JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

Limonoids from the Fruits of *Aphanamixis polystachya* (Meliaceae) and Their Biological Activities

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Supporting Information

ABSTRACT: Seven new prieurianin-type limonoids, aphapolynins C−I (**1**−**7**), and a new aphanamolide-type limonoid, aphanamolide B (**8**), along with seventeen known compounds, were isolated from the fruits of *Aphanamixis polystachya*. The structures of these compounds were established on the basis of spectroscopic studies. The absolute configurations were determined by combination of electronic circular dichroism (ECD) calculation, CD exciton chirality method, and single crystal X-ray diffraction. All these isolates were evaluated for their cytotoxicities against three human cancer cell lines, for their inhibitory effects on lipopolysaccharide (LPS) induced RAW264.7 murine macrophages, and for their fungicidal, herbicidal, and insecticidal activities. Compounds **1**, **14**, **16**, and **17** exhibited significant fungicidal activities; **1** and **25** in particular showed good insecticidal activities. The α,β-unsaturated lactone and 14,15-epoxy ring moieties were essential for the insecticidal activity.

KEYWORDS: *limonoids, fungicidal activity, insecticidal activity, Aphanamixis polystachya, Meliaceae*

INTRODUCTION

Ecologically acceptable methods to protect our food supply from plant diseases and phytophagous insect attack are urgently needed. Plant extracts for pest management can be a good alternative to common insecticides that are detrimental to the environment. The neem tree and the chinaberry tree have received much attention in this context, owing their importance to the presence of phytochemicals that have considerable potential as antifeedants or biopesticides.[1] Meliaceous limonoids have earned global recognition because of their effects against insects, especially those of agricultural importance. The azadirachtin from the neem tree shows strong insecticidal activities against a broad spectrum of insect species with favorable nontoxicity toward mammalian organisms.[2,3]

Previous chemical investigations on *Aphanamixis polystachya* revealed many structurally complex rings A,B-*seco* limonoids (prieurianin-type);[4−9] of them, prieurianin displayed moderate antifeedant activity to *Helicoverpa armigera* with EC_{50} at 18.8 ppm, and epoxyprieurianin with EC_{50} at 3.2 ppm.[10] In our recent primary screening, the $CHCl_3$-soluble fraction from the tropical plant *A. polystachya*[11] showed insecticidal activity, which prompted us to further investigate the limonoids from this plant and their agricultural importance. As a result, from the fruits of *A. polystachya*, 25 compounds (Figure 1), including seven new prieurianin-type limonoids, aphapolynins C−I (**1**−**7**), and one new C_3−C_6 connected aphanamolide-type limonoid aphanamolide B (**8**), along with seventeen known compounds (**9**−**25**), were isolated. These isolates were elucidated on the basis of extensive 1D and 2D NMR experiments. The absolute configuration of the known compound aphanamolide A was unambiguously determined by single X-ray diffraction using a mirror Cu Kα radiation; those of **1**−**3** were established by the CD exciton chirality method, including the calculation of the electronic circular dichroism (ECD) spectrum for **3**. Herein we describe the isolation and structure elucidation of these new compounds, along with the biological evaluation of all the isolates, considering both their safety (cytotoxicities and anti-inflammatory activities) and agricultural applications (fungicidal, herbicidal, and insecticidal activities).

MATERIALS AND METHODS

General Experimental Procedures. Melting points were measured on an X-4 instrument and are uncorrected (Beijing Tech Instrument Co., Ltd., Beijing, China). Optical rotations were measured with a Jasco P-1020 polarimeter (Jasco, Tokyo, Japan). Circular dichroism (CD) spectra were obtained on a Jasco 810 spectrometer (Jasco, Tokyo, Japan). Ultraviolet (UV) spectra were recorded on a UV-2450 UV/vis spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra (KBr disks, in cm^{-1}) were recorded on a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany). Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance III NMR instrument (^1H, 500 MHz; ^{13}C, 125 MHz) (Bruker, Karlsruhe, Germany), with tetramethylsilane (TMS) as the internal standard. Chemical shift values (δ) are given in parts per million (ppm) and coupling constants in hertz. The following abbreviations are used to designate multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Electrospray ionization (ESI) and high-resolution (HR)-ESI mass spectral data were acquired on an Agilent 1100 series LC/MSD ion trap mass spectrometer and an Agilent 6520B UPLC-Q-TOF instrument (Agilent Technologies, Santa Clara,

Received: November 22, 2012
Revised: February 6, 2013
Accepted: February 7, 2013
Published: February 7, 2013

dx.doi.org/10.1021/jf3049774 | J. Agric. Food Chem. 2013, 61, 2171−2182

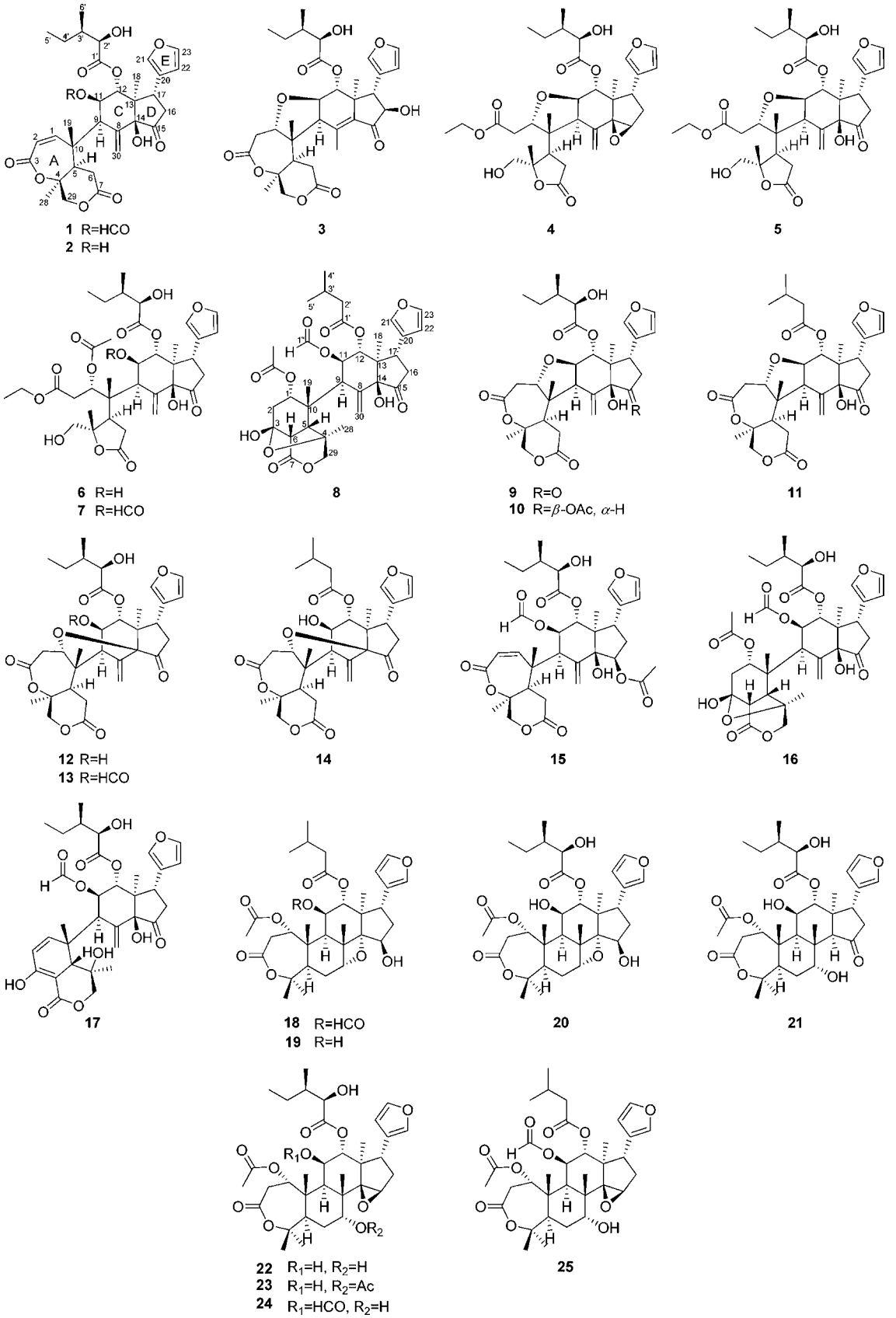

Figure 1. Structures of limonoids **1**−**25** from the fruits of *Aphanamixis polystachya*.

CA, USA), respectively. Preparative high-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-8A system (Shimadzu, Tokyo, Japan) equipped with a Shim-pack RP-C_{18} column (200 mm × 20 mm i.d., 10 μm, Shimadzu, Tokyo, Japan) with flow

dx.doi.org/10.1021/jf3049774 | J. Agric. Food Chem. 2013, 61, 2171−2182

Table 1. ^{1}H NMR and ^{13}C NMR Data of Compounds 1−3

	1[a]		2[b]		3[b]	
position	δ_H, mult (*J*, Hz)	δ_C	δ_H, mult (*J*, Hz)	δ_C	δ_H, mult (*J*, Hz)	δ_C
1	7.43, d (12.0)	152.9, CH	6.57, d (12.0)	155.0, CH	4.09, dd (10.0, 5.5)	81.1, CH
2a	5.96, d (12.0)	119.2, CH	6.04, d (12.0)	121.7, CH	3.03, dd (13.5, 10.0)	39.1, CH_2
2b					2.88, dd (13.5, 5.5)	
3		166.9, C		171.1, C		170.6, C
4		79.7, C		91.0, C		81.1, C
5	2.37, br s	49.9, CH	3.32, dd (10.0, 2.0)	51.7, CH	2.69, dd (10.5, 6.5)	45.3, CH
6*α*	2.56, dd (15.5, 10.0)	29.8, CH_2	2.57, dd (18.5, 2.0)	35.9, CH_2	2.89, dd (15.0, 6.5)	33.0, CH_2
6*β*	2.70, dd (15.5, 7.5)		3.20, dd (18.5, 10.0)		3.07, dd (15.0, 10.5)	
7		172.7, C		178.8, C		174.0, C
8		138.8, C		140.4, C		148.6, C
9	3.00, d (7.5)	51.9, CH	3.50, d (9.0)	58.4, CH	3.41, d (8.0)	56.8, CH
10		43.4, C		44.8, C		53.3, C
11	5.42, dd (10.5, 7.5)	71.6, CH	4.66, dd (11.0, 9.0)	81.3, CH	4.39, dd (10.5, 8.0)	78.1, CH
12	6.04, d (10.5)	74.7, CH	6.29, d (11.0)	76.1, CH	5.31, d (10.5)	76.0, CH
13		49.1, C		50.7, C		44.2, C
14		79.2, C		80.8, C		139.3, C
15		207.5, C		209.5, C		204.7, C
16*α*	2.47, dd (19.5, 9.5)	41.2, CH_2	2.43, dd (19.5, 10.0)	42.2, CH_2	4.37, d (12.0)	78.6, CH
16*β*	2.76, dd (19.5, 9.0)		2.79, dd (19.5, 9.0)			
17	3.79, t (9.5)	34.9, CH	3.79, t (9.5)	37.4, CH	2.83, d (12.0)	51.2, CH
18	0.86, s	12.4, CH_3	0.89, s	12.9, CH_3	1.18, s	18.7, CH_3
19	1.02, s	23.1, CH_3	1.20, s	22.7, CH_3	1.21, s	19.2, CH_3
20		123.0, C		124.8, C		122.9, C
21	7.39, s	140.5, CH	7.31, s	142.0, CH	7.40, s	142.6, CH
22	6.49, s	111.1, CH	6.35, d (0.5)	112.2, CH	6.44, s	112.0, CH
23	7.55, s	142.8, CH	7.44, t-like (1.5)	144.3, CH	7.49, s	144.3, CH
28	1.53, s	25.3, CH_3	1.41, s	22.3, CH_3	1.70, s	26.8, CH_3
29a	4.23, d (11.5)	73.6, CH_2	3.37, s	71.8, CH_2	4.42, d (11.0)	74.4, CH_2
29b	4.16, d (11.5)		3.37, s		4.04, d (11.0)	
30a	5.78, s	120.6, CH_2	5.84, s	121.2, CH_3	2.43, s	20.2, CH_3
30b	5.35, s		5.31, s			
1′		173.3, C		175.7, C		175.7, C
2′	3.07, dd (5.5, 3.5)	74.2, CH	3.34, d (3.5)	75.6, CH	3.57, d (3.5)	75.8, CH
3′	1.40, m	37.2, CH	1.59, m	41.0, CH	1.67, m	40.8, CH
4′a	1.08, m	22.8, CH_2	1.41, m	25.4, CH_2	1.45, m	25.4, CH_2
4′b	0.96, m		1.20, m		1.23, m	
5′	0.71, t (7.5)	11.3, CH_3	0.82, t (7.5)	12.5, CH_3	0.84, t (7.5)	12.4, CH_3
6′	0.72, d (7.0)	15.4, CH_3	0.90, d (7.0)	15.5, CH_3	0.93, d (7.0)	15.6, CH_3
HCOO	8.22, s	160.7, CH				
14-OH	6.64, s					
2′-OH	4.96, d (5.5)					

[a]Data were measured in DMSO-d_6 at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b]Data were measured in CD_3OD at 500 MHz (^{1}H) and 125 MHz (^{13}C).

rate at 10.0 mL/min and column temperature at 25 °C, detected by a binary channel UV detector at 210 and 230 nm. Silica gel (100−200 mesh and 200−300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (40−70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-Gel RP-C_{18} (50 μm; YMC, Milford, MA, USA) were used for column chromatography (CC). Thin-layer chromatography (TLC) was performed on precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China) and detected by spraying with 10% H_2SO_4 in EtOH (*v*/*v*). All solvents used were of analytical grade (Jiangsu Hanbang Science and Technology Co., Ltd., Nanjing, China).

Plant Material. The fruits of *A. polystachya* (Wall.) R. N. Parker, family Meliaceae, were collected from Xishuangbanna, Yunnan Province, China, in March 2010, and authenticated by Prof. Jing-Yun Cui of Xishuangbanna Tropical Garden, Chinese Academy of Sciences. A voucher specimen (No. AP-Fruits-2010-03-ZY) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. Air-dried fruits of the plant material (5.0 kg) were extracted three times with 95% EtOH (10.0 L each, 3 h, 3 h, 2 h) under reflux to give 875.0 g of crude extract, which was suspended in 1.5 L of water and then extracted with petroleum ether (PE, 60−90 °C) to remove the fatty components completely. The defatted crude extracts were further partitioned with $CHCl_3$ to give a $CHCl_3$-soluble fraction (90.0 g). The $CHCl_3$ fraction was subjected to a silica gel column (100−200 mesh, 900.0 g, ⌀ 8.0 cm × 60.0 cm) eluted successively with PE/ethyl acetate (EtOAc) gradient (20:1 to 0:1, *v*/*v*) to obtain six fractions (A−F). Fraction C (15.3 g) was applied to a silica gel column (200−300 mesh, 153.0 g, ⌀ 5.0 cm × 50.0 cm) eluted with $CHCl_3$/MeOH (50:1 to 5:1, *v*/*v*, gradient system) as eluant to give three fractions. Fraction CB (5.3 g) was separated over YMC reversed-phase C_{18} column (50 μm; 100.0 g, ⌀ 3.5 cm × 40.0 cm) (MeOH/H_2O, 65:35 to 100:0, *v*/*v*) to afford three major fractions; CB1 (2.1 g) was purified by a silica gel column (200−300 mesh, 63.0 g, ⌀ 2.5 cm × 50.0 cm) to afford rohituka-14 (**13**, 358.5 mg, yield 0.00717%), rohituka-3 (**9**, 810.1 mg, yield 0.01620%),

dx.doi.org/10.1021/jf3049774 | J. Agric. Food Chem. 2013, 61, 2171−2182

Table 2. ^{1}H NMR Spectroscopic Data of Compounds 4−8

	4[a]	5[b]	6[a]	7[a]	8[a]
position	δ_H, mult (J, Hz)	δ_H, mult (J, Hz)	δ_H, mult (J, Hz)	δ_H, mult (J, Hz)	δ_H, mult (J, Hz)
1	4.22, dd (10.5, 1.5)	4.20, dd (9.5, 3.0)	4.39, br d (11.0)	nd[c]	5.01, d (5.0)
2a	2.70, dd (15.0, 2.0)	2.44, dd (14.5, 9.5)	3.29, overlapped	3.06, d (14.5)	2.46, dd (15.5, 5.0)
2b	2.46, dd (15.0, 11.0)	2.40, dd (14.5, 3.0)	3.15, m	2.70, dd (14.5, 11.5)	2.02, d (15.5)
5	2.86, dd (11.0, 2.0)	2.87, dd (12.5, 8.0)	3.78, overlapped	3.20, br s	2.94, s
6a	2.83, dd (15.0, 10.5)	2.73, dd (18.0, 8.0)	2.95, dd (17.0, 14.0)	2.82 (br s)	3.26 (s)
6b	2.73, dd (15.0, 1.5)	2.49, dd (18.0, 12.5)	2.45, dd (17.0, 8.0)	2.80 (br s)	
9	3.75, d (9.5)	3.47, d (9.0)	3.04, d (10.0)	3.29, d (9.5)	4.01, d (8.0)
11	4.30, dd (9.5, 7.5)	4.05, dd (9.0, 7.0)	5.61, t (10.0)	5.43, dd (10.5, 9.5)	5.51, dd (10.5, 8.0)
12	5.57, d (7.5)	5.87, d (7.0)	6.15, d (10.0)	6.04, d (10.5)	6.15, d (10.5)
15	4.00, s				
16a	2.21, dd (14.0, 7.5)	2.89, dd (19.5, 9.0)	2.79, dd (19.0, 9.0)	2.85, dd (19.5, 9.0)	2.86, dd (19.5, 8.5)
16b	2.00, dd (14.0, 11.0)	2.36, dd (19.5, 10.0)	2.36, dd (19.0, 10.0)	2.43, dd (19.5, 10.0)	2.39, dd (19.5, 9.5)
17	3.03, dd (11.0, 7.5)	3.88, dd (10.0, 9.0)	3.93, t (9.5)	3.94, t (9.5)	3.94, t (9.5)
18	0.82, s	0.85, s	0.97, s	0.97, s	0.99, s
19	1.19, s	1.09, s	1.34 (s)	1.45, overlapped	1.71, s
21	7.23, s	7.18, s	7.38 (s)	7.33, s	7.29, s
22	6.28, d (1.0)	6.20, d (1.0)	6.44, d (1.0)	6.41, d (1.0)	6.39, d (1.0)
23	7.40, t (1.5)	7.35, t (1.5)	7.47, t (1.5)	7.43, t (1.5)	7.44, t (1.5)
28	1.52, s	1.47, s	1.43, s	1.57, s	1.79, s
29a	3.64, d (12.5)	3.78, d (12.5)	3.76, d (13.0)	3.79, d (13.5)	4.08, d (11.5)
29b	3.51, d (12.5)	3.56, d (12.5)	3.66, d (13.0)	3.73, d (13.5)	3.98, d (11.5)
30a	5.50, s	6.05, s	5.85, s	5.80, s	5.95, d (1.5)
30b	5.44, s	5.34, s	5.34, s	5.49, s	5.71, s
1′					
2′	3.72, d (4.0)	3.42, d (3.5)	3.10, d (3.0)	3.20, d (2.0)	1.75, dd (16.0, 7.0)
					1.68, dd (16.0, 7.0)
3′	1.71, m	1.62, m	1.49, m	1.46, m	1.83, dd (13.0, 7.0)
4′a	1.45, m	1.27, m	1.15, m	1.14, m	0.87, d (7.0)
4′b	1.21, m	1.31, m			
5′	0.88, t (7.5)	0.81, t (7.0)	0.83, t (7.5)	0.80, t (7.5)	0.84, d (7.0)
6′	0.94, d (7.0)	0.89, d (6.5)	0.83, d (7.0)	0.84, d (7.0)	
OEt	4.18, q (7.0)	4.15, qd (7.5, 2.0)	4.23, q (7.0)	4.09, qd (7.5, 3.5)	
	1.31, t (7.0)	1.31, t (7.5)	1.34, t (7.0)	1.25, t (7.5)	
OAc			2.11, s	2.03, s	2.06, s
HCOO				8.13, s	7.90, s

[a]Data were measured in CD_3OD at 500 MHz. [b]Data were measured in $CDCl_3$ 500 MHz. [c]nd = not detectable.

rohituka-12 (**12**, 26.4 mg, yield 0.00053%), and dregenana-1 (**15**, 56.5 mg, yield 0.00113%) using CH_2Cl_2/MeOH (25:1 to 10:1, v/v). Fraction CB2 (2.4 g) was separated over a reversed-phase C_{18} column (50 μm; 100.0 g, ⌀ 3.5 cm × 40.0 cm) (CH_3CN/H_2O, 50:50 to 90:10, v/v) to afford four major fractions; CB21 (50.0 mg) was purified by preparative HPLC to afford two minor compounds, **3** (2.5 mg, yield 0.00005%, t_R = 28.4 min) and **4** (9.2 mg, yield 0.00018%, t_R = 33.1 min), using 65% methanol in water. Compounds **1** (150.4 mg, yield 0.00301%), **5** (6.2 mg, yield 0.00012%), and **7** (54.3 mg, yield 0.00109%) were purified following the same procedure from fraction CB22 (1.5 g) by silica gel CC (200−300 mesh, 45.0 g, ⌀ 2.0 cm × 30.0 cm) using PE/EtOAc (2:1, v/v). Fraction D (18.5 g) was separated over a reverse-phased silica gel column (50 μm; 200.0 g, ⌀ 5.0 cm × 50.0 cm) ($MeOH/H_2O$, 50:50 to 100:0, v/v) to afford five major fractions D1−D5. Fraction D1 (1.3 g) was separated over a silica gel column (200−300 mesh, 39.0 g, ⌀ 2.0 cm × 30.0 cm) eluted with CH_2Cl_2/Me_2CO (5:1, v/v) to give two major subfractions and then purified by preparative HPLC eluted with $MeOH/H_2O$ (60:40, v/v) to yield **2** (4.5 mg, yield 0.00009%, t_R = 25.5 min). Fraction D2 (2.1 g) was chromatographed over a silica gel column (200−300 mesh, 42.0 g, ⌀ 2.0 cm × 30.0 cm) ($CHCl_3$/MeOH, 60:1 to 10:1, v/v) to afford four parts (D2A−D2D). Fractions D2A (374.1 mg) (200−300 mesh, 6.0 g, ⌀ 1.0 cm × 30.0 cm) and D2C (1.1 g) (200−300 mesh, 33.0 g, ⌀ 2.0 cm × 30.0 cm) were subjected to CC of silica gel (CH_2Cl_2/Me_2CO, 5:1, v/v) to give **6** (132.2 mg, yield 0.00264%) and aphanamolide A (**16**, 650.2 mg, yield 0.01300%), respectively. Fraction D3 (4.2 g) was chromatographed over a silica gel column (200−300 mesh, 84.0 g, ⌀ 2.5 cm × 60 cm) ($CHCl_3$/MeOH, 20:1 to 5:1, v/v) to afford three parts (D3A−D3C). D3B (320 mg) was finally purified by preparative HPLC (CH_3CN/H_2O, 55:45, v/v) to give **8** (18.4 mg, yield 0.00037%, t_R = 36.3 min). Fraction D4 (5.1 g) was further chromatographed over a silica gel column (200−300 mesh, 102.0 g, ⌀ 2.5 cm × 60 cm) (PE/Me_2CO, 10:1 to 2:1, v/v) to give three parts (D4A−D4C). D4B (352.5 mg) was separated by preparative HPLC (CH_3CN/H_2O, 60:40, v/v) to give rohituka-9 (**11**, 13.4 mg, yield 0.00027%, t_R = 15.3 min), rohituka-15 (**14**, 50.2 mg, yield 0.00100%, t_R = 24.4 min), and rohituka-7 (**10**, 14.1 mg, yield 0.00028%, t_R = 30.8 min). The isolation procedures for compounds **17**−**25** were reported previously.[12,13]

Aphapolynin C (1). White amorphous powder: mp 187−188 °C; $[\alpha]_D^{26}$ −113.9 (c = 0.34, MeOH); CD (CH_3CN) λ ($\Delta\varepsilon$) 190 (−4.79), 223 (+6.07), 257 (−4.79) nm; UV (CH_3CN) λ_{max} (log ε) 193 (4.23), 213 (4.16) nm; IR (KBr) λ_{max} 3470, 2968, 2938, 2879, 1745, 1633, 1504, 1464, 1383, 1279, 1209, 1141, 1071, 1027, 989, 897, 874, 797, 730, 664, 604, 548 cm^{-1}; for ^{1}H NMR and ^{13}C NMR data, see Table 1; ESI-MS (positive mode) m/z 646.3 $[M + NH_4]^+$; ESI-MS (negative mode) m/z 627.4 $[M - H]^-$, 663.4 $[M + Cl]^-$; HR-ESI-MS (negative mode) m/z 663.2219 $[M + Cl]^-$ (calcd for $C_{33}H_{40}ClO_{12}$ 663.2214).

dx.doi.org/10.1021/jf3049774 | J. Agric. Food Chem. 2013, 61, 2171−2182

*Aphapolynin D (**2**).* White amorphous powder: mp 215−216 °C; $[\alpha]_D^{26}$ −94.6 (*c* = 0.18, MeOH); CD (CH_3CN) λ (Δε) 204 (−14.64), 226 (+18.26), 255 (−7.33) nm; UV (CH_3CN) λ_{max} (log ε) 193 (4.21), 213 (4.06) nm; IR (KBr) λ_{max} 3445, 2964, 2878, 1743, 1635, 1504, 1463, 1412, 1386, 1224, 1166, 1135, 1065, 1029, 988, 956, 900, 874, 800, 723, 655, 603, 453 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 1; ESI-MS (positive mode) *m/z* 601.0 $[M + H]^+$, 618.2 $[M + NH_4]^+$; ESI-MS (negative mode) *m/z* 599.0 $[M - H]^-$, 635.1 $[M + Cl]^-$; HR-ESI-MS (negative mode) $[M + Cl]^-$ *m/z* 635.2275 (calcd for $C_{32}H_{40}ClO_{11}$ 635.2265).

*Aphapolynin E (**3**).* White amorphous powder: mp 199−200 °C; $[\alpha]_D^{26}$ −30.7 (*c* = 0.09, MeOH); CD (CH_3CN) λ (Δε) 196 (−10.68), 224 (+5.57), 259 (+7.09) nm; UV (CH_3CN) λ_{max} (log ε) 193 (4.12), 211 (3.91), 255 (3.98) nm; IR (KBr) λ_{max} 3442, 2932, 2873, 1739, 1619, 1456, 1398, 1294, 1212, 1136, 1076, 1024, 873, 802, 762, 602, 527, 419 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 1; ESI-MS (positive mode) *m/z* 601.1 $[M + H]^+$, 618.1 $[M + NH_4]^+$; ESI-MS (negative mode) *m/z* 599.1 $[M - H]^-$, 635.1 $[M + Cl]^-$; HR-ESI-MS (negative mode) *m/z* 599.2493 $[M - H]^-$ (calcd for $C_{32}H_{39}O_{11}$ 599.2498).

*Aphapolynin F (**4**).* White amorphous powder: mp 167−168 °C; $[\alpha]_D^{26}$ −9.1 (*c* = 0.14, MeOH); UV (CH_3CN) λ_{max} (log ε) 196 (4.02), 213 (3.62) nm; IR (KBr) λ_{max} 3456, 2969, 1739, 1641, 1503, 1461, 1385, 1269, 1201, 1137, 1068, 1030, 961, 924, 874, 833, 797, 736, 685, 656, 618, 603, 419 cm^{-1}; for ^1H NMR data, see Table 2, and for ^{13}C NMR data, see Table 3; ESI-MS (positive mode) *m/z* 648.3 $[M + NH_4]^+$; ESI-MS (negative mode) *m/z* 665.5 $[M + Cl]^-$; HR-ESI-MS (negative mode) *m/z* 665.2752 $[M + Cl]^-$ (calcd for $C_{34}H_{46}ClO_{11}$ 665.2734).

*Aphapolynin G (**5**).* White amorphous powder: mp 177−178 °C; $[\alpha]_D^{26}$ −84.3 (*c* = 0.28, MeOH); UV (CH_3CN) λ_{max} (log ε) 194 (4.14), 210 (3.79) nm; IR (KBr) λ_{max} 3445, 2968, 2935, 2879, 1743, 1634, 1504, 1462, 1387, 1270, 1212, 1137, 1061, 1028, 960, 901, 874, 795, 734, 663, 603, 569 cm^{-1}; for ^1H NMR data, see Table 2, and for ^{13}C NMR data, see Table 3; ESI-MS (positive mode) *m/z* 647.2 $[M + H]^+$, 664.4 $[M + NH_4]^+$; ESI-MS (negative mode) *m/z* 645.3 $[M - H]^-$, 681.4 $[M + Cl]^-$; HR-ESI-MS (negative mode) *m/z* 681.2675 $[M + Cl]^-$ (calcd for $C_{34}H_{46}ClO_{12}$ 681.2683).

*Aphapolynin H (**6**).* White amorphous powder: mp 157−158 °C; $[\alpha]_D^{26}$ −58.4 (*c* = 0.11, MeOH); UV (CH_3CN) λ_{max} (log ε) 194 (4.31) nm; IR (KBr) λ_{max} 3480, 2969, 2930, 2881, 2352, 2323, 1749, 1720, 1644, 1503, 1466, 1399, 1301, 1243, 1204, 1167, 1140, 1089, 1067, 1022, 954, 873, 805, 744, 664, 618, 517, 419 cm^{-1}; for ^1H NMR data, see Table 2, and for ^{13}C NMR data, see Table 3; ESI-MS (positive mode) *m/z* 724.4 $[M + NH_4]^+$; ESI-MS (negative mode) *m/z* 705.3 $[M - H]^-$, 741.4 $[M + Cl]^-$; HR-ESI-MS (negative mode) *m/z* 741.2899 $[M + Cl]^-$ (calcd for $C_{36}H_{50}ClO_{14}$ 741.2895).

*Aphapolynin I (**7**).* White amorphous powder: mp 207−209 °C; $[\alpha]_D^{26}$ −46.0 (*c* = 0.23, MeOH); UV (CH_3CN) λ_{max} (log ε) 194 (4.08), 216 (3.80) nm; IR (KBr) λ_{max} 3513, 2970, 2880, 1746, 1730, 1632, 1504, 1462, 1369, 1271, 1239, 1202, 1135, 1113, 1069, 1026, 961, 896, 874, 843, 795, 844, 795, 739, 666, 628, 604, 586, 478 cm^{-1}; for ^1H NMR data, see Table 2, and for ^{13}C NMR data, see Table 3; ESI-MS (positive mode) *m/z* 752.4 $[M + NH_4]^+$; ESI-MS (negative mode) *m/z* 769.5 $[M + Cl]^-$; HR-ESI-MS (negative mode) *m/z* 769.2848 $[M + Cl]^-$ (calcd for $C_{37}H_{50}ClO_{15}$ 769.2844).

*Aphanamolide B (**8**).* A white amorphous solid: mp 202−203 °C; $[\alpha]_D^{26}$ −57.2 (*c* = 0.20, MeOH); CD (CH_3CN) λ (Δε) 198 (+1.36), 207 (−1.35), 222 (+6.21), 306 (−3.20) nm; UV (CH_3CN) λ_{max} (log ε) 194 (4.08), 214 (3.81) nm; IR (KBr) λ_{max} 3437, 2962, 1730, 1633, 1467, 1369, 1255, 1160, 1120, 1062, 1028, 983, 961, 926, 894, 874, 799, 732, 666, 604, 411 cm^{-1}; for ^1H NMR data, see Table 2, and for ^{13}C NMR data, see Table 3; ESI-MS (positive mode) *m/z* 659.1 $[M + H]^+$, 676.2 $[M + NH_4]^+$; ESI-MS (negative mode) *m/z* 657.0 $[M - H]^-$, 693.2 $[M + Cl]^-$; HR-ESI-MS (negative mode) *m/z* 693.2314 $[M + Cl]^-$ (calcd for $C_{34}H_{42}ClO_{13}$ 693.2319).

*X-ray Data of Aphanamolide A (**16**).* A colorless crystal of aphanamolide A crystallized from methanol, mp 229−230 °C, orthorhombic space group $P2_12_12$. Crystal data: formula $C_{35}H_{44}O_{14}$, *M* = 688.70, crystal size = 0.42 × 0.37 × 0.34 mm^3, *a* = 27.9258 (2) Å, *b* = 12.2466 (2) Å, *c* = 10.53920 (10) Å, α = β = γ = 90°, *V* = 3604.36 (7) $Å^3$, *T* = 291 K, *Z* = 4, *d* = 1.269 $g·cm^{-3}$, μ(Cu Kα) = 1.54184 Å, *F* (000) = 1464.0. Intensity data were collected at room temperature on an Oxford Diffraction Gemini-S Ultra CCD diffractometer with Cu Kα radiation. Cell refinement and data reduction were performed with CrysAlisPro. The structures were solved by direct methods using SHELXS-97.[14] Refinements were performed with SHELXL-97[15] using full-matrix least-squares. All non-hydrogen atoms were refined anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. Crystallographic data for aphanamolide A (**16**) have been deposited at the Cambridge Crystallographic Data Centre (deposition No. CCDC-844939). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K. [fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk].

Table 3. ^{13}C NMR Spectroscopic Data of Compounds 4−8

position	4[a]	5[b]	6[a]	7[a]	8[a]
1	85.6, CH	83.3, CH	73.6, CH	73.7, CH	75.9, CH
2	36.5, CH_2	32.8, CH_2	38.9, CH_2	37.6, CH_2	41.7, CH_2
3	173.0, C	170.8, C	174.8, C	172.9, C	104.1, C
4	91.7, C	89.8, C	93.1, C	93.2, C	82.5, C
5	44.4, CH	34.7, CH	41.9, CH	43.9, CH	51.3, CH
6	34.1, CH_2	32.8, CH_2	33.5, CH_2	35.6, CH_2	53.5, CH
7	178.6, C	175.5, C	177.3 C	177.9, C	172.0, C
8	139.7, C	140.0, C	139.6, C	141.9, C	141.0, C
9	52.4, CH	49.5, CH	53.5, CH	50.3, CH	50.3, CH
10	50.3, C	48.2, C	51.4, C	55.6, C	45.9, C
11	81.2, CH	80.0, CH	73.7, CH	71.9, CH	73.5, CH
12	81.7, CH	78.2, CH	79.3, CH	75.8, CH	74.6, CH
13	46.1, C	48.1, C	50.9, C	51.3, C	50.7, C
14	73.8, C	80.2, C	82.0, C	81.8 C	81.9, C
15	61.1, CH	209.6, C	210.0, C	209.3, C	208.4, C
16	34.8, CH_2	42.0 CH_2	42.8, CH_2	42.9, CH_2	42.5, CH_2
17	39.9, CH	34.9, CH	37.2, CH	36.9, CH	36.8, CH
18	14.6, CH_3	13.5, CH_3	14.0, CH_3	13.6, CH_3	13.6, CH_3
19	19.4, CH_3	19.8, CH_3	21.7, CH_3	nd[c]	23.4, CH_3
20	124.5, C	122.6, C	125.1, C	124.7, C	124.8, C
21	141.8, CH	140.5, CH	142.3, CH	142.4, CH	142.1, CH
22	112.6, CH	110.4, CH	112.1, CH	112.1, CH	112.2, CH
23	143.9, CH	142.9, CH	144.3, CH	144.2, CH	144.1, CH
28	20.6, CH_3	20.0, CH_3	21.7, CH_3	20.2, CH_3	22.3, CH_3
29	69.0, CH_2	67.7, CH_2	67.3, CH_2	67.5, CH_2	80.4, CH_2
30	122.0, CH_2	120.3, CH_2	124.0, CH_2	123.1, CH_2	124.9, CH_2
1′	175.5, C	174.8, C	175.6, C	175.1, C	174.1 C
2′	76.0, CH	74.2, CH	76.5, CH	76.3, CH	44.1, CH_2
3′	40.9, CH	39.2, CH	39.3, CH	39.5, CH	26.1, CH
4′	25.4, CH_2	23.9, CH_2	24.8, CH_2	24.4, CH_2	22.9, CH_3
5′	12.4, CH_3	11.9, CH_3	12.1, CH_3	12.1, CH_3	23.0, CH_3
6′	15.6, CH_3	14.9, CH_3	16.1, CH_3	16.0, CH_3	
OEt	62.3, CH_2	61.2, CH_2	61.8, CH_2	62.2, CH_2	
	14.8, CH_3	14.1, CH_3	14.8, CH_3	14.6, CH_3	
OAc			172.5, C	172.2, C	172.0, C
			22.0, CH_3	21.3, CH_3	21.8, CH_3
HCOO				163.4, CH	162.2, CH

[a]Data were measured in CD_3OD at 125 MHz. [b]Data were measured in $CDCl_3$125 MHz. [c]nd = not detectable.

Biological Assays. The cytotoxicities and anti-inflammatory activities of **1**−**25** were evaluated at China Pharmaceutical University, while the fungicidal, herbicidal, and insecticidal activities were screened at Syngenta.

Cytotoxicity Assay. Three human cancer cell lines (Chinese Academy of Science Cell Bank, Shanghai, China) were used for *in*

Table 4. Fungicidal, Herbicidal, and Insecticidal Activities of Compounds 1−25[a]

	fungicide[b]							herbicide[b]		insecticide[b]			
compd	Pi 200/60	St[c] 100	Uv 100	Pd 20/2	As 20/2	Bf 20/2	Gz 20/2	At 10	Pa 32	Sa[c] 1000	Px[c] 500	Db[c] 500	Ce 50
1	0/0	0	99	55/0	0/0	0/0	0/0	0	0	33	33	99	66
2	0/0	0	33	27/0	55/0	0/0	0/0	0	0	33	33	66	0
3	0/0	0	55	0/0	0/0	0/0	0/0	0	0	0	0	0	0
4	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	33	0
5	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
6	0/0	0	77	0/0	0/0	0/0	0/0	0	0	0	0	0	0
7	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
8	0/0	18	0	27/0	0/0	0/0	0/0	0	0	0	0	0	0
9	0/0	0	77	55/0	0/0	0/0	0/0	0	0	0	0	0	0
10	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
11	0/0	33	0	55/0	0/0	0/0	0/0	0	0	0	0	0	0
12	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
13	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
14	0/0	0	55	99/0	0/0	0/0	0/0	0	0	0	0	0	0
15	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	33	33	0
16	0/0	51	27	99/0	0/0	0/0	0/0	0	0	0	0	0	0
17	0/0	18	99	55/0	0/0	0/0	0/0	0	0	0	66	33	0
18	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
19	0/0	33	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
20	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
21	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	0	0
22	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	33	0
23	0/0	0	0	0/0	0/0	0/0	0/0	0	0	0	0	33	0
24	0/0	0	0	27/0	0/0	0/0	0/0	0	0	0	0	66	0
25	0/0	0	0	55/0	55/0	0/0	0/0	0	0	0	33	99	0

[a]Data are presented as means of the assessment scores across two replicates unless otherwise stated, the rate in ppm. [b]Pi, *Phytophthora infestans* (on tomato leaf pieces); St, *Septoria tritici* (on wheat leaf pieces); Uv, *Uromyces viciae-fabae* (on bean leaf pieces); Pd, *Pythium dissimile* (in artificial media); As, *Alternaria solani*; Bf, *Botryotinia fuckeliana* (in artificial media); Gz, *Gibberella zeae* (in artificial media); At, *Arabidopsis thaliana*; Pa, *Poa annua*; Sa, *Sitobion avenae* (leaf disk); Px, *Plutella xylostella* (artificial diet); Db, *Diabrotica balteata* (artificial diet); Ce, *Caenorhabditis elegans* (liquid culture). [c]The data are the mean of three replicates.

vitro cytotoxicity evaluation: MCF-7 (human breast cancer), Bel-7402 (human hepatocellular carcinoma), and BCG-823 (human gastric carcinoma), which were tested using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, USA) method[16] in 96-well microplates according to previously described protocol.[17]

Anti-inflammatory Activity Assay. The anti-inflammatory activities of compounds **1**−**25** were evaluated by testing their inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in mouse macrophage cell line, RAW264.7, bought from the Chinese Academy of Science Cell Bank (Shanghai, China). The level of NO was determined using the NO kit according to the previously described protocol.[18]

Fungicide Assays. Compounds **1**−**25** were evaluated against several pathogens on leaf-piece assays,[19] at the rate of 200 and 60 ppm for *Phytophthora infestans* on tomato, and 100 ppm for *Septoria tritici* on wheat and *Uromyces viciae-fabae* on bean. The compounds were also evaluated in mycelial growth tests in artificial media against *Pythium dissimile*, *Alternaria solani*, *Botryotinia fuckeliana*, and *Gibberella zeae*, at rates of 20 and 2 ppm. Each assay was performed with two replicates at each rate, except the *S. tritici* assay, which was performed with three replicates. Chemicals were applied to leaf pieces prior to inoculation with spores of the pathogen, or in the case of the artificial media assays. The plates were first stored in controlled environment cabinets spanning from 4 to 14 days, depending on the assay; mycelial growth or disease inhibition was then assessed. Each well was scored using a three-banded system, with complete inhibition of mycelial growth or disease symptoms as 99, partial inhibition as 55, and no inhibition as 0. Azoxystrobin and prochloraz were included in the test as positive control. The biological data presented in Table 4 are the mean scores for each treatment across replicates.

Herbicide Assays. Compounds **1**−**25** were tested for herbicidal activity[20] against *Arabidopsis thaliana* at 10 ppm and *Poa annua* at 32 ppm, with two replicates for each treatment. Test plates were incubated for 7 days in a controlled environment cabinet before assessment. The plates were then assessed, scored as either 0 (for no observable effect) or 99 (where an herbicidal effect was observed). Norflurazon was tested as a positive control. The data presented in Table 4 are the mean scores of the two replicates.

Insecticide Assays.[21] The compounds were tested for activity against *Sitobion avenae* (1000 ppm), *Plutella xylostella* (500 ppm), and *Diabrotica balteata* (500 ppm) in a leaf-disk assay. The compounds were also evaluated against the nematode species *Caenorhabditis elegans* in liquid culture at 50 ppm. Chemicals were applied to feeding *S. avenae*, prior to infestation with *P. xylostella* larvae, or diluted into the *C. elegans* culture. Thiamethoxam and indoxacarb were included as positive control. The assay plates were stored in controlled environment cabinets for 5 to 9 days (depending on the species). Mortality was then assessed relative to untreated control wells, with wells showing significant levels of mortality scored as 99, and wells without significant mortality scored as 0. The data presented in Table 4 are the mean scores of the two or three replicates.

RESULTS AND DISCUSSION

Structure Elucidation of New Compounds. Aphapolynin C (**1**), a white, amorphous powder, had the molecular formula of $C_{33}H_{40}O_{12}$ by HR-ESI-MS. Its IR spectrum displayed OH (3470 cm^{-1}) and carbonyl (1745 cm^{-1}) absorptions. Its ^1H NMR spectrum (Table 1) showed two singlet proton signals of an exocyclic olefinic methylene group at δ_H 5.78 and 5.35, three singlet methyl signals at δ_H 1.53, 1.02,

dx.doi.org/10.1021/jf3049774 | J. Agric. Food Chem. 2013, 61, 2171−2182

and 0.86, a formyl proton signal at δ_H 8.22 (1H, s) correlated with the carbon signal at δ_C 160.7 (CH) in the HSQC spectrum, two *cis*-olefinic proton signals at δ_H 7.43 (1H, d, J = 12.0 Hz) and 5.96 (1H, d, J = 12.0 Hz), and a 2-hydroxy-3-methylpentanoyl moiety established with the help of ^{1}H−^{1}H COSY [δ_H 3.07 (1H, dd, J = 5.5, 3.5 Hz), 1.40 (1H, m), 1.08 (1H, m), 0.96 (1H, m), 0.71 (3H, t, J = 7.5 Hz), and 0.72 (3H, d, J = 7.0 Hz)]. In addition to two acyl groups, its ^{13}C NMR spectrum (Table 1) exhibited 26 signals, including a $\Delta^{8(30)}$ exocyclic double bond [δ_C 138.8 (C) and 120.6 (CH_2)] and one β-substituted furanyl ring [δ_C 142.8 (CH), 140.5 (CH), 123.0 (C), and 111.1 (CH)], which suggested that compound **1** is a typical prieurianin-type (ring A lactone, ring B-cleaved) limonoid.[4]

Comparison of its ^{1}H and ^{13}C NMR spectroscopic data (Table 1) with those of rohituka-7[9] implied that their structures

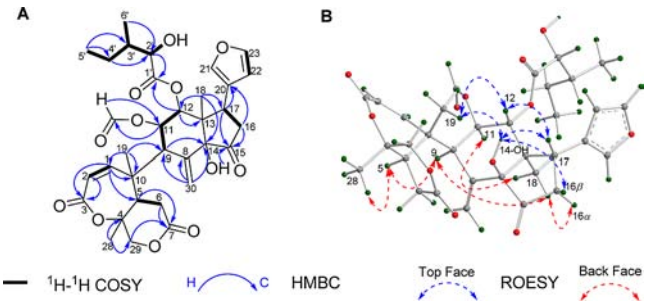

Figure 2. Key ^{1}H−^{1}H COSY, HMBC, and ROESY correlations of **1**.

were closely related, with the marked difference being a ketone carbonyl group at C-15, which was confirmed by the HMBC cross peaks from δ_H 6.64 (14-OH), 2.76, and 2.47 (H_2-16) to δ_C 207.5 (C-15) (Figure 2A). The formyl group was located at C-11 by the HMBC correlations between the formyl proton signal at δ_H 8.22 and δ_C 71.6 (C-11), and between δ_H 5.42 (H-11) and the carbonyl of the formyl group at δ_C 160.7. The long-chain 2-hydroxy-3-methylpentanoyl moiety was assigned at C-12 by the HMBC cross peak from δ_H 6.04 (H-12) to δ_C 173.3 (C-1′). The key HMBC correlations from H-1 to C-3, C-5, and C-10; from H-2 to C-3 and C-10; and from H_3-19 to C-1 indicated an α,β-unsaturated lactone in ring A; the HMBC correlations of two mutually coupled doublet proton signals at δ_H 4.23 and 4.16 (H_2-29, coupling constants at 11.5 Hz) to a carbonyl carbon at δ_C 172.7 (C-7), which in turn correlated with H-5 and H_2-6, respectively, in the HMBC spectrum, inferred the existence of a six-membered lactone. Moreover, the protons of the exocyclic olefinic protons exhibited HMBC cross peaks with C-8, C-9, and C-14, allowing the assignment of itself at C-8. The furan ring was connected between C-17 and C-20, which was determined by the key HMBC correlations from H-17 to C-20, and also from H_2-16 to C-20. The planar structure of compound **1** was thus established. Interestingly, its ^{1}H NMR signals broadened and some ^{13}C NMR resonances attenuated or disappeared when recorded in $CDCl_3$, but were well exhibited in CD_3OD or DMSO-d_6, which might due to the hindered rotation around the C-9 and C-10 bond in $CDCl_3$.

The relative configuration of compound **1** was mainly determined by a ROESY experiment (Figure 2B), in which the correlations of H_3-18/H-16α, H_3-18/H-9, H_3-18/H-11, H-9/H-5, and H_3-28/H-5 indicated that they were cofacial, and were arbitrarily assigned as an α-orientation. The ROESY cross peaks of H-17/H-16β, H-17/H-12, 14-OH/H-12, and H-12/H_3-19 indicated that H-12, 14-OH, H-17, and H_3-19 were β-configured. The above-mentioned stereochemistry of compound **1** was in accordance with that of rohituka-7 that was established by X-ray diffraction analysis,[6] thus the structure of **1** was elucidated as shown.

Compound **2** was obtained as a white, amorphous powder, with a molecular formula of $C_{32}H_{40}O_{11}$ determined by HR-ESI-MS. The NMR data of **2** were nearly superimposable on those of **1**, except for the absence of the characteristic signals for a formyl group, and instead the presence of an additional hydroxyl group, resulting in the upfield shifted H-11 signal ($\Delta\delta$ −0.76) and the severely downfield shifted C-11 signal ($\Delta\delta$ +9.7), suggested the loss of a formyl group at C-11.

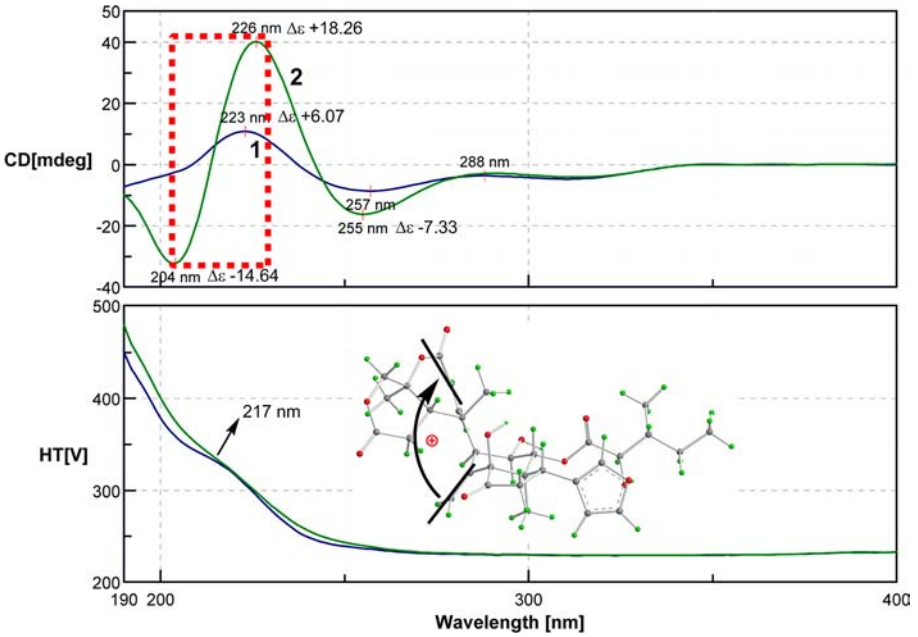

Figure 3. CD spectrum and the exciton chirality of **1** and **2**; the bold lines denote the electric transition dipole of the chromophores for **2**.

dx.doi.org/10.1021/jf3049774 | J. Agric. Food Chem. 2013, 61, 2171−2182

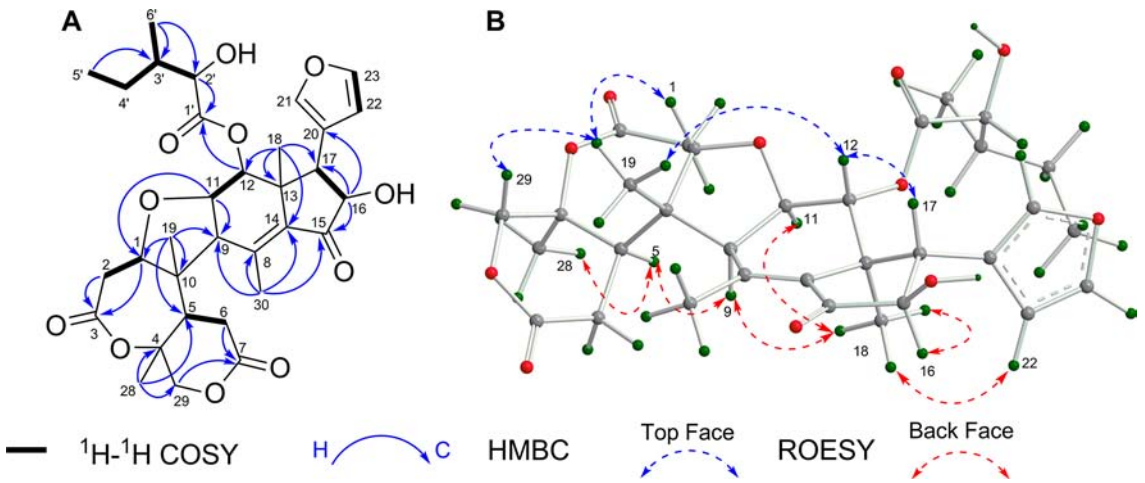

Figure 4. Key ^{1}H−^{1}H COSY, HMBC, and ROESY correlations of **3**.

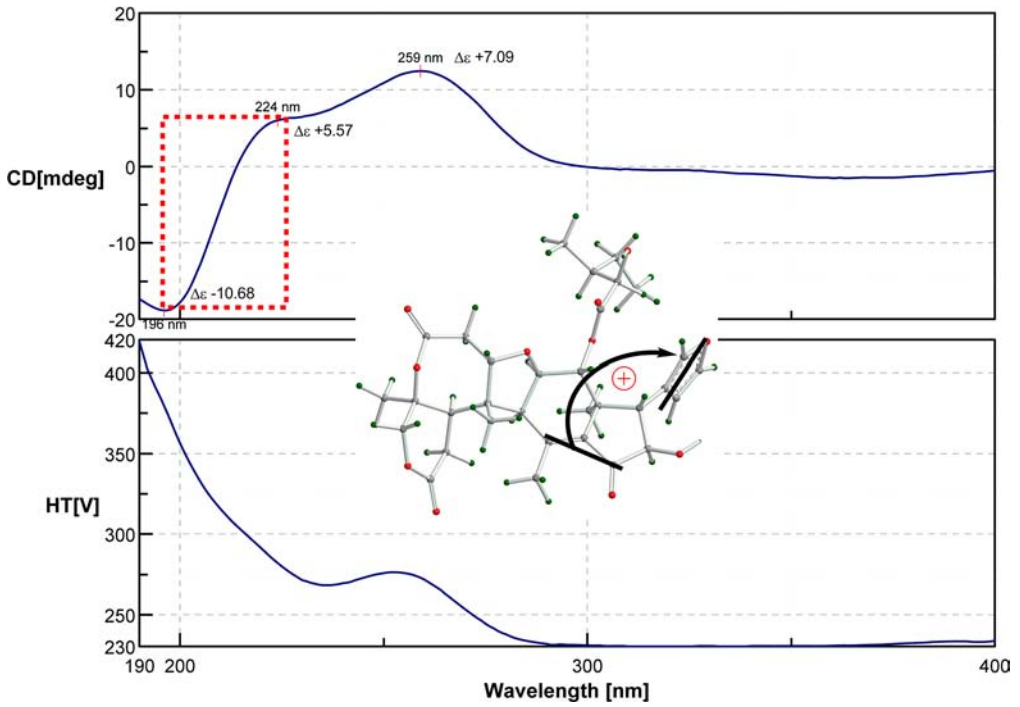

Figure 5. CD spectrum and the exciton chirality of **3**; the bold lines denote the electric transition dipole of the chromophores for **3**.

The absolute configurations of aphapolynins C (**1**) and D (**2**) were established by the CD exciton chirality method, and they shared the same CD Cotton effect. The CD spectra of **2** exhibited positive chirality resulting from the exciton coupling between two different chromophores of the α,β-unsaturated lactone at 226 nm ($\Delta\varepsilon$ +18.26, $\pi-\pi^*$ transition)[22] and the $\Delta^{8(30)}$ double bond at 204 nm ($\Delta\varepsilon$ −14.64, $\pi-\pi^*$ transition),[23−25] respectively, with a maximum at 217 nm in its UV spectrum. The clockwise manner of two chromophores in space thus defined the absolute configurations of **1** and **2** (4*S*,5*R*,9*R*,10*R*,11*R*,12*R*,13*R*,14*S*,17*S*,2′*R*,3′*R*) as depicted (Figure 3).

Aphapolynin E (**3**) was isolated as a white, amorphous powder. Its molecular formula was determined to be $C_{32}H_{40}O_{11}$ by HR-ESI-MS in negative mode, indicating 13 degrees of unsaturation. The IR absorptions indicated the presence of hydroxyl (3442 cm^{-1}) and carbonyl (1739 cm^{-1}) groups. Its ^{1}H NMR spectrum exhibited signals for four tertiary methyls (δ_H 2.43, 1.70, 1.21, and 1.18, each 3H, s), one secondary methyl (δ_H 0.93, 3H, d, J = 7.0 Hz), one primary methyl (δ_H 0.84, 3H, t, J = 7.5 Hz), and one β-substituted furanyl ring (δ_H 7.49, 7.40, and 6.44, each 1H, s). The ^{13}C NMR spectrum displayed 32 carbon resonances, which were categorized by DEPT and HSQC experiments into six methyls, four sp^3 methylenes, nine sp^3 methines (six oxygenated), three sp^3 quaternary carbons, four carbonyl resonances, a double bond, and four monosubstituted furan carbons. Deducting seven degrees of unsaturation accounted by four carbonyls, and three double bonds, the remaining six degrees of unsaturation suggested the presence of hexacyclic limonoid in **3**.

Comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **3** (Table 1) with those of prieurianin-type limonoids[9] implied that their structures were closely related, with the significant differences being at rings C and D. The common $\Delta^{8(30)}$

dx.doi.org/10.1021/jf3049774 | J. Agric. Food Chem. 2013, 61, 2171−2182

exocyclic double bond[26] in prieurianin-type limonoids was absent in compound **3**, and instead, a downfield methyl (δ_H 2.43) and a typical α,β-unsaturated ketone unit (δ_C 204.7) were present, whose positions were determined by 2D NMR (HSQC and HMBC). The HMBC correlations between H_3-30 and C-8 assigned the severely deshielded methyl to the conjugated double bond. The ketone group was located at C-15 by the HMBC cross peak between H-16 and C-15. The HMBC correlations of H-16 with C-17, and H-17 with C-16, indicated that the C-16 was oxygenated. The complete structure of **3** was confirmed by the analysis of its HMBC spectrum (Figure 4A), in which the key HMBC correlations from H-1 and H_2-2 to C-3, from H_3-19 to C-5 and C-10, and from H_3-28 to C-4 and C-5 indicated the presence of a seven-membered lactone in ring A (lactonized at C-3); from H_2-29 and H_2-6 to C-7 implied a six-membered lactone between C-7 and C-29, characteristic of a ring A,B-*seco* limonoid. The HMBC correlations from H-11 to C-1 and H-1 to C-11 established a tetrahydrofuran ring between C-1 and C-11. The existence of the 2-hydroxy-3-methylpentanoyl was revealed by the $^1H-^1H$ COSY spectrum, and its location at C-12 was determined by the key correlation between H-12 and C-1′ in the HMBC spectrum. Thus, the gross structure of **3** was established.

The relative configuration of **3** was mainly deduced from the analysis of its ROESY correlations. As shown in Figure 4B, the observed correlations of H-12 with H_3-19 and H-17, together with the cross peak of H_3-19 with H-1, indicated that H-1, H-12, H-17, and H_3-19 were cofacial and arbitrarily assigned as in β-orientation, and accordingly, the α-orientation of the long-chain ester at C-12 and the furan ring. In consequence, the ROESY correlations of H-9 with H-5 and H_3-18; H-16 with H_3-18 and H-22 (furan); and H-11 with H_3-18 showed that they were in the same plane and determined to be α-directed. The large coupling constant ($J_{11,12}$ = 10.5 Hz) between H-11 and H-12 suggested a 1,3-diaxial relationship, consistent with the assignments of H-11α and H-12β.[22] Likewise, the large coupling constant between H-16 and H-17 ($J_{16,17}$ = 12.0 Hz) supported the assignments of H-16α and H-17β. The relative configurations of 2′-OH and H_3-6′ in ester chain at C-12 were assigned as in β-orientation by comparison of their NMR data with those of the same moiety in aphapolynin A that was reported previously, the absolute configuration of which was established by single-crystal X-ray diffraction.[12]

The CD spectrum of **3** exhibited positive chirality resulting from the exciton coupling between the two different chromophores of the furan ring at 196 nm ($\Delta\varepsilon$ −10.68, $\pi-\pi^*$ transition)[27] and the $\Delta^{8(14)}$ enone at 224 nm ($\Delta\varepsilon$ +5.57, $\pi-\pi^*$ transition),[23] indicating that the transition dipole moments of these two chromophores were oriented in a clockwise manner (Figure 5). Moreover, for further confirmation of the absolute configuration of compound **3**, its ECD spectroscopy was calculated using time-dependent density functional theory (TDDFT),[28] which was in good agreement with the experimental one (Figure 6). Therefore, the absolute configuration of **3** was assigned as 1*S*,4*S*,5*R*,9*S*,10*R*,11*R*,12*R*,13*S*,15*R*,17*S*,2′*R*,3′*R*.

Compound **4**, a white powder, had a molecular formula of $C_{34}H_{46}O_{11}$ determined by the HR-ESI-MS, indicative of 12 degrees of unsaturation. The IR spectrum exhibited absorption bands at 3456 and 1739 cm^{-1} evidencing the presence of hydroxyl and ester functionalities, respectively. The presence of three tertiary methyls at δ_H 1.52 (s), 1.19 (s), and 0.82 (s); one secondary methyl at δ_H 0.94 (d, J = 7.0 Hz); one primary

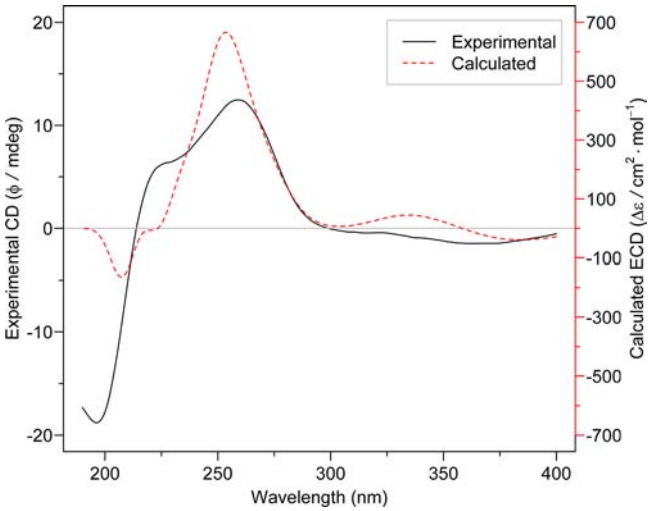

Figure 6. Assignment of the absolute configuration of **3** (1*S*,4*S*,5*R*,9*S*,10*R*,11*R*,12*R*,13*S*,15*R*,17*S*,2′*R*,3′*R*) by comparison of the experimental CD spectra with the spectra calculated using TDDFT methods.

methyl at δ_H 0.88 (t, J = 7.5 Hz); one ethoxy group at δ_H 4.18 (q, J = 7.0 Hz) and 1.31 (t, J = 7.0 Hz); a $\Delta^{8(30)}$ exocyclic double bond at δ_H 5.50 (s) and 5.44 (s); and a β-substituted furanyl ring at δ_H 7.40 (t-like, J = 1.5 Hz), 7.23 (s), and 6.28 (d, J = 1.0 Hz) was readily revealed by the ^1H NMR spectrum (Table 2). Aided with the HSQC experiments, the ^{13}C NMR spectrum showed 34 carbon resonances, categorized into six sp^3 methyls, nine sp^3 methines, three sp^2 methines, six sp^3 methylenes, one sp^2 methylene, and nine quaternary carbons. The aforementioned evidence indicated that it was a prieurianin-type limonoid.[5] Comparison of the ^1H and ^{13}C NMR data of **4** (Tables 2 and 3) with those of **3** indicated that they were structurally related, possibly by cleavage of the lactones in **3** and formation of a new five membered lactone in **4** (instead of a six membered lactone), as shown by the observed significantly upfield shifted C-29 methylene protons and, as a consequence, the absence of once strong HMBC correlations from H_2-29 to C-7 in **3**. The ethoxy group was placed at C-3 by the HMBC correlation from the proton signal at δ_H 4.17 to the ester carbonyl carbon at δ_C 173.0, which also correlated with H-1 and H_2-2. The epoxy ring between C-14 and C-15 was suggested by the chemical shifts of C-14 at δ_C 73.8 (C) and C-15 at δ_C 61.1 (CH), and was established with the aid of the HMBC correlations from H_3-18 and H_2-30 to C-14, and from a singlet proton signal at δ_H 4.00 (H-15) to C-16 and C-17. Other fragments of compound **4** were linked by using a combination of 2D NMR spectra.

The relative configuration of the skeleton core was established mostly by the ROESY experiment, in which the cross peaks of H-11/H_3-18, H_3-18/H-22, H-5/H-9, and H-16α/H-30a (δ_H 5.50) indicated that they were cofacial and toward α-face. In consequence, the 14,15-epoxide ring was in β-configuration, which also supported by comparison of its chemical shifts and the proton coupling pattern with those containing such moiety.[5] Likewise, the ROESY correlations of H-1/H_3-19, H-1/H_3-28, H-12/H-17, and H-12/H_3-19 suggested that they were β-oriented. Thus, the structure of compound **4** was established.

The molecular formula of **5** was determined to be $C_{34}H_{46}O_{12}$ by the HR-ESI-MS, indicating the presence of 12 degrees of

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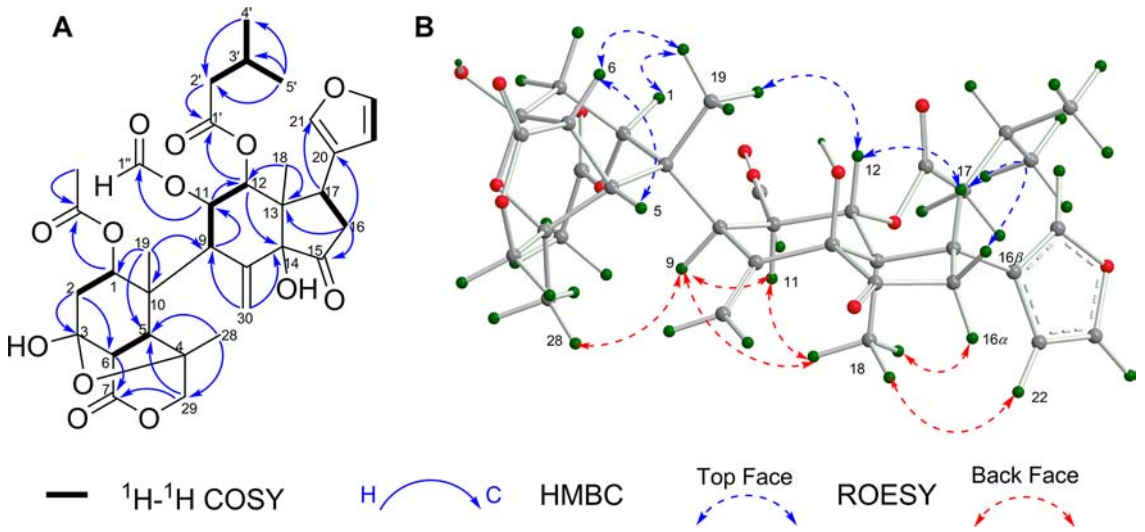

Figure 7. Key HMBC and ROESY correlations of **8**.

unsaturation. Careful comparison of its NMR data with those of **4** indicated that they shared the same rings except for the absence of the 14,15-epoxide in ring D present in the NMR spectra of **4**. The 14-hydroxyl-15-oxo fragment was established by the HMBC correlations from H-9 and H_2-30 to C-14, and from both H_2-16 and H-17 to a carbonyl carbon signal at C-15. The structure of **5** was established as shown by 2D NMR experiments, including HSQC, HMBC, and ROESY spectra.

Compound **6** was obtained as a white amorphous powder, determined to have molecular formula of $C_{36}H_{50}O_{14}$ by HR-ESI-MS. Its NMR data resembled those of **5** except for the downfield shifted H-1 ($\Delta\delta$ +0.19) and H-11 ($\Delta\delta$ +1.56) signals, and upfield shifted C-1 ($\Delta\delta$ −9.7) and C-11 ($\Delta\delta$ −6.3) signals, and the presence of the characteristic signals for an acetoxyl group [δ_H 2.11 (s); δ_C 22.0 (CH_3) and 172.5 (C═O)], suggesting a cleavage of the tetrahydrofuran ring between C-1 and C-11. The acetoxyl group was attached to the C-1 by the key HMBC from H-1 to the carbonyl carbon of the acetoxyl group.

The molecular formula of **7** was determined to be $C_{37}H_{50}O_{15}$ by the HR-ESI-MS, 28 mass units more than that of **6**. Its ^{1}H and ^{13}C NMR spectra exhibited characteristic signals for a formyl group [δ_H 8.13 (s) and δ_C 163.4 (CH)], which was assigned to be at C-11 by the HMBC cross peak from δ_H 5.43 (H-11) to the formyl carbonyl carbon signal at δ_C 163.4 (C-1′) and from δ_H 8.13 to δ_C 71.9 (C-11).

The four *O*-ethyl-bearing compounds (**4**−**7**) seemed to be artifacts, formed in the extraction process using EtOH as the extract solvent. However, they were confirmed to exist in the title plant by HPLC analysis of its acetone extract.

Compound **8** was obtained as a white amorphous solid. The HR-ESI-MS exhibited a molecular formula of $C_{34}H_{42}O_{13}$, indicating 14 degrees of unsaturation. The IR spectrum exhibited absorption bands for hydroxyl (3437 cm^{-1}), carbonyl (1730 cm^{-1}), and double bond (1633 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra displayed 34 carbon resonances comprising six methyls, five methylenes (an olefinic carbon), eleven methines (three olefinic carbons), and twelve quaternary carbons (including five carbonyls and two olefinic ones). The three carbon−carbon double bonds and five carbonyl groups accounted for 8 degrees of unsaturation, and the remaining 6 degrees of unsaturation suggested that the structure contained six rings. Comparison of the ^{1}H and ^{13}C NMR data of **8** with those of C_3−C_6 connected compound aphanamolide A[29] suggested a great structural resemblance between them. Further analysis of the spectroscopic data of these two compounds revealed that the only difference was the presence of an additional 3-methylbutanoyl group in **8** instead of the 2-hydroxy-3-methylpentanoyl moiety at C-12 in aphanamolide A. The 3-methylbutanoyl group was established mainly by the ^{1}H−^{1}H COSY and HMBC spectra. The ^{1}H−^{1}H COSY spectrum combined with the HSQC spectrum allowed the assignment of the partial structure of the modified ester chain (C-2′, C-4′, and C-5′ to C-3′), whose linkage was determined to be at C-12 by the observed HMBC cross peak of δ_H 6.15 (1H, d, J = 10.5 Hz, H-12) to the ester carbonyl carbon at C-1′ (Figure 7A).

The relative configuration of **8** was deduced from the analysis of its ROESY correlations in combination with molecular modeling studies. As shown in Figure 7B, the observed correlations of H-9 with H-11, H_3-18, and H_3-28, together with the correlations of H_3-18 with H-16α and H-22, indicated that H-9, H-11, H_3-18, H_3-28 and the furan ring were cofacial and thus were arbitrarily assigned to be in α-orientation. In consequence, the configurations of H-1, H-6, H-12, H-17, H_3-19, and H_2-29 were β-orientated by the key ROESY correlations from H_3-19 to H-1, H-6, and H-12, from H-12 to H-17, and H_3-19 and H_2-29. The β-orientation of H-5 was deduced from the ROESY correlation between H-6 and H-5, in accordance with the proposed biosynthesis:[12] the common six-membered lactone in prieurianin-type limonoids flipped nearly 180° around the bond between C-5 and C-10 to form a new six-membered lactone in compound **8**. However, the orientations of 3-OH and 14-OH could not be determined directly by the ROESY spectrum as no valuable correlations were observed arising from them. To confirm our conjectures and remove uncertainties in structure, a single crystal of the major compound **16** was obtained and subjected to an X-ray diffraction experiment with Cu Kα (λ = 1.54184 Å) radiation, allowing the determination of the absolute configuration of **16** (Figure 8). The absolute configuration of **8** was established by comparison of its CD spectra with that of **16**. Both of them exhibited a remarkable positive Cotton effect at λ_{max} = 222 nm and negative Cotton effect at λ_{max} = 306 nm, indicating that

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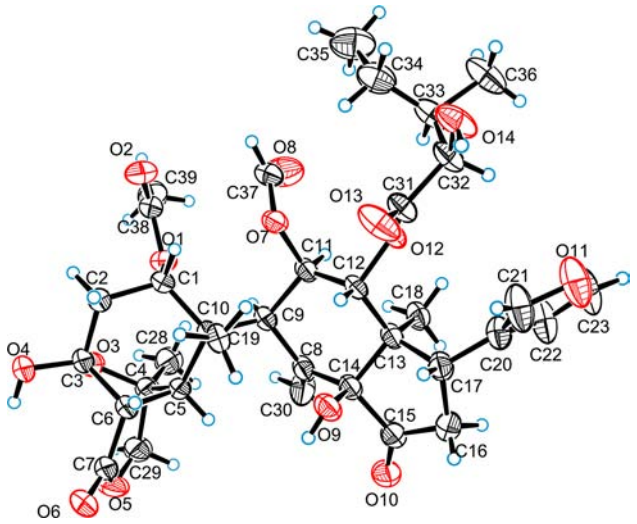

Figure 8. X-ray crystal structure of **16** (ORTEP drawing); displacement ellipsoids are shown at the 30% probability level.

their absolute configurations were identical. Therefore, the structure of **8** was established with configurations of 1*S*,3*R*,4*S*,5*R*,6*S*,9*R*,10*R*,11*R*,12*R*,13*R*,14*S*,17*S*.

The other 17 known compounds were identified by comparison of their ^{1}H NMR, ^{13}C NMR, and ESI-MS data with those reported as rohituka-3 (**9**),[9] rohituka-7 (**10**),[9] rohituka-9 (**11**),[9] rohituka-12 (**12**),[9] rohituka-14 (**13**),[9] rohituka-15 (**14**),[9] dregenana-1 (**15**),[9] aphanamolide A (**16**),[29] aphapolynin A (**17**),[12] and aphanalides A–H (**18**–**25**),[13] respectively.

Cytotoxic Activities. Limonoids **1**–**25** were evaluated for their cytotoxicities against MCF-7, Bel-7402, and BCG-823 cell lines by the MTT method. None of compounds showed cytotoxicity against tested cell lines (IC_{50} > 20 μM).

Anti-inflammatory Activities. All the isolates were also submitted for initial screening of their inhibitory effects on lipopolysaccharide-stimulated inflammation factor release for NO, which also showed negative results: each compound with concentration at 50 μM inhibited the NO production by less than 50%.

The above-mentioned results showed that the tested limonoids displayed low toxicities toward mammalian organism.

Fungicidal Activities. These isolates were further tested for their antifungal activities against seven phytopathogenic fungi (Table 4). Most compounds lack noticeable fungicidal activity except for **1**, **6**, **9**, and **17** showing strong fungicidal activity against *U. viciae-fabae* and **14** and **16** exhibiting complete control over *P. dissimile*. In addition, compound **16** showed moderate activity against *S. tritici*, while compounds **8** and **11** showed weak activity against *S. tritici*. Compounds **3** and **14** showed moderate activity against *U. viciae-fabae*, while compounds **2** and **16** showed weak activity against *U. viciae-fabae*. Compounds **1**, **9**, **11**, **17**, and **25** showed moderate activity against *P. dissimile*, while compounds **2**, **8**, and **24** showed weak activity against *P. dissimile*. To *A. solani*, only compounds **2** and **25** displayed moderate activity. All the isolates displayed no activity to three fungi: *P. infestans*, *B. fuckeliana*, and *G. zeae*. The fungicidal activities of the isolated 25 limonoids were strain specific with a narrow spectrum. Therefore, it was difficult to draw clear structure–activity relationships of the 25 isolates.

Herbicidal Activities. All the compounds were also screened in order to find potential herbicidal activity, however, none of the compounds exhibited such activity.

Insecticidal Activities. Compounds **1**–**25** were also tested for insecticidal activity against four pest species (*S. avenae*, *P. xylostella*; *D. balteata*, and *C. elegans*). The mean assessment scores are given in Table 4. Most of the compounds were inactive, except for compounds **1**, **2**, **4**, **15**, and **22**–**25**. The α,β-unsaturated lactone and 14,15-epoxy moieties seem essential for the insecticidal activity, which was in accordance with the reported literature.[10,30] The potential insecticidal compounds **1** and **25** were further selected for testing their modes of action. Both compounds inhibited the nicotine response with IC_{50} at 3.13 ppm (**1**) and 1.59 ppm (**25**), respectively, and **25** also inhibited the GABA response with IC_{50} at 8.00 ppm.

In summary, 25 limonoids were isolated from Meliaceae family tree *A. polystachya*. Considering both safety (cytotoxicities and anti-inflammatory activities) and agricultural applications (fungicidal, herbicidal, and insecticidal activities), these isolates were evaluated for a series of biological activities, which would be helpful for the research and development of new natural agents for pest control.

■ ASSOCIATED CONTENT

Supporting Information

HR-ESI-MS, ^{1}H and ^{13}C NMR, and 2D NMR spectra of compounds **1**–**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This work was financially supported by the Project of National Natural Science Foundation of China (21272275), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), the Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT-IRT1193), and the Fundamental Research Funds for the Central Universities (JKY2011011). The Syngenta Postgraduate Fellowship awarded to Y.Z. (Series No.: SPF-048-1) was also appreciated.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Ntalli, N. G.; Caboni, P. Botanical nematicides: a review. *J. Agric. Food Chem.* **2012**, *60*, 9929–9940.

(2) Charleston, D. S.; Kfir, R.; Dicke, M.; Vet, L. E. M. Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth. *Biol. Control* **2005**, *33*, 131–142.

(3) Veitch, G. E.; Boyer, A.; Ley, S. V. The azadirachtin story. *Angew. Chem., Int. Ed.* **2008**, *47*, 9402–9429.

(4) Brown, D. A.; Taylor, D. A. H. Limonoid extractives from *Aphanamixis polystacha*. *Phytochemistry* **1978**, *17*, 1995–1999.

(5) Maclachlan, L. K.; Taylor, D. A. H. A revision of the structures of three limonoids. *Phytochemistry* **1982**, *21*, 2426–2427.

(6) King, T. J.; Taylor, D. A. H. The structure of the limonoid rohituka substance 7 from *Aphanamixis polystacha*. *Phytochemistry* **1983**, *22*, 307.

(7) Mulholland, D. A.; Naidoo, N. Limonoids from *Aphanamixis polystacha*. *Phytochemistry* **1999**, *51*, 927–930.

(8) Zhang, H. P.; Wu, S. H.; Luo, X. D.; Ma, Y. B.; Wu, D. G. A new limonoid from the seed of *Aphanamixis polystachya*. *Chin. Chem. Lett.* **2002**, *13*, 341−342.
(9) Zhang, H. P.; Chen, F.; Wang, X.; Wu, D. G.; Chen, Q. Complete assignments of ^{1}H and ^{13}C NMR data for rings A,B-*seco* limonoids from the seed of *Aphanamixis polystachya*. *Magn. Reson. Chem.* **2007**, *45*, 189−192.
(10) Koul, O.; Daniewski, W. M.; Multani, J. S.; Gumulka, M.; Singh, G. Antifeedant effects of the limonoids from *Entandrophragma candolei* (Meliaceae) on the gram pod borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Agric. Food Chem.* **2003**, *51*, 7271−7275.
(11) Chen, S. K.; Chen, B. Y.; Li, H. In *Flora Reipublicae Popularis Sinicae (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, China, 1997; Vol. *43*, p 79.
(12) Zhang, Y.; Wang, J. S.; Wang, X. B.; Wei, D. D.; Luo, J. G.; Luo, J.; Yang, M. H.; Kong, L. Y. Aphapolynins A and B, two new limonoids from the fruits of *Aphanamixis polystachya*. *Tetrahedron Lett.* **2011**, *52*, 2590−2593.
(13) Wang, J. S.; Zhang, Y.; Wang, X. B.; Kong, L. Y. Aphanalides A−H, ring A-*seco* limonoids from the fruits of *Aphanamixis polystachya*. *Tetrahedron* **2012**, *68*, 3963−3971.
(14) Sheldrick, G. M. *SHELXS-97: program for crystal structure resolution*; University of Göttingen: Göttingen, Germany, 1997.
(15) Sheldrick, G. M. *SHELXL-97: Program for crystal structure refinement*; University of Göttingen: Göttingen, Germany, 1997.
(16) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(17) Wang, W.; Liu, J. Z.; Han, J.; Xu, Z. R.; Liu, R. X.; Liu, P.; Wang, W. X.; Ma, X. C.; Guan, S. H.; Guo, D. A. New triterpenoids from *Kadsura heteroclita* and their cytotoxic activity. *Planta Med.* **2006**, *72*, 450−457.
(18) Guo, C.; Wang, J. S.; Zhang, Y.; Yang, L.; Wang, P. R.; Kong, L. Y. Relationship of chemical structure to *in vitro* anti-inflammatory activity of tirucallane triterpenoids from the stem barks of *Aphanamixis grandifolia*. *Chem. Pharm. Bull.* **2012**, *60*, 1003−1010.
(19) Lin, L.; Mulholland, N.; Wu, Q. Y.; Beattie, D.; Huang, S. W.; Irwin, D.; Clough, J.; Gu, Y. C.; Yang, G. F. Synthesis and antifungal activity of novel sclerotiorin analogues. *J. Agric. Food Chem.* **2012**, *60*, 4480−4491.
(20) Zhao, H. P.; Liu, Y. X.; Cui, Z. P.; Beattie, D.; Gu, Y. C.; Wang, Q. M. Design, synthesis, and biological activities of arylmethylamine substituted chlorotriazine and methylthiotriazine compounds. *J. Agric. Food Chem.* **2011**, *59*, 11711−11717.
(21) Wang, H.; Yang, Z. K.; Fan, Z. J.; Wu, Q. J.; Zhang, Y. J.; Mi, N.; Wang, S. X.; Zhang, Z. C.; Song, H. B.; Liu, F. Synthesis and insecticidal activity of *N*-tert-butyl-*N*,*N*′-diacylhydrazines containing 1,2,3-thiadiazoles. *J. Agric. Food Chem.* **2011**, *59*, 628−634.
(22) Lin, B. D.; Chen, H. D.; Liu, J.; Zhang, S.; Wu, Y.; Dong, L.; Yue, J. M. Mulavanins A−E: limonoids from *Munronia delavayi*. *Phytochemistry* **2010**, *71*, 1596−1601.
(23) Wang, X. N.; Yin, S.; Fan, C. Q.; Wang, F. D.; Lin, L. P.; Ding, J.; Yue, J. M. Turrapubesins A and B, first examples of halogenated and maleimide-bearing limonoids in nature from *Turraea pubescens*. *Org. Lett.* **2006**, *8*, 3845−3848.
(24) Wang, X. N.; Yin, S.; Fan, C. Q.; Lin, L. P.; Ding, J.; Yue, J. M. Eight new limonoids from *Turraea pubescens*. *Terahedron* **2007**, *63*, 8234−8241.
(25) Yuan, T.; Yang, S. P.; Zhang, C. R.; Zhang, S.; Yue, J. M. Two limonoids, khayalenoids A and B with an unprecedented 8-oxa-tricyclo [4.3.2.$0^{2,7}$] undecane motif, from *Khaya senegalensis*. *Org. Lett.* **2009**, *11*, 617−620.
(26) Chen, H. D.; Yang, S. P.; Liao, S. G.; Zhang, B.; Wu, Y.; Yue, J. M. Limonoids and sesquiterpenoids from *Amoora tsangii*. *J. Nat. Prod.* **2008**, *71*, 93−97.
(27) Fan, C. Q.; Wang, X.; Yin, S.; Zhang, C. R.; Wang, F. D.; Yue, J. M. Tabularisins A−D, phragmalin *ortho* esters with new skeleton isolated from the seeds of *Chukrasia tabularis*. *Terahedron* **2007**, *63*, 6741−6747.
(28) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A. Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C. J.; Ochterski, W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision B.01*; Gaussian, Inc.: Wallingford, CT, 2010.
(29) Yang, S. P.; Chen, H. D.; Liao, S. G.; Xie, B. J.; Miao, Z. H.; Yue, J. M. Aphanamolide A, a new limonoid from *Aphanamixis polystachya*. *Org. Lett.* **2011**, *13*, 150−153.
(30) Luo, X. D.; Wu, S. B.; Wu, D. G.; Ma, Y. B.; Qi, S. H. Novel antifeeding limonoids from *Dysoxylum hainanense*. *Tetrahedron* **2002**, *58*, 7797−7804.