Food Chem. 2017, 65, 6608−6614