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COMMUNICATION

Five New Oxylipins from *Chaenomeles sinensis*

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Abstract In the course of our continuing search for bioactive constituents of Korean medicinal plants, we isolated five new oxylipins, chaenomic acid A–E (**1**–**5**), and six known ones (**6**–**11**) from the twigs of *Chaenomeles sinensis*. The structures of the new compounds (**1**–**5**) were determined by spectroscopic methods, including 1D and 2D NMR (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY), olefin cross-metathesis, and LC/MS analysis. The known compounds (**6**–**11**) were identified by comparison of their spectroscopic data and specific optical rotation with the reported data. The isolated compounds (**1**–**11**) were tested for their inhibitory effects on nitric oxide production in lipopolysaccharide-activated murine microglial cells and for their cytotoxic activities against four human cancer cell lines (A549, SK-OV-3, A498, and HCT-15).

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Keywords *Chaenomeles sinensis* · Rosaceae · Oxylipins · NO · NGF

Abbreviations

COSY	Correlation spectroscopy
DMEM	Dulbecco's modified Eagle's medium
FAB	Fast atom bombardment
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
HPLC	High-performance liquid chromatography
HR	High resolution
LPLC	Low-pressure liquid chromatography
LPS	Lipopolysaccharide
MS	Mass spectrometry
L-NMMA	N^{G}-Monomethyl-L-arginine
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
NO	Nitric oxide
SRB	Sulforhodamine B
TLC	Thin-layer chromatography

Introduction

Chaenomeles sinensis Koehne (=*Cydonia sinensis* Thouin, Rosaceae) is a deciduous tree widely distributed throughout Korea, Japan, and China. The fruits of this tree have been used in Korean traditional medicine for treating a cough, the common cold, pain, and diarrhea [1, 2]. In a previous phytochemical investigation, triterpenes and flavonoids were isolated from this plant, and antioxidant, antiviral, and antihyperlipidemic properties were reported in biological activity studies [1, 3–5]. In the course of our

continuing search for bioactive constituents in Korean medicinal plants, we found that the EtOAc fraction of the MeOH extract of *C. sinensis* inhibited NO production in a lipopolysaccharide (LPS)-activated microglia BV-2 cell line. The EtOAc fraction was subjected to repeated column chromatography on silica gel, Sephadex LH-20, and semi-preparative HPLC separation to yield five new oxylipins, chaenomic acid A–E (**1**–**5**), and six known compounds (**6**–**11**). The structures of the new compounds (**1**–**5**) were determined by spectroscopic methods, including 1D and 2D NMR (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY), olefin cross-metathesis, and LC/MS analysis. The known compounds (**6**–**11**) were identified by comparison of their spectroscopic data and specific optical rotation with the reported data. The isolated compounds (**1**–**11**) were tested for their inhibitory effects on NO production in LPS-activated murine microglial cells and for their cytotoxic activities against four cultured human tumor cell lines: A549 (non-small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), A498 (renal cell carcinoma), and HCT-15 (colon cancer cells).

Materials and Methods

General Experimental Procedures

Optical rotations were measured on a Jasco P-1020 polarimeter (Jasco, Easton, MD, USA). IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrophotometer (Bruker, Karlsruhe, Germany). High resolution fast atom bombardment mass spectrometry (HRFABMS) spectra were obtained on a JEOL JMS700 mass spectrometer (JEOL, Tokyo, Japan). LC/MS was performed with an Agilent 1100 LC/MS system (Agilent, Santa Clara CA, USA). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE III 700 NMR spectrometer (Bruker, Billerica, MA, USA) operating at 700 (^{1}H) and 175 MHz (^{13}C), with chemical shifts given in ppm (δ). Preparative high-performance liquid chromatography (HPLC) was performed by a Gilson 306 pump (Gilson, Middleton, WI, USA) with a Shodex refractive index detector (Shodex, New York, NY, USA). Low-pressure liquid chromatography (LPLC) was carried out over a LiChroprep Lobar-A RP-18 column (240 mm × 10 mm i.d.; Merck, Darmstadt, Germany) with an FMI QSY-0 pump (Teledyne Isco, Lincoln, NE, USA). Column chromatography was performed with silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany). The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Merck precoated silica gel F_{254} plates and reversed-phase (RP)-18 F_{254s} plates (Merck, Darmstadt, Germany) were used for thin-layer chromatography (TLC). Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde–sulfuric acid.

Plant Material

Twigs of *C. sinensis* were collected in Seoul, Korea in January 2012, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU-NPL 1206) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation

Twigs of *C. sinensis* (7.0 kg) were extracted three times with 80 % aqueous MeOH (each 10 L × 1 day) under reflux and filtered. The filtrate was evaporated under vacuum to obtain a crude MeOH extract (320 g), which was suspended in distilled water and successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, to yield 3, 15, 6, and 30 g of each residue, respectively. The EtOAc-soluble layer (6 g) was separated on a Sephadex LH-20 column chromatography [300 g, 3 × 70 cm, eluted with MeOH–H_2O, 9:1 (2.0 L)] to yield five fractions [Fr. A, 0.4 L; Fr. B, 0.4 L; Fr. C, 0.4 L; Fr. D, 0.4 L; and Fr. E, 0.4 L]. Fraction A (3.8 g) was subjected to silica gel (230–400 mesh) column chromatography [100 g, 3 × 30 cm, eluted with $CHCl_3$–MeOH, 10:1 (1.0 L) and 5:1 (1.0 L)] to yield seven fractions [Fr. A1, 10:1, 0.3 L; Fr. A2, 10:1, 0.3 L; Fr. A3, 10:1, 0.4 L; Fr. A4, 5:1, 0.25 L; Fr. A5, 5:1, 0.25 L; Fr. A6, 5:1, 0.25 L; and Fr. A7, 5:1, 0.25 L]. Subfraction A2 (80 mg) was purified by semi-preparative reversed-phase HPLC using an Econosil RP-C_{18} column (10 μm column; 250 × 10 mm; 10 μm particle size; Shodex refractive index detector) with a solvent system of MeCN–H_2O (3:2, each 700 mL; flow rate, 2 mL/min) to obtain compounds **7** (8 mg) and **9** (10 mg). Subfraction A3 (300 mg) was applied to LPLC on a LiChroprep Lobar-A RP-18 column (240 mm × 10 mm i.d., 40–63 μm) with a solvent system of MeOH–H_2O (1:1, 800 mL) and purified by semi-preparative HPLC with a solvent system of MeCN–H_2O (1:4, each 800 mL; flow rate, 2 mL/min) to yield compounds **3** (3 mg), **5** (5 mg), **10** (5 mg), and **11** (5 mg). Subfraction A4 (400 mg) was applied to LPLC on a LiChroprep Lobar-A RP-18 column (240 mm × 10 mm i.d., 40–63 μm) with a solvent system of MeOH–H_2O (3:2, 800 mL) and purified by semi-preparative HPLC with a solvent system of MeOH–H_2O (3:2, each 800 mL; flow rate, 2 mL/min) to yield compounds **6** (30 mg) and **8** (20 mg). Subfraction A6 (350 mg) was separated by LPLC on a LiChroprep Lobar-A RP-18 column (240 mm × 10 mm i.d., 40–63 μm) with a solvent system of MeOH–H_2O (1:1, 800 mL) and further purified

Table 1 ^{1}H NMR [ppm, mult (*J* in Hz)] data of **1–5** in CD_3OD (700 MHz)

Position	1	2	3	4	5
2	2.30, br s	2.31, br s	2.31, t (6.9)	2.29, t (7.3)	2.30, t (7.3)
3	1.63, overlap	1.63, overlap	1.62, quin (6.9)	1.61, overlap	1.62, overlap
4	1.36, overlap	1.36, overlap	1.35, overlap	1.36, overlap	1.37, overlap
5	1.36, overlap	1.36, overlap	1.35, overlap	1.36, overlap	1.37, overlap
6	1.36, overlap	1.36, overlap	1.35, overlap	1.36, overlap	1.37, overlap
7	1.36, overlap	1.36, overlap	1.37, overlap	1.38, overlap	1.37, overlap
8	1.53, m	1.53, m	1.52, m	1.53, m	1.54, m
9	4.05, q (6.1)	4.05, q (6.2)	4.06, q (6.2)	4.06, q (6.1)	4.08, m
10	5.74, dd (15.8, 6.1)	5.74, dd (15.8, 6.2)	5.76, ddd (15.5, 6.2, 0.8)	5.74, dd (15.9, 6.1)	5.81, overlap
11	5.70, dd (15.8, 5.2)	5.69, dd (15.8, 5.2)	5.71, ddd (15.5, 5.9, 0.8)	5.70, dd (15.9, 6.0)	5.81, overlap
12	3.98, overlap	3.97, overlap	4.11, t (5.9)	3.94, t (5.8)	4.18, overlap
13	3.98, overlap	3.97, overlap	4.24, ddd (5.9, 4.5, 1.7)	3.75, ddd (9.8, 5.8, 2.7)	4.16, overlap
14a	5.70, dd (15.8, 5.1)	5.69, dd (15.8, 5.1)	6.94, dd (16.0, 4.5)	1.64, ddd (14.2, 9.8, 2.7)	2.40, ddd (13.7, 8.9, 5.7)
14b				1.51, ddd (14.2, 9.8, 3.0)	1.82, ddd (13.7, 5.0, 2.0)
15	5.74, dd (15.8, 5.9)	5.75, dd (15.8, 5.9)	6.36, dd (16.0, 1.7)	4.28, m	3.97, ddd (8.9, 5.0, 4.2)
16	3.99, overlap	3.98, overlap		5.54, ddq (15.3, 6.6, 1.5)	3.44, dt (8.4, 4.2)
17	1.55, m	1.55, m	2.67, q (7.3)	5.69, dqd (15.3, 6.4, 1.1)	1.57, overlap
18	0.95, t (7.4)	0.95, t (7.4)	1.09, t (7.3)	1.71, ddd (6.4, 1.5, 0.6)	1.01, t (7.4)

by semi-preparative HPLC with a solvent system of MeOH–H_2O (3:2, each 800 mL; flow rate, 2 mL/min) to yield compounds **1** (8 mg), **2** (5 mg), and **4** (3 mg).

Chaenomic Acid A (**1**)
Colorless gum, [α] +98.6 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3,354 (OH), 2,942, 1,720 (C=O), 1,282, 1,028 cm^{-1}; ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 345.2277 $[M + H]^+$ (calcd. for $C_{18}H_{33}O_6$, 345.2277).

Chaenomic Acid B (**2**)
Colorless gum, [α] +20.4 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3,352 (OH), 2,941, 1,721 (C=O), 1,280, 1,029 cm^{-1}; ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 345.2278 $[M + H]^+$ (calcd. for $C_{18}H_{33}O_6$, 345.2277).

Chaenomic Acid C (**3**)
Colorless gum, [α] +6.6 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3,355 (OH), 2,943, 1,721 (C=O), 1,280, 1,028 cm^{-1}; ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 343.2121 $[M + H]^+$ (calcd. for $C_{18}H_{31}O_6$, 343.2121).

Chaenomic Acid D (**4**)
Colorless gum, [α] +66.8 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3,354 (OH), 2,942, 1,720 (C=O), 1,282, 1,028 cm^{-1}; ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 345.2277 $[M + H]^+$ (calcd. for $C_{18}H_{33}O_6$, 345.2277).

Table 2 ^{13}C NMR data of **1–5** in CD_3OD (175 MHz)

Position	1	2	3	4	5
1	176.6	176.6	176.4	176.7	176.6
2	33.7	33.7	33.8	33.8	33.9
3	25.2	25.1	24.8	24.8	24.8
4	28.9	28.9	28.8	28.8	28.8
5	29.1	29.0	29.2	29.2	29.2
6	28.9	28.9	29.0	29.0	29.0
7	25.0	25.0	25.0	25.1	25.0
8	36.8	36.8	36.9	36.9	36.7
9	71.6	71.6	71.5	71.7	71.7
10	134.8	134.9	135.7	135.3	136.6
11	129.1	129.1	128.7	129.7	126.3
12	74.9	74.8	74.3	75.1	83.9
13	74.9	75.0	73.8	70.9	72.9
14	129.4	129.2	145.8	39.8	37.3
15	135.1	135.0	129.1	68.6	80.0
16	73.0	73.0	202.0	134.5	74.6
17	29.5	29.6	32.7	124.7	26.1
18	8.7	8.7	7.0	16.4	9.3

Chaenomic Acid E (**5**)
Colorless gum, [α] +160.2 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3,356 (OH), 2,942, 1,721 (C=O), 1,280, 1,028 cm^{-1}; ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data, see Tables 1 and 2, respectively; HRFABMS *m/z* 345.2277 $[M + H]^+$ (calcd. for $C_{18}H_{33}O_6$, 345.2277).

Olefin Cross-Metathesis of **1–5**

Compounds **1–5** (each 0.5 mg) with 50 μL of methyl acrylate, 50 μg of 2nd generation Hoveyda-Grubbs catalyst, and 500 μL of CH_2Cl_2 were stirred for 3 h. The reaction mixture was analyzed by LC/MS under the following conditions: column, ACQUITY UPLC® BEH C18 1.7 μm 2.1 × 50 mm (Waters); elution solvent, 15–100 % aqueous MeOH with 0.1 % formic acid over 20 min and MeOH with 0.1 % formic acid from 20 to 25 min, with a flow rate of 0.4 mL/min. The product **1a** was detected at t_R 9.77 min and identified by the pseudomolecular ion peak $[M + Na]^+$ at *m/z* 281 in ESIMS.

Measurement of NO Production and Cell Viability

BV-2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5 % FBS and 1 % penicillin–streptomycin. To measure NO production, BV-2 cells were plated onto a 96-well plate (3×10^4 cells/well). After 24 h, the cells were pretreated with compounds for 30 min and stimulated with 100 ng/mL LPS for 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture media using the Griess reaction. The supernatant was harvested and mixed with an equal volume of Griess reagent (1 % sulfanilamide, 0.1 % *N*-1-naphthylethylenediamine dihydrochloride in 5 % phosphoric acid). After 10 min, the absorbance at 540 nm was measured using an Emax microplate reader (Molecular Devices, Sunnyvale). Sodium nitrite was used as a standard to calculate the nitrite concentration. The cell viability was measured using the MTT assay. N^G-Monomethyl-L-arginine (L-NMMA; Sigma), a well-known NO synthase inhibitor, was used as a positive control.

Cytotoxicity Testing

A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each compound against four cultured human tumor cell lines [6]. The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549, SK-OV-3, A498, and HCT-15. The cancer cell lines cells were provided by the National Cancer Institute (NCI). Doxorubicin (purity ≥98 %, Sigma) was used as a positive control. The IC_{50} values of doxorubicin against the A549, SK-OV-3, A498, and HCT-15 cell lines were 0.0014, 0.0217, 0.0025, and 0.1077 μM, respectively. Tested compounds were demonstrated to be pure as evidenced by NMR and HPLC analyses (purity ≥95 %).

Results and Discussion

Compound **1** was isolated as a colorless gum and the molecular formula $C_{18}H_{32}O_6$ was determined from the pseudomolecular ion peak $[M + H]^+$ at *m/z* 345.2277 (calcd. for $C_{18}H_{33}O_6$, 345.2277) in HRFABMS. The IR spectrum of **1** indicated that **1** possessed hydroxyl (3,354 cm^{-1}) and carbonyl (1,720 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) of **1** showed the presence of two *trans* double bonds [δ_H 5.74 (1H, dd, $J = 15.8$, 6.1 Hz, H-10), 5.74 (1H, dd, $J = 15.8$, 5.9 Hz, H-15), 5.70 (1H, dd, $J = 15.8$, 5.2 Hz, H-11), and 5.70 (1H, dd, $J = 15.8$, 5.1 Hz, H-14)], four oxymethines [δ_H 4.05 (1H, q, $J = 6.1$ Hz, H-9), 3.99 (1H, overlap, H-16), and 3.98 (2H, overlap, H-12 and H-13)], and a methyl group [δ_H 0.95 (3H, t, $J = 7.4$ Hz, H-18)]. The ^{13}C NMR spectrum of **1** revealed 18 carbon signals including a carbonyl carbon (δ_C 176.6), two double bonds (δ_C 135.1, 134.8, 129.4, and 129.1), and four oxygenated carbons [δ_C 74.9 (×2), 73.0, and 71.6]. These spectral data were similar to that of pinellic acid (**6**) [7], except that the proton and carbon resonances of a double bond (δ_H 5.74 and 5.70; δ_C 135.1 and 129.4) and a hydroxyl group (δ_H 3.99; δ_C 73.0) were present in **1** (Fig. 1). The planar structure of **1** was confirmed through analysis of ^1H–^1H COSY and HMBC data (Fig. 2). Olefin cross-metathesis of **1** with methyl acrylate/2nd generation Hoveyda-Grubbs catalyst yielded product **1a** (Fig. 3), which was identified by the molecular ion $[M + Na]^+$ at *m/z* 281 in LC/MS analysis [8]. This data indicated the presence of a double bond at C-10 of **1**. The relative configuration of **1** was determined by the peak pattern of H-10 and H-11. Shirahata et al. [7] synthesized four stereoisomers of pinellic acid (**6**) and found that the chemical shift of H-10 was more downfield shifted than that of H-11 in the *syn*-diol at C12/C13. The same peak pattern was observed in the ^1H NMR of **1** [δ_H 5.74 (H-10) and 5.70 (H-11)] and the relative configuration at C12–C13 was determined as a *syn*-form. Moreover, a relatively small difference of chemical shifts between H-10 and H-11 ($\Delta\delta$ 0.04) of **1** confirmed the same configurations of C-9 and C-12 (each *S**; $\Delta\delta$ 0.05, 9*S*,12*S* form of pinellic acid; $\Delta\delta$ 0.06, 9*S*,12*R* form of pinellic acid) [7]. The same configurations of C-13 and C-16 (each *S**) were also determined by a small difference of chemical shift between H-14 and H-15 ($\Delta\delta$ 0.04) [7]. Furthermore, the chemical shifts and coupling constants, which were all very similar to **6**, plus the consideration from a biosynthetic aspect, suggested that **1** shared the same configurations as **6** in C-9, C-12, and C-13 positions. Thus, the structure of **1** was established as (9*S**,10*E*,12*S**,13*S**,14*E*,16*S**)-9,12,13,16-tetrahydroxy-10,14-octadecadienoic acid, and named chaenomic acid A.

Compound **2** had the molecular formula $C_{18}H_{32}O_6$ and inspection of ^1H and ^{13}C NMR of **2** revealed that it was a stereoisomer of **1**. A larger difference of the chemical shift between H-14 and H-15 ($\Delta\delta$ 0.06) than that of **1** ($\Delta\delta$ 0.04) was observed, confirming that the configurations of C-13 and C-16 were different (*S** and *R**, respectively) [7]. The

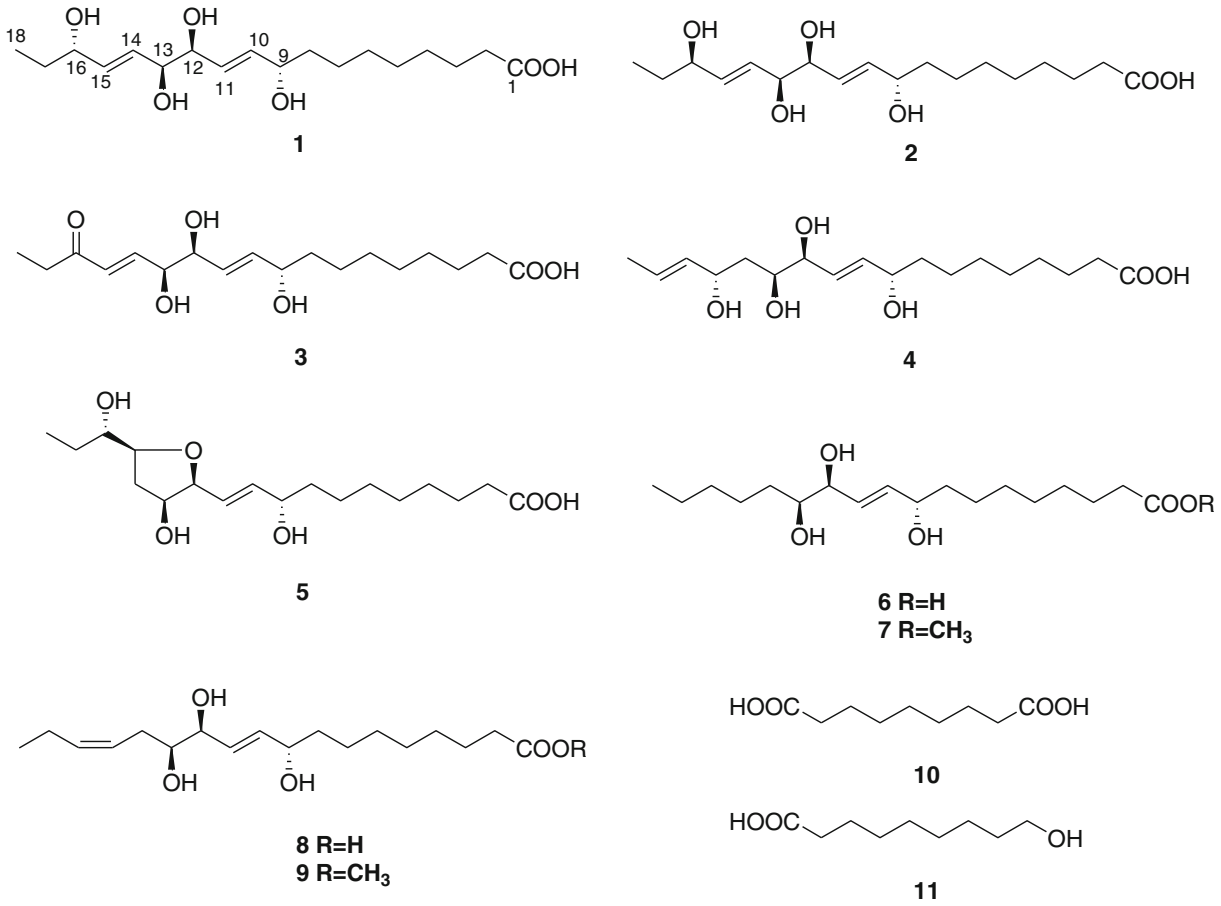

Fig. 1 The structures of compounds **1**–**11**

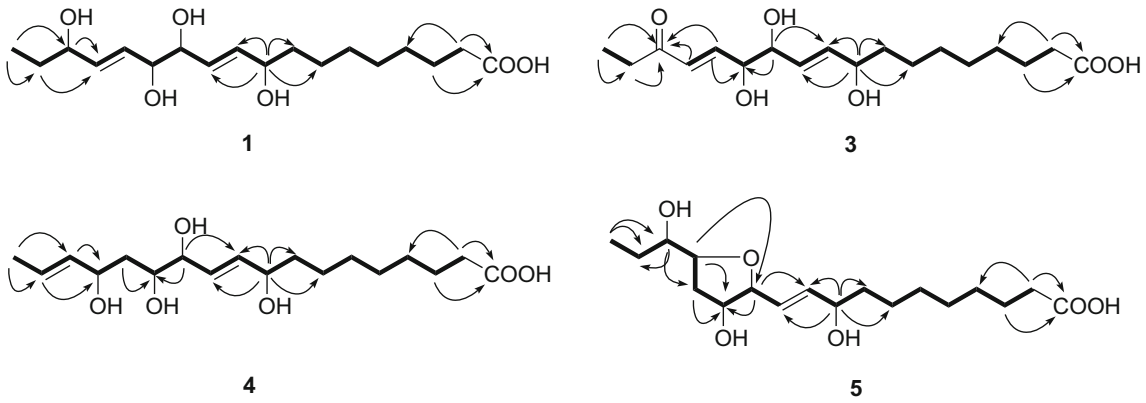

Fig. 2 ^{1}H–^{1}H COSY (*bold*) and HMBC (*plain arrow*) correlations of **1** and **3**–**5**

full NMR analysis established the structure of **2** as (9*S**,10*E*,12*S**,13*S**,14*E*,16*R**)-9,12,13,16-tetrahydroxy-10,14-octadecadienoic acid, named chaenomic acid B.

Compound **3** was obtained as a colorless gum. The molecular formula was determined to be $C_{18}H_{30}O_6$ from the $[M + H]^+$ pseudomolecular ion peak in the positive ion HRFABMS. The ^{1}H and ^{13}C NMR data of **3** were quite similar to that of **1**, except for the presence of a signal attributable to a carbonyl carbon (δ_C 202.0) instead of an oxygenated methine carbon in **1**. The HMBC correlations of H-14, H-15, H-17, and H-18 to C-16 (Fig. 2) confirmed the location of carbonyl group at C-16. The structure of **3** was corroborated by analysis of 2D NMR experiments (^{1}H–^{1}H COSY, HMQC, and HMBC). As described for **1**,

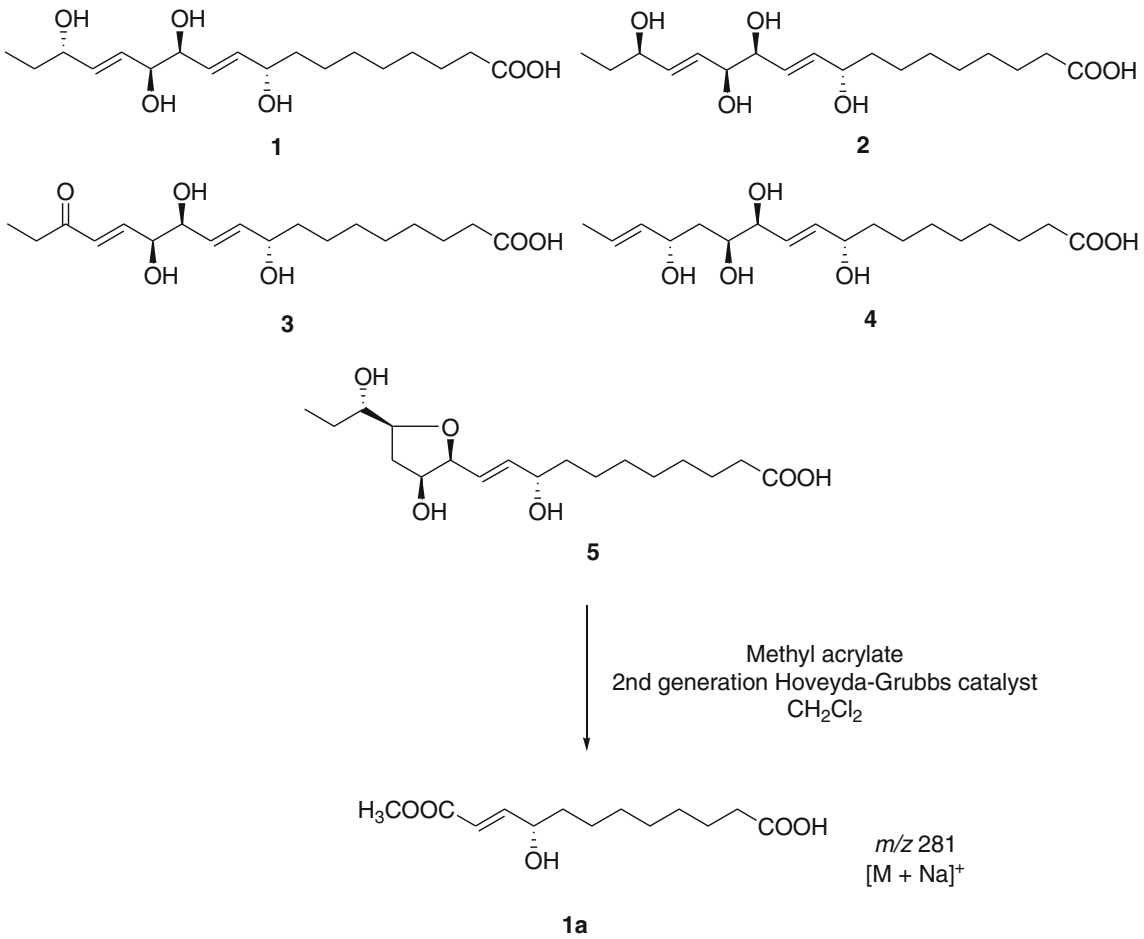

Fig. 3 Olefin cross-metathesis of **1**–**5**

the relative configurations of C-9, C-12, and C-13 were confirmed as all *S** by the peak pattern of H-10 (δ_H 5.76) and H-11 (δ_H 5.71) and their small difference ($\Delta\delta$ 0.05) [7]. Therefore, the structure of **3** was determined as (9*S**,10*E*,12*S**,13*S**,14*E*)-9,12,13-trihydroxy-16-oxo-10,14-octadecadienoic acid, and named chaenomic acid C.

Compound **4** had the molecular formula $C_{18}H_{32}O_6$. The NMR data of **4** were similar to that of **1** with the major differences being the chemical shifts and splitting patterns of *trans* double bond signals [δ_H 5.69 (1H, dqd, J = 15.3, 6.4, 1.1 Hz, H-17) and 5.54 (1H, ddq, J = 15.3, 6.6, 1.5 Hz, H-16)] and the terminal methyl proton [δ_H 1.71 (1H, ddd, J = 6.4, 1.5, 0.6 Hz, H-18)] in the ^{1}H NMR spectrum. The HMBC cross-peaks of H-18 (δ_H 1.71) to C-17 (δ_C 124.7) and C-16 (δ_C 134.5), and H-17 (δ_H 5.69) and H-16 (δ_H 5.54) to C-15 (δ_C 68.6) confirmed that a double bond and a hydroxyl group were located at C-16 and C-15, respectively. Through extensive NMR analysis including 2D NMR, the planar structure of **4** was determined. The *S** configurations of C-9, C-12, and C-13 were confirmed by the same application as **1** [δ_{H10} 5.74, δ_{H11} 5.70; $\Delta\delta_{H10\text{-}H11}$ 0.04)]. The NOESY correlations of H-13/H-14a and H-14b/H-15 and their small J values ($J_{H13\text{-}H14a}$ = 2.7 Hz, $J_{H14b\text{-}H15}$ = 3.0 Hz) confirmed the 15*S** configuration (Fig. 4) [9]. Thus, the structure of **4** was established as (9*S**,10*E*,12*S**,13*S**,15*S**,16*E*)-9,12,13,15-tetrahydroxy-10,16-octadecadienoic acid, and named chaenomic acid D.

Compound **5** was isolated as a colorless gum with the molecular formula of $C_{18}H_{32}O_6$. Compounds **5** and **1** had very similar ^{1}H and ^{13}C NMR spectra, but marked differences included the absence of double bond signals and the presence of an oxymethine proton and carbon signals [δ_H 3.97 (1H, ddd, J = 8.9, 5.0, 4.2 Hz); δ_C 80.0]. The HMBC cross-peak of H-15 to C-12 indicated **5** had a tetrahydrofuran ring moiety and its relative configuration was confirmed as 12*S**,13*S**,15*S**, and 16*S** through the NOESY correlations of H-12/H-14a and H-15, H-13/H-15, H-14b/H-16, and H-15/H-17 (Fig. 5). Through the consideration from a biosynthetic aspect, **5** might share the same configurations as **1**–**4** and **6**–**9** in C-9, C-12, and C-13 positions. Thus, the relative configuration at

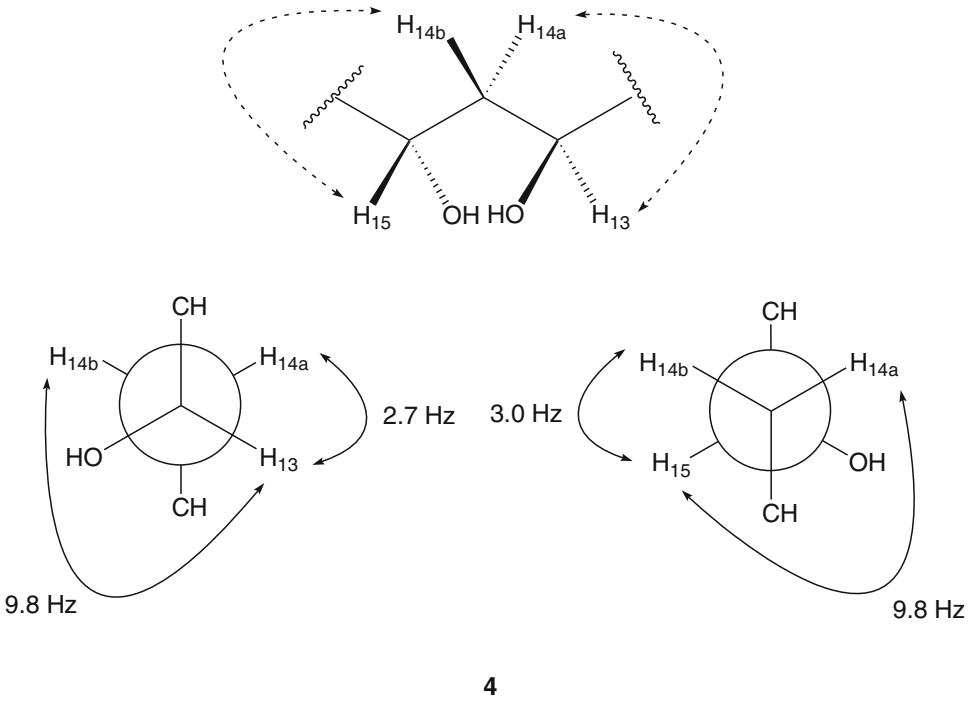

Fig. 4 NOESY correlations (*dashed arrows*) of C-13 to C-15 and their *J* values of **4**

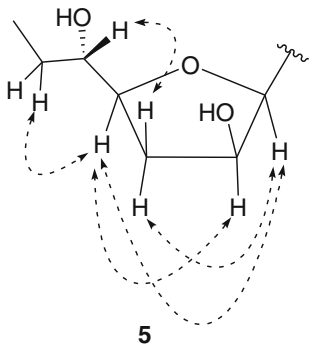

Fig. 5 NOESY correlations (*dashed arrows*) of **5**

C-9 was suggested as 9*S** and the structure of **5** was determined as (9*S**,10*E*,12*S**,13*S**,15*S**,16*S**)-9,13,16-trihydroxy-12,15-oxy-10-octadecenoic acid, and named chaenomic acid E.

Biosynthesis of **1**–**5** from corchorifatty acid F (**8**) is proposed (Fig. 6). Thus, **1/2** could be synthesized via hydroxylation at C-16 and double bond shift to C-14 in **8**, and **3** further dehydrogenation at C-16. Compound **4** could be also synthesized via hydroxylation at C-15 and double bond shift to C-16 in **8**. Epoxide (**i**) is to be synthesized by hydroxylation at C-15/C-16 in the double bond and then dehydration in **8**. Thus, **5** could be synthesized through the ring closure to tetrahydrofuran in **i**.

The six known compounds (**6**–**11**) were identified as pinellic acid (**6**) [7], methyl (9*S*,12*S*,13*S*)-9,12,13-trihydroxy-10*E*-octadecenoate (**7**) [10], corchorifatty acid F (**8**) [11], methyl 9,12,13-trihydroxyoctadeca-10*E*,15*Z*-dienoate (**9**) [12], azelaic acid (**10**) [13], and 9-hydroxynonanoic acid (**11**) [14] by comparing their spectroscopic data and specific optical rotation with the reported data.

Anti-neuroinflammatory effects of the isolates (**1**–**11**) were evaluated by examining NO production in LPS-activated BV-2 microglia cell line. The activity of each compound was expressed as the concentration that inhibited NO production by 50 % (IC_{50}). As shown in Table 3, compound **10** significantly inhibited LPS-stimulated NO production with an IC_{50} value of 15.08 μM, which displayed more potency than that of positive control L-NMMA (IC_{50} 28.44 μM). Compounds **1**, **2**, **5**, **6**, and **8** showed moderate activity with IC_{50} values of 54.52, 47.79, 66.54, 40.95, and 39.32 μM, respectively. None of the isolates (**1**–**11**) had any effect on cell viability. Interestingly, we observed that pinellic acid (**6**) and corchorifatty acid F (**8**) displayed moderate activity (IC_{50} 40.95 and 39.32 μM, respectively), but their methyl esters **7** and **9** showed weak or no activity (IC_{50} 222.32 and >500 μM, respectively). These data suggest that the presence of a free carboxylic acid is important for inhibiting NO production in LPS-activated BV-2 cells. Also, compounds **1**–**11** were evaluated for their antiproliferative activities against four human cancer cell lines (A549, SK-OV-3, A498, and HCT-15) using the SRB bioassay. Compound **4** showed weak inhibitory activity against A549 and A498 cell lines (IC_{50} 76.81 and 68.44 μM, respectively) and compounds **8** and **11** exhibited selective cytotoxicity against A498 cell lines

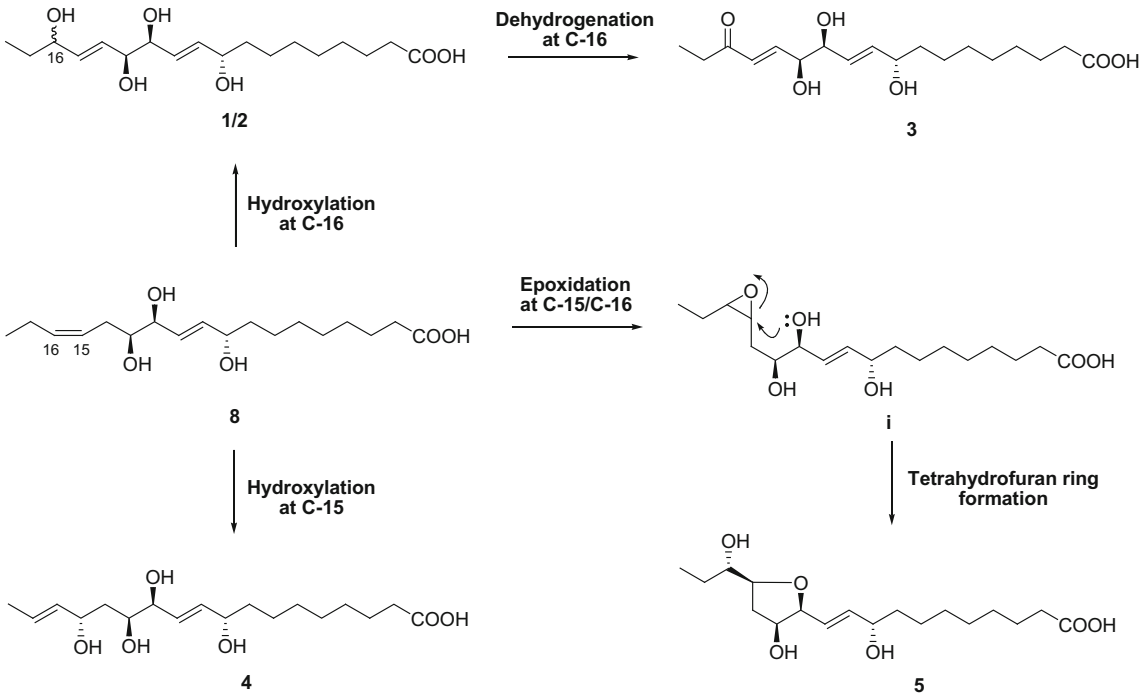

Fig. 6 Hypothetical biogenetic pathway of **1**–**5**

Table 3 Inhibitory effect on NO production of compounds **1**–**11** in LPS-activated BV-2 Cells

Comp.	IC_{50} (μM)[a]	Cell viability (%)[b]
1	54.52	124.70 ± 3.72
2	47.79	101.19 ± 4.83
3	93.30	109.09 ± 6.73
4	>500	102.97 ± 4.47
5	66.54	107.43 ± 4.72
6	40.95	108.04 ± 1.64
7	222.32	103.30 ± 6.09
8	39.32	115.82 ± 3.15
9	>500	119.94 ± 6.45
10	15.08	182.51 ± 5.06
11	87.48	111.91 ± 5.61
L-NMMA[c]	28.44	96.41 ± 3.41

[a] IC_{50} value of each compound was defined as the concentration (μM) that caused 50 % inhibition of NO production in LPS-activated BV-2 cells

[b] Cell viability after treatment with 20 μM of each compound was determined by MTT assay and is expressed in percentage (%). The results are averages of three independent experiments, and the data are expressed as mean ± SD

[c] L-NMMA as a positive control

(IC_{50} 88.91 and 94.28 μM, respectively). Other compounds were inactive ($IC_{50} > 100$ μM, respectively).

In conclusion, we isolated five new oxylipins, chaenomic acid A–E (**1**–**5**), and six known compounds (**6**–**11**) from the twigs of *C. sinensis*. Among them, compound **10** showed potent anti-neuroinflammatory activity and compounds **4**, **8**, and **11** displayed weak cytotoxicity against A549 and A498 cell lines.

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Conflict of interest All authors declare that there are no conflicts of interest.

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