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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · OCTOBER 2014

Impact Factor: 3.8 · DOI: 10.1021/np500542x · Source: PubMed

JOURNAL OF NATURAL PRODUCTS

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Isolation and Total Syntheses of Cytotoxic Cryptolactones A_1, A_2, B_1, and B_2: α,β-Unsaturated δ-Lactones from a *Cryptomyzus* sp. Aphid

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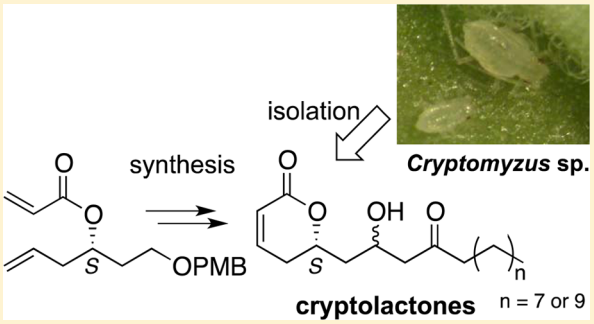
Supporting Information

ABSTRACT: The cryptolactones A_1, A_2, B_1, and B_2, which are α,β-unsaturated δ-lactones, were isolated from a *Cryptomyzus* sp. aphid. The structures were established by 1-D and 2-D NMR spectra and CI-HRMS. Their absolute configurations were determined with the Kusumi−Mosher method, combined with asymmetric total syntheses. The syntheses were accomplished with the Mukaiyama aldol reaction and olefin metathesis, which utilized the second-generation Grubbs catalyst for the key steps. These compounds exhibited cytotoxic activity against human promyelocytic leukemia HL-60 cells with IC_{50} values of 0.97−5.3 μM.



We previously studied the chemical structures of substances derived from aphids, and we found that colored aphids produced novel, polyketide pigments.[1] These pigments possessed interesting biological activities, such as cytotoxicity[1a,c] and antibacterial activity.[1c] As part of our continuing efforts toward determining chemical structures of substances derived from aphids, we focused on a colorless aphid, *Cryptomyzus* sp., which was observed feeding on *Ribes fasciculatum* (family; Saxifragaceae, Japanese common name: yabusanzashi), in Tokushima Prefecture, Japan. This tiny aphid is 0.5−1 mm long, has a transparent skin, and lives in obscurity on the abaxial side of leaves. Although we did not expect to find polyketide pigments, due to the lack of body color, we isolated colorless polyketides, cryptolactones A_1, A_2, B_1, and B_2 (**1**−**4**) from this aphid (Figure 1). Here, we aimed to elucidate the structures, determine the cytotoxicity, and perform the total synthesis of these cryptolactones.

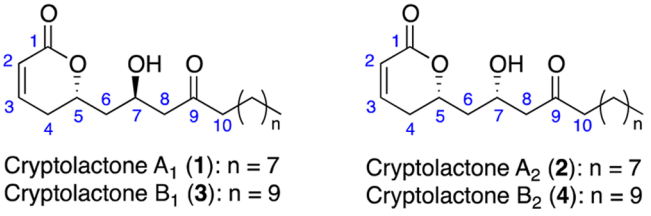


Figure 1. Cryptolactones isolated from a colorless aphid. (Top) Photograph of *Cryptomyzus* sp. aphids; (bottom) structures of cryptolactones A_1, A_2, B_1, and B_2 (**1**−**4**, respectively).

RESULTS AND DISCUSSION

The aphids were removed from the plant with a soft paintbrush and collected in a plastic Erlenmeyer flask equipped with a plastic funnel. In the laboratory, the aphids were crushed with a pestle and washed repeatedly with ether. The ether extract of *Cryptomyzus* sp. was subjected to silica gel column chromatography (*n*-hexane/EtOAc = 2:1), reversed-phase preparative TLC (MeOH/H_2O = 5:1), and HPLC to afford cryptolactones **1**−**4** as colorless solids. The separation of **1**−**4** was quite difficult, even with HPLC; consequently, only small amounts of pure, natural **1**−**4** could be isolated.[2]

The molecular formula of **1** was established as $C_{18}H_{30}O_4$ by CI-HRMS. The ^{13}C NMR spectrum of **1** clearly exhibited 18 carbon resonances, which were classified as one methyl, 11 methylenes, four methines, and two carbonyl carbons, based on a DEPT ^{13}C NMR analysis (Table 1). NMR studies, including HMQC and HMBC experiments, suggested the presence of two oxygen-bearing methine carbons [δ_C/δ_H 74.8/4.74 and 63.7/4.39] and two olefinic carbons [δ_C/δ_H 121.4/6.03 and 145.2/6.89], which were conjugated to a lactone carbonyl carbon [δ_C 164.2]. Furthermore, the double bond had *Z*

Received: July 3, 2014
Published: October 29, 2014

Table 1. ^{13}C and ^{1}H NMR Data for Compounds 1−4 in $CDCl_3$

	1[a]		2[b]		3[a]		4[b]	
position	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)
1	164.2, C		164.2, C		164.2, C		164.2, C	
2	121.4, CH	6.03, ddd (9.9, 2.6, 1.1)	121.3, CH	6.03, dt (9.8, 1.6)	121.4, CH	6.03, ddd (9.7, 2.6, 1.1)	121.3, CH	6.03, dt (9.8, 1.7)
3	145.2, CH	6.89, ddd (9.9, 5.7, 2.6)	145.2, CH	6.91, dt (9.8, 4.5)	145.1, CH	6.89, ddd (9.7, 5.8, 2.6)	145.2, CH	6.90, dt (9.8, 4.5)
4	29.9, CH_2	2.34, ddt (18.4, 11.5, 2.6)	29.1, CH_2	2.42−2.46, m (overlapped)	29.9, CH_2	2.34, ddt (18.4, 11.5, 2.6)	29.1, CH_2	2.42−2.46, m (overlapped)
		2.41, dddd (18.4, 5.7, 4.4, 1.1)				2.41, dddd (18.4, 5.8, 4.3, 1.1)		
5	74.8, CH	4.74, dddd (11.5, 9.1, 4.4, 3.3)	75.4, CH	4.72, m	74.8, CH	4.74, dddd (11.5, 9.1, 4.3, 3.2)	75.4, CH	4.72, m
6	41.5, CH_2	1.75, ddd (14.3, 9.9, 3.3)	40.5, CH_2	2.02, ddd (14.5, 8.0, 6.7)	41.5, CH_2	1.75, ddd (14.4, 9.9, 3.2)	40.5, CH_2	2.02, ddd (14.5, 8.0, 6.6)
		1.82, ddd (14.3, 9.1, 2.7)		1.81, ddd (14.5, 5.8, 3.9)		1.82, ddd (14.4, 9.1, 2.9)		1.81, ddd (14.5, 5.9, 4.0)
7	63.7, CH	4.39, dddd (9.9, 9.3, 2.7, 2.6)	64.3, CH	4.29, m	63.7, CH	4.39, dddd (9.9, 9.2, 2.9, 2.6)	64.3, CH	4.30, m
8	48.8, CH_2	2.53, dd (17.9, 9.3)	48.5, CH_2	2.64, dd (18.0, 8.0)	48.8, CH_2	2.53, dd (17.7, 9.2)	48.5, CH_2	2.64, dd (17.9, 7.8)
		2.66, dd (17.9, 2.6)		2.69, dd (18.0, 4.1)		2.66, dd (17.7, 2.6)		2.69, dd (17.9, 4.2)
9	212.2, C		212.4, C		212.2, C		212.4, C	
10	43.6, CH_2	2.43, t (6.5)	43.6, CH_2	2.42−2.46, m (overlapped)	43.6, CH_2	2.43, t (6.5)	43.6, CH_2	2.42−2.46, m (overlapped)
11	23.6, CH_2	1.57, quin (7.1)	23.6, CH_2	1.58, brs	23.6, CH_2	1.57, quin (7.1)	23.6, CH_2	1.56, brs
12−17 (12−19)[c]	22.6, 29.1, 29.2, 29.3, 29.4, 31.8, CH_2	1.26−1.32, m	22.6, 29.1, 29.2, 29.4, 29.4, 31.8, CH_2	1.26−1.30, m	22.7, 29.1, 29.3, 29.4, 29.4, 29.6, 29.6, 31.9, CH_2	1.26−1.32, m	22.7, 29.1, 29.3, 29.4, 29.4, 29.6, 29.6, 31.8, CH_2	1.26−1.30, m
18 (20)[c]	14.1, CH_3	0.88, t (7.0)	14.1, CH_3	0.88, t (6.9)	14.1, CH_3	0.88, t (7.0)	14.1, CH_3	0.88, t (7.0)
7-OH				3.35, d (2.8)		3.38, brs		3.34, d (2.9)

[a]Data from ^{13}C (150 MHz) and ^{1}H NMR (600 MHz). [b]Data from ^{13}C (125 MHz) and ^{1}H NMR (500 MHz). [c]Data for compounds **3** and **4**.

dx.doi.org/10.1021/np500542x | J. Nat. Prod. 2014, 77, 2459−2464

geometry [δ_H 6.03 and 6.89 with J = 9.9 Hz]. The HMBC experiment revealed that one oxymethine moiety was located at the C-5 position (Figure 2). Finally, on the basis of the

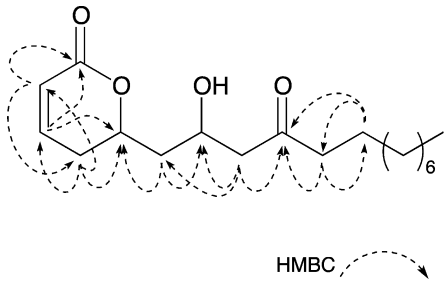
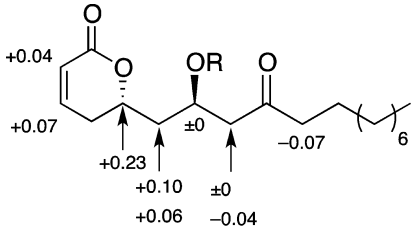


Figure 2. HMBC correlations of compound **1** in $CDCl_3$.

chemical shifts of this oxymethine signal [δ_C/δ_H 74.8/4.74][3] and other NMR data, we determined that **1** was an α,β-unsaturated δ-lactone with a side chain containing a β-hydroxy ketone moiety. However, the relative configuration (*syn* or *anti*) of the oxygen functions between the C-5 and C-7 positions and their absolute configurations could not be determined at this stage.

Therefore, the Kusumi–Mosher method[4] was applied to determine the absolute configuration at the C-7 position. We prepared (*R*)- and (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) esters of **1** (i.e., **5a** and **5b**, respectively; Figure 3)



5a: R = (*R*)-MTPA
5b: R = (*S*)-MTPA

Figure 3. $\Delta\delta$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for the (*R*)- and (*S*)-MTPA esters (**5a** and **5b**, respectively) of cryptolactone A_1 (**1**).

and compared the chemical shifts in their ^{1}H NMR spectra. The $\Delta\delta^{SR}$ ($\delta_S - \delta_R$) of H-2, H-3, H-5, and H_2-6 showed positive values, but those of H_2-8 and H_2-10 were negative. These observations suggested that the C-7 carbon was in the *R*-configuration. However, the absolute configuration at the C-5 position of **1** could not be determined. Furthermore, the molecular formula of **2** was same as that of **1**, and the IR and NMR spectra of **2** were also quite similar to those of **1** (Table 1). Therefore, **2** was most likely a stereoisomer of **1**, but the configurations at the C-5 and C-7 positions remained unclear.

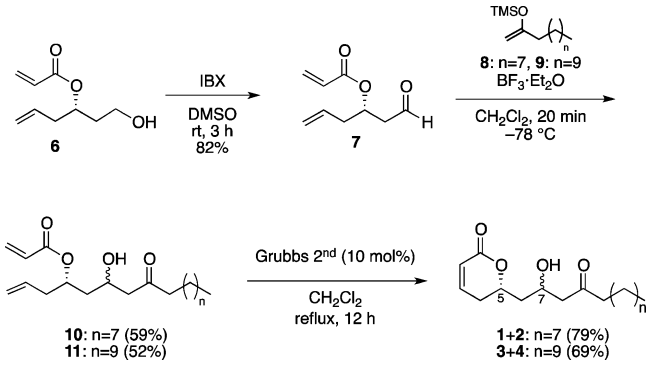
The molecular formulas of **3** and **4** were both established as $C_{20}H_{34}O_4$ with CI-HRMS. The IR and NMR spectra of these compounds were very similar to those of **1** and **2**; the only difference between **1**/**2** and **3**/**4** was the presence of two additional methylene units in **3**/**4**. Furthermore, the chemical shifts in the ^{1}H NMR spectra at the C-7 position were similar between **1**/**2** and **3**/**4**. These similarities suggested that compounds **3** and **4** were longer chain analogues of **1** and **2**.

To determine the absolute configurations of **1**–**4**, we conducted total syntheses of these cryptolactones. This would also provide additional material for future investigations of their biological activities, including antiviral, antibacterial, and antifungal activities.

The known, optically active alcohol **6**, which was prepared from (3*R*)-1-*p*-methoxybenzyloxy-3,4-epoxybutane via reported procedures,[5] was oxidized with 2-iodoxybenzoic acid (IBX) to give aldehyde **7**. Aldehyde **7** was treated with silyl enol ether **8**, under the conditions of the Mukaiyama aldol reaction.[6] This reaction nonselectively yielded an inseparable, diastereomeric mixture of the aldol adduct **10**, in 48% yield (two steps; ratio 1:1.4). The mixture of **10** was subjected to a ring-closing olefin metathesis, which employed the second-generation Grubbs catalyst, to afford a mixture of two α,β-unsaturated δ-lactones in 79% yield. The isomers were separated by HPLC; they were identified as **1** and **2** by comparing their physical data with those of the natural products **1** and **2**. The specific rotations of the synthetic ($[\alpha]^{21}_D$ −55.6 (*c* 0.63, $CHCl_3$)) and the natural **1** ($[\alpha]^{20}_D$ −53.5 (*c* 2.22, $CHCl_3$)) were both negative, which indicated that the absolute configuration of natural **1** must be 5*S*, 7*R*. Similarly, the absolute configuration of natural **2** was determined to be 5*S*, 7*S*, as shown in Figure 1.

Compounds corresponding to **3** and **4** were also synthesized in the same manner, starting with **7** and utilizing the silyl enol ether **9** (Scheme 1). Compound **3** had the same (5*S*, 7*R*)

Scheme 1



configuration as that of **1**. Although the specific rotation of the natural **4** could not be observed, due to the very small amount of sample obtained from the aphid, we propose that the 5*S*, 7*S* configuration should be assigned to the structure of **4** as it was for **2**. Thus, the structures of these four compounds isolated from the colorless aphid, *Cryptomyzus* sp., were determined synthetically.

These cryptolactones were α-pyrone derivatives isolated for the first time from aphids. Other α-pyrones isolated from nature have exhibited interesting biological activities. For example, gamahonolide A inhibited the growth of *C. herbarum*;[7] kurzilactone was cytotoxic against KB cells;[8] goniodiol was cytotoxic against human lung tumor cells (A-549);[9] and cryptocaryols were found to rescue Pdcd4 from TPA-induced degradation.[10] Therefore, we tested the cytotoxicity of **1**–**4** against human promyelocytic leukemia HL-60 cells.[11] We found that **1**, **2**, **3**, and **4** exhibited cytotoxic activities with IC_{50} values of 1.2, 5.3, 0.97, and 4.9 μM, respectively. The IC_{50} value of the positive control compound, doxorubicin, was 0.14 μM.[12]

CONCLUSIONS

In this study, we isolated cryptolactones **1**–**4** from a *Cryptomyzus* sp. aphid, we examined the structures with detailed 1-D

dx.doi.org/10.1021/np500542x | *J. Nat. Prod.* 2014, 77, 2459–2464

and 2-D NMR spectra, and we finally determined the structures by synthesis. We achieved the total asymmetric syntheses of these cryptolactones by performing the Mukaiyama aldol reaction, with olefin metathesis in the key steps. Cryptolactones are C-18/C-20 fatty acid derivatives that consist of an α-pyrone moiety. This type of carbon skeleton has never been obtained previously from aphids. Moreover, these compounds exhibited cytotoxic activity against human promyelocytic leukemia HL-60 cells, with IC_{50} values of 0.97−5.3 μM. This finding supported our hypothesis that aphid polyketides, including pigments, may represent chemopreventive agents that aid in resisting infection or attack.[1c] Currently, we are conducting further investigations of the biological activities of compounds **1**−**4** in our laboratory, and the data will be reported in due course.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were measured on a JASCO V-650 spectrophotometer. Optical rotations were measured on JASCO P-1030 polarimeters. IR spectra were measured on a JASCO FT/IR-410 spectrophotometer. ^{1}H NMR spectra were acquired with Varian Unity-600 (600 MHz), Varian Unity-500 (500 MHz), and Varian Mercury-300 (300 MHz) spectrophotometers with TMS as the internal standard in $CDCl_3$. ^{13}C NMR spectra were measured on Varian Unity-600 (150 MHz) and Varian Unity-500 (125 MHz) spectrophotometers; chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_C 77.0 ppm). Signal multiplicities were established with DEPT experiments.

Mass spectra, including high-resolution mass spectra, were acquired with a JEOL JMS-700 spectrophotometer. The TLC analysis was performed with Merck precoated silica gel plates (60F). Column chromatography was conducted with silica gel 60N (Kanto Chemical Co. Inc., 63-210 μm (micro meter)). Preparative TLC was performed with Merck precoated silica gel plates (60 RP-18 WF_{254S}). Preparative HPLC was carried out on a JASCO 880-PU pump unit equipped with an 875-UV detector (λ 220 nm) and a CHIRALPACK AD column (20 × 250 mm); the column was eluted with *n*-hexane/2-propanol (9:1) at a flow rate of 8.0 mL/min. For analysis, two CHIRALPACK AD columns (4.6 × 250 mm, two columns connected together) were eluted with *n*-hexane/2-propanol (9:1) at a flow rate of 1.0 mL/min.

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), 4-(*N*,*N*-dimethylamino)pyridine (DMAP), *N*,*N*-diisopropylethylamine (DIPEA), and triethylamine were purchased from Nacalai Tesque Inc. Dimethyl sulfoxide (DMSO), tetrahydrofuran (THF, dehydrated stabilizer free: super plus), and CH_2Cl_2 (dehydrated: super) were purchased from Kanto Chemical Co., Inc. Acryloyl chloride was purchased from Wako Pure Chemical Industries, Ltd. Boron trifluoride etherate ($BF_3{\cdot}Et_2O$) and pyridinium *p*-toluene sulfonate (PPTS) were purchased from Tokyo Chemical Industry Co., Ltd. The second-generation Grubbs catalysts, (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl), were purchased from Sigma-Aldrich Co., Inc. All of these solvents and reagents were used without further purification. Silyl enol ethers **8**[13] and **9**[14] were prepared according to reported procedures.

Biological Material. The aphids, *Cryptomyzus* sp., were collected as they fed on *Ribes fasciculatum* in Tokushima Prefecture, Japan, in June 2007. The species was authenticated by one of the authors (S.T.), according to the following features. The aphid had the first and second abdominal spiracles closely spaced, and the first and seventh abdominal segments lacked marginal tubercles. These taxonomical points suggested that the aphid belonged to the *Macrosiphini* tribe. The aphid was distinguished by the following: (1) the apex was siphuncular, not reticulated; (2) the cauda was tongue-shaped; (3) the prothorax had two setae dorso-mesially; (4) the antennal tubercles were developed; (5) the antenna had secondary rhinaria on the third segment; (6) the head was smooth; antennal tubercles were divergent; (7) the siphunclus was swollen; (8) the spiracles were round; and (9) the dorsal setae of the body were long and capitate. In addition, the cauda was short, at most, about 1.5 times as long as it was wide. The antenna had secondary rhinaria bunched on the fourth segment. Many of these features showed that the aphid was *Cryptomyzus* sp.

A voucher specimen was not preserved from the original collection, and subsequent attempts to collect the aphid have not been successful.

Extraction, Isolation, and Spectroscopic Analyses of Cryptolactones. The colorless aphids *Cryptomyzus* sp. were removed from the plant with a soft paintbrush and collected into a plastic Erlenmeyer flask equipped with a plastic funnel. The aphids (517 mg) were crushed in diethyl ether with a mortar, and then, the ethereal supernatant solution was separated by decantation. The residue was washed with several portions of fresh ether (total: 30 mL). The combined ethereal solutions were dried over Na_2SO_4 and evaporated to produce an extract (94 mg). The extract was subjected to repeated chromatographic purifications over silica gel (*n*-hexane/EtOAc = 2:1), reversed-phase preparative TLC ($MeOH/H_2O$ = 5:1, three times), and HPLC (three times) to afford cryptolactones **1** (13.8 mg), **2** (2.2 mg), **3** (3.2 mg), and **4** (0.5 mg), as colorless solids.

*Cryptolactone A$_1$ (**1**):* colorless solid; $[\alpha]^{20}_D$ −53.5 (*c* 2.22, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 203 (4.02); IR (ATR) ν_{max} 3407 (−OH), 2920, 1712 (C═O) cm^{-1}; NMR data, see Table 1; CI-MS *m*/*z* 311 $[M + H]^+$, 293, 275; CI-HRMS *m*/*z* 311.2221 $[M + H]^+$ (calcd for $C_{18}H_{31}O_4$, 311.2222).

*Cryptolactone A$_2$ (**2**):* colorless solid; $[\alpha]^{20}_D$ −44.1 (*c* 0.42, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 202 (4.11); IR (ATR) ν_{max} 3447 (−OH), 2924, 1709 (C═O) cm^{-1}; NMR data, see Table 1; CI-MS *m*/*z* 311 $[M + H]^+$, 293, 275; CI-HRMS *m*/*z* 311.2220 $[M + H]^+$ (calcd for $C_{18}H_{31}O_4$, 311.2222).

*Cryptolactone B$_1$ (**3**):* colorless solid; $[\alpha]^{22}_D$ −46.1 (*c* 0.71, $CHCl_3$); UV (CH_3CN) λ_{max} (log ε) 203 (4.05); IR (ATR) ν_{max} 3407 (−OH), 2918, 1711 (C═O) cm^{-1}; NMR data, see Table 1; CI-MS *m*/*z* 339 $[M + H]^+$, 321; CI-HRMS *m*/*z* 339.2526 $[M + H]^+$ (calcd for $C_{20}H_{35}O_4$, 339.2535).

*Cryptolactone B$_2$ (**4**):* colorless solid; IR (ATR) ν_{max} 3460 (−OH), 2918, 1709 (C═O) cm^{-1}; NMR data, see Table 1; CI-MS *m*/*z* 339 $[M + H]^+$, 321; CI-HRMS *m*/*z* 339.2539 $[M + H]^+$ (calcd for $C_{20}H_{35}O_4$, 339.2535).

MTPA Esters of 1. *(R)-MTPA Ester of 1 (**5a**).* A suspension of **1** (1.8 mg) in CH_2Cl_2 (6 mL) was combined with (*S*)-(+)-MTPACl (total: 25 μL), DMAP (total: 13 mg), and DIPEA (total: 14 μL) in three portions, and the mixture was stirred at ambient temperature for 3 days. The progress of the reaction was monitored by TLC. The mixture was washed with 0.1 N HCl (3 mL × 3), saturated $NaHCO_3$ solution (3 mL × 3), and saturated NaCl solution (3 mL × 3). The organic layer was dried over Na_2SO_4 and evaporated. The crude residue was purified with silica gel column chromatography (1.5 g, *n*-hexane/EtOAc = 3:1) to give 1.3 mg (43%) of ester **5a** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (2H, d, *J* = 7.3 Hz), 7.35−7.42 (3H, m), 6.77 (1H, ddd, *J* = 9.9, 5.4, 3.3 Hz), 5.97 (1H, ddd, *J* = 9.9, 2.4, 1.2 Hz), 5.62 (1H, dddd, *J* = 7.8, 5.1, 6.6, 6.0 Hz), 4.20 (1H, dddd, *J* = 11.1, 9.0, 4.8, 3.3 Hz), 3.54 (3H, q, *J* = 1.2 Hz), 2.92 (1H, dd, *J* = 16.8, 6.6 Hz), 2.84 (1H, dd, *J* = 16.8, 6.0 Hz), 2.47 (1H, dt, *J* = 17.1, 7.5 Hz), 2.39 (1H, dt, *J* = 17.1, 7.5 Hz), 2.25 (1H, dddd, *J* = 18.3, 11.1, 3.3, 2.4 Hz), 2.16 (1H, dddd, *J* = 18.3, 5.4, 4.8, 1.2 Hz), 2.07 (1H, ddd, *J* = 14.7, 9.0, 5.1 Hz), 1.98 (1H, ddd, *J* = 14.7, 7.8, 3.3 Hz), 1.56 (2H, brs), 1.25 (12H, brs), 0.88 (3H, t, *J* = 6.8 Hz); FAB-MS *m*/*z* 549 $[M + Na]^+$; FAB-HRMS *m*/*z* 549.2438 $[M + Na]^+$ (calcd for $C_{28}H_{37}F_3NaO_6$, 549.2440).

(S)-MTPA Ester of 1 (5b). The ester **5b** was prepared by the same procedure and provided a 51% yield, as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (2H, d, *J* = 7.2 Hz), 7.38−7.42 (3H, m), 6.84 (1H, ddd, *J* = 9.9, 5.1, 3.3 Hz), 6.01 (1H, ddd, *J* = 9.9, 2.4, 1.5 Hz), 5.63 (1H, ddt, *J* = 7.8, 5.4, 6.0 Hz), 4.43 (1H, tdd, *J* = 9.3, 6.3, 3.3 Hz), 3.48 (3H, q, *J* = 0.9 Hz), 2.93 (1H, dd, *J* = 16.8, 6.0 Hz), 2.79 (1H, dd, *J* = 16.8, 6.0 Hz), 2.25−2.43 (4H, m), 2.17 (1H, ddd, *J* = 14.7, 9.3, 5.4 Hz), 2.04 (1H, ddd, *J* = 14.7, 7.8, 3.3 Hz), 1.56 (2H, brs), 1.25 (12H, brs), 0.88 (3H, t, *J* = 6.8 Hz); FAB-MS *m*/*z* 549 $[M + Na]^+$; FAB-HRMS *m*/*z* 549.2438 $[M + Na]^+$ (calcd for $C_{28}H_{37}F_3NaO_6$, 549.2440).

Syntheses of 1−4. *(1S,3RS)-3-Hydroxy-5-oxo-1-(prop-2-enyl)-tetradecanyl Acrylate (**10**).* A solution of **6**[5b] (102 mg, 0.59 mmol) in

dx.doi.org/10.1021/np500542x | J. Nat. Prod. 2014, 77, 2459−2464

DMSO was mixed with IBX (330 mg, 1.18 mmol) at ambient temperature. The mixture was stirred for 3 h and then treated with H_2O (2.2 mL). The resulting suspension was filtered, and the remaining solid was washed with ether (70 mL).

The ethereal filtrate was washed with a saturated aqueous $NaHCO_3$ solution (20 mL × 2), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10:1, then 5:1) to give aldehyde **7** in 82% yield (81 mg) as a pale yellow oil. Because this aldehyde was easily dehydrated and polymerized, the Mukaiyama aldol reaction was carried out promptly.

A solution of silyl enol ether **8** (159 mg, 0.65 mmol) in dry CH_2Cl_2 (0.3 mL) was mixed with $BF_3{\cdot}Et_2O$ (60 μL, 0.48 mmol) at −78 °C; then, a solution of aldehyde **7** (73 mg, 0.43 mmol) in dry CH_2Cl_2 (0.8 mL) was added dropwise to the mixture over 15 min at −78 °C. After adding a solution of pyridine (70 μL, 0.87 mmol) and PPTS (220 mg, 0.87 mmol) in dry CH_2Cl_2 (1.0 mL), the mixture was stirred for 20 min at −78 °C, then treated with a saturated aqueous $NaHCO_3$ solution (1.5 mL, then 30 mL). The resulting mixture was extracted with CH_2Cl_2 (20 mL × 3), and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10:1, then 5:1) to give a diastereomeric mixture of aldol adduct **10** (ratio 1:1.4) in 59% yield (89 mg) as a pale yellow oil.

The ^1H NMR chart of diastereomeric mixture **10** can be found in the Supporting Information. CI-MS *m*/*z* 339 $[M + H]^+$; CI-HRMS *m*/*z* 339.2521 $[M + H]^+$ (calcd for $C_{20}H_{35}O_4$, 339.2535).

*Cryptolactone A_1 (**1**) and Cryptolactone A_2 (**2**).* A solution of aldol adduct **10** (105 mg, 0.31 mmol) in dry CH_2Cl_2 (3 mL) was combined with the second-generation Grubbs catalyst (26 mg, 0.031 mmol), and the mixture was refluxed for 12 h under an Ar atmosphere. The resulting mixture was concentrated and purified by silica gel column chromatography (*n*-hexane/EtOAc = 10:1, then 5:1, then 1:1) to give a diastereomeric mixture of cryptolactone A in 79% yield (76 mg) as a pale yellow solid. Each diastereomer was isolated by HPLC with a chiral-phase column [CHIRALPACK AD, 20 × 250 mm, *n*-hexane/2-propanol (9:1), 8 mL/min], with a UV (220 nm) detector. The retention times of **1** and **2** were 52.3 and 45.8 min, respectively. Synthetic cryptolactone A_1 (**1**) was obtained as a colorless solid; $[\alpha]^{21}_D$ −55.6 (*c* 0.63, $CHCl_3$). Synthetic cryptolactone A_2 (**2**) was also obtained as a colorless solid; $[\alpha]^{22}_D$ −44.4 (*c* 0.62, $CHCl_3$).

*Cryptolactone B_1 (**3**) and Cryptolactone B_2 (**4**).* Starting from **6**, compounds corresponding to **3** and **4** were synthesized in the Mukaiyama aldol reaction (52%) with silyl enol ether **9**. This reaction was followed by a ring-closing olefin metathesis, performed with the second-generation Grubbs catalyst (69%). Each diastereomer was isolated by HPLC, with a chiral-phase column [CHIRALPACK AD, 20 × 250 mm, *n*-hexane/2-propanol (9:1), 8 mL/min], equipped with a UV (220 nm) detector. The retention times of **3** and **4** were 37.9 and 33.9 min, respectively. Synthetic cryptolactone B_1 (**3**) was obtained as a colorless solid; $[\alpha]^{23}_D$ −48.4 (*c* 0.89, $CHCl_3$). Synthetic cryptolactone B_2 (**4**) was also obtained as a colorless solid; $[\alpha]^{23}_D$ −33.9 (*c* 0.85, $CHCl_3$).

MTT Assay for Cytotoxic Activity. Human promyelocytic leukemia (HL-60) cells were grown in suspension culture and incubated at 37 °C in RPMI-1640 medium supplemented with 10% FBS and glutamine (2 mM). The cytotoxicities of **1**−**4** for HL-60 cells were analyzed with the colorimetric 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, with some modifications.[11] HL-60 cells (1×10^4) were plated in 96-well plates and incubated at 37 °C in 5% CO_2/95% air for 1 h. Then, 10 μL of serially diluted test compound solutions of **1**−**4** were added, and the cells were incubated for 24 h. The final concentrations of **1**−**4** in the sample wells ranged from 0.63 to 100 μM. After 24 h, 10 μL of MTT (5 mg/mL stock solution) was added, and the cells were incubated for an additional 4 h. Next, the cells were treated with 100 μL of 20% sodium dodecyl sulfate in 0.01 N HCl to solubilize the intracellular formazan crystals. The optical density (OD) of each well was measured with a microplate spectrophotometer equipped with a 570 nm filter. Then, we calculated the percentage of absorbance from the sample-treated cells compared to that of the vehicle control (0.5% DMSO in standard medium). The resulting cytotoxic activities are expressed as IC_{50} values. The IC_{50} value of the positive control compound, doxorubicin, was 0.14 μM.[12]

■ ASSOCIATED CONTENT

Supporting Information

Materials include photographs of the aphid and the host plant; 1-D NMR (^1H and ^{13}C), mass, and IR spectra for natural and synthetic **1**−**4**, **6**, and the precursor of **6**; UV spectra for natural **1**−**3**; and ^1H NMR spectra for **5a** and **5b**. Detailed procedures for the preparation of **6** are also described and presented with spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported partially by a Grant-in-Aid for Scientific Research (C, 22590032 and 25460030) from MEXT (the Ministry of Education, Culture, Sports, Science and Technology of Japan). We are also thankful to the MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2008−2012.

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(2) The ^1H NMR, ^{13}C NMR, UV, IR, and mass spectra and the specific rotation could be acquired with these pure compounds. Although 2-D NMR spectra of **1** were obtained with a pure sample of **1**, the spectra of **3** were obtained with a mixture of **3** and **4** (4.7:1). The 2-D NMR spectra of **2** and **4** could not be acquired.

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