Regular Article

Total Syntheses and Cytotoxic Evaluations of Cryptolactones A₁, A₂, B₁, B₂, and Their Derivatives

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The cryptolactones A₁, A₂, B₁, and B₂ isolated from a *Cryptomyzus* sp. aphid were synthesized via the Mukaiyama aldol reaction and olefin metathesis. Their antipodes and derivatives were also synthesized by the same strategy to investigate structure–activity relationships. These compounds exhibited cytotoxic activity against human promyelocytic leukemia HL-60 cells with IC₅₀ values of 2.1–42 μM.

Key words cryptolactones; asymmetric synthesis; cytotoxicity

Introduction

Over the recent decades, we have studied colored substances derived from colored aphids. We found that aphids have a wide variety of novel polyketide pigments, including uroleuconaphines^{1,2)} (yellow and red pigments), viridaphin A₁ (green pigment),³⁾ furanaphin (yellow pigment having fluorescence)⁴⁾ and megouraphin glucosides (yellow pigments having fluorescence).⁵⁾ These pigments exhibit interesting biological activities, such as cytotoxicity and antibacterial activity.^{6,7)} We also accomplished total syntheses of two pigments, furanaphin⁸⁾ and xanthouroleuconaphin.⁹⁾

Recently, we also focused on bioactive organic compounds in the colorless aphid, *Cryptomyzus* sp., which was observed feeding on *Ribes fasciculatum* (family; Saxifragaceae, Japanese common name: yabusanzashi), and we obtained four

colorless polyketides, cryptolactones A₁ (**1**), A₂ (**2**), B₁ (**3**), and B₂ (**4**)¹⁰⁾ (Fig. 1). These compounds were 5-substituted α,β-unsaturated δ-lactone derivatives, whose stereochemistry and absolute configuration were determined by the Kusumi–Mosher method and total syntheses.

Furthermore, since it was well-known that the α,β-unsaturated δ-lactone moiety displayed a broad range of potent biological activities such as inhibition of human immunodeficiency virus (HIV) proteases, induction of apoptosis, and antileukemic activities, we investigated the cytotoxicity of **1**–**4** against human promyelocytic leukemia HL-60 cells. We found that **1**, **2**, **3**, and **4** exhibited cytotoxic activities with IC₅₀ values of 1.2, 5.3, 0.97, and 4.9 μM, respectively.¹⁰⁾ The IC₅₀ value of doxorubicin, a positive control compound, was 0.14 μM. These results may indicate that the length of the side chain at the 5-position influences the activity. Therefore, we reconfirmed this hypothesis using cryptolactones **1**–**4** and newly synthesized **5** and **6**, and investigated structure–activity relationships among **1**–**6** and their antipodes.¹¹⁾

Results and Discussion

Our synthetic plan for cryptolactones **1**–**4** and their analogs **5** and **6** is illustrated in Chart 1. The construction of α,β-

Fig. 1. Structures of the Cryptolactone Family

Chart 1. Retrosynthetic Plan for Cryptolactones

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unsaturated δ -lactone could be carried out by ring-closing metathesis reaction of **7** at the late stage of the synthesis as described in our previous paper.¹⁰ Since elongating the side chains with various lengths could be suitable for Mukaiyama aldol reaction with silyl enol ether,^{12,13} aldehyde **8** could serve as a common intermediate. The aldehyde **8** would be synthesized from known optically active epoxide **9** by the addition of a vinyl unit and subsequent acylation.^{14–16}

Our synthesis commenced with preparation of common intermediate (*S*)-**8** from (*R*)-**9** as previously reported.¹⁰ With the common intermediate (*S*)-**8** in hand, we next examined the construction of the α,β -unsaturated δ -lactone moiety. Mukaiyama aldol reaction of silyl enol ether **10a–c** with (*S*)-**8** using $\text{BF}_3 \cdot \text{OEt}_2$ gave **7a–c** in 59% ($n=7$), 52% ($n=9$) and 40% ($n=4$) yield, respectively, with around 1:1 mixture of the diastereomers in all case. Finally, the ring-closing metathesis reaction of **7a–c** using Grubbs' second-generation catalyst afforded cryptolactones (**1–4**) and analogs **5** and **6** in 60–79% yield (Chart 2). The isomers were separated by HPLC. In order to compare the biological activity of both enantiomers, we also synthesized antipodes of **1–6** (*ent*-**1–6**) starting from (*S*)-**9** by a similar procedure (Chart 3).

All compounds were evaluated for their cytotoxicity towards human promyelocytic leukemia HL-60 cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. We also investigate the structure–activity relationships between the both enantiomers of cryptolactones and

length of side chain¹⁷ (Table 1).

First, we focused on compounds **1–6**, whose absolute configurations at the C-5 position were *S*. Comparing **1** vs. **2**, **3** vs. **4**, and **5** vs. **6**, compounds **1**, **3**, and **5** with (7*R*) configuration were more cytotoxic than compounds **2**, **4**, and **6** with (7*S*) configuration, respectively. Thus, the cytotoxicity of compounds with anti-1,3-dioxy relationships at C-5 and C-7 positions in a zigzag chain configuration were more enhanced than the syn-isomers **2**, **4**, and **6**. Furthermore, compounds **5** and **6**, bearing shorter carbon chains, were less cytotoxic than the others. Interestingly, *ent*-**1–6** exhibited similar cytotoxic properties towards HL-60 cell lines as follows: 1) The cytotoxicity values of anti-isomers *ent*-**1**, **3**, and **5** were more active than those of syn-isomers *ent*-**2**, **4**, and **6**, respectively, and 2) compounds *ent*-**5** and **6** with shorter carbon chains were less cytotoxic than the others.

Conclusion

In summary, we synthesized cryptolactones (**1–4**) and their derivatives, which exhibited cytotoxic activity against human promyelocytic leukemia HL-60 cells with IC_{50} values of 2.1–42 μM . Recently, we reported that uroleuconaphins isolated from the red goldenrod aphid *Uroleucon nigrotuberculatum* aid in the resistance of infection by entomopathogenic fungi at the level of the individual aphid and/or at the species level, and we hypothesized that a large majority of aphids have polyketides that may function as chemopreventive agents.⁷ Since cryptolactones exhibited cytotoxicity, we expect that cryptolactones also preserve the aphid from infection by insect pathogens. Further experiments along these lines are in progress.

Experimental

General Experimental Procedures Melting points were determined on a Yanaco MP-3 or Büchi B-545 apparatus, and were uncorrected. Optical rotations were measured on JASCO P-1030 polarimeters. IR spectra were measured on a JASCO FT/IR-410 spectrophotometer. ^1H -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 estab-

Chart 2. Synthesis of Cryptolactones (**1–4**) and Their Derivatives (**5**, **6**)

Chart 3. Synthesis of Antipodes of Cryptolactones (*ent*-**1–4**) and Their Derivatives (*ent*-**5**, **-6**)

Table 1. Cytotoxicity against HL-60 Cell Lines

Compounds	HL-60 (IC_{50} , μM)
1	2.7
2	8.6
3	2.1
4	9.4
5	16.2
6	38.5
<i>ent</i> - 1	4.7
<i>ent</i> - 2	5.5
<i>ent</i> - 3	2.2
<i>ent</i> - 4	4.0
<i>ent</i> - 5	15.7
<i>ent</i> - 6	41.6

lished 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 pre-coated silica gel plates (60F). Column chromatography was conducted with silica gel 60N (Kanto Chemical Co. Inc., Japan, 63–210mm). Preparative TLC was performed with a Merck pre-coated silica gel (60 RP-18 WF254S). Preparative HPLC was carried out on a JASCO 880-PU pump unit equipped with an 875-UV detector (λ 220nm) and a CHIRALPACK AD column (20 $\times$ 250mm); the column was eluted with *n*-hexane/2-propanol (9/1) at a flow rate of 8.0mL/min. For analysis, two CHIRALPACK AD columns (4.6 $\times$ 250mm, two columns connected together) were eluted with *n*-hexane–2-propanol (9 : 1) at a flow rate of 1.0mL/min.

Triethylamine was purchased from Nacalai Tesque Inc. (Japan). 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 Corporation (Japan). Boron trifluoride etherate ($\text{BF}_3 \cdot \text{Et}_2\text{O}$) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). The Second generation Grubbs catalyst was purchased from Sigma-Aldrich Co., Inc. (U.S.A.). All of these solvents and reagents were used without further purification. Silyl enol ethers **10a–c**^{18,19)} 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 professor Shigeru Takahashi in Utsunomiya University, according to the following features. The aphid had the 1st and 2nd abdominal spiracles closely spaced, and the 1st and 7th abdominal segments lacked marginal tubercles. These taxonomical points suggested that the aphid belonged to the *Macrosiphini* tribe. The aphid was distinguished by: (1) the apex was siphuncular, not reticulated; (2) the cauda was tongue-shaped; (3) the prothorax had 2 setae dorso-mesially; (4) the antennal tubercles were developed; (5) the antenna had secondary rhinaria on the 3rd segment; (6) the head was smooth; antennal tubercles were divergent; (7) The siphunculus was swollen. (8) The spiracles were round; and (9) the dorsal setae of 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 4th 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.

Synthesis and Characterization of Cryptolactone A₁, A₂, B₁, and B₂ and Their Antipodes Title compounds and their antipodes were synthesized according to our previous synthetic strategy in Chart 1.¹⁰⁾

Analytical Data for Cryptolactone A₁, A₂, B₁, B₂, and Their Antipode

(–)-**Cryptolactone A₁** ((–)-**1**): colorless solid; mp 41–45°C; $[\alpha]_{\text{D}}^{21}$ –55.6 (*c* 0.63, CHCl_3) (lit.¹⁰⁾ $[\alpha]_{\text{D}}^{20}$ –53.5 (*c* 2.22, CHCl_3); All spectral data (IR, Mass, HRMS, ¹H- and ¹³C-NMR,) are in agreement with natural product (–)-**1**.

(+)-**Cryptolactone A₁** ((+)-**1**): colorless solid; mp 43–45°C; $[\alpha]_{\text{D}}^{21}$ +52.9 (*c* 0.99, CHCl_3). The NMR spectral data (¹H-NMR) of (+)-**1** were in agreement with (–)-**1**.

(–)-**Cryptolactone A₂** ((–)-**2**): colorless solid; mp 44–51°C; $[\alpha]_{\text{D}}^{22}$ –44.4 (*c* 0.62, CHCl_3) (lit.¹⁰⁾ $[\alpha]_{\text{D}}^{20}$ –44.1 (*c* 0.42, CHCl_3); All spectral data (IR, Mass, HRMS, ¹H- and ¹³C-NMR,) are in agreement with natural product (–)-**2**.

(+)-**Cryptolactone A₂** ((+)-**2**): colorless solid; mp 48–51°C; $[\alpha]_{\text{D}}^{21}$ +42.4 (*c* 1.00, CHCl_3). The NMR spectral data (¹H-NMR) of (+)-**2** were in agreement with (–)-**2**.

(–)-**Cryptolactone B₁** ((–)-**3**): colorless solid; mp 50–55°C; $[\alpha]_{\text{D}}^{23}$ –48.4 (*c* 0.89, CHCl_3) (lit.¹⁰⁾ $[\alpha]_{\text{D}}^{22}$ –46.1 (*c* 0.71, CHCl_3); All spectral data (IR, Mass, HRMS, ¹H- and ¹³C-NMR,) are in agreement with natural product (–)-**3**.

(+)-**Cryptolactone B₁** ((+)-**3**): colorless solid; mp 55–57°C; $[\alpha]_{\text{D}}^{21}$ +49.0 (*c* 0.99, CHCl_3). The NMR spectral data (¹H-NMR) of (+)-**3** were in agreement with (–)-**3**.

(–)-**Cryptolactone B₂** ((–)-**4**): colorless solid; mp 53–58°C; $[\alpha]_{\text{D}}^{23}$ –33.9 (*c* 0.85, CHCl_3); All spectral data (IR, Mass, HRMS, ¹H- and ¹³C-NMR,) are in agreement with natural product (–)-**4**.

(+)-**Cryptolactone B₂** ((+)-**4**): colorless solid; mp 54–57°C; $[\alpha]_{\text{D}}^{21}$ +37.0 (*c* 1.00, CHCl_3). The NMR spectral data (¹H-NMR) of (+)-**4** were in agreement with (–)-**4**.

Synthesis and Analytical Data for New Compounds (**5** and **6**)

(1*S*,3*RS*)-3-Hydroxy-5-oxo-1-(prop-2-enyl)undecanyl Acrylate (**7c**)

A solution of silyl enol ether **10c** (144mg, 0.72mmol) in dry CH_2Cl_2 (1.5mL) was mixed with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (67.0 μL , 0.53mmol) at –78°C; then, a solution of aldehyde (*S*)-**8** (81mg, 0.48mmol) in dry CH_2Cl_2 (1.6mL) was added dropwise to the mixture over 15min at –78°C. After adding a solution of pyridine (90 μL , 0.98mmol) and pyridinium *p*-toluenesulfonate (PPTS) (242mg, 0.96mmol) in dry CH_2Cl_2 (1.0mL), the mixture was stirred for 5min at –78°C, then treated with saturated aqueous NaHCO_3 solution (1.5mL, then 30mL). The resulting mixture was extracted with CH_2Cl_2 (3 $\times$ 20mL), 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 = 20 : 1, then 10 : 1 v/v%) to give a diastereomeric mixture of aldol adduct **7c** (ratio approx. 1 : 1.2) in 40% yield (57.6mg), as a pale yellow oil; chemical ionization mass spectrometry (CI-MS) *m/z* 297 [*M* + *H*]⁺; CI-HRMS *m/z* 297.2075 [*M* + *H*]⁺ (Calcd for $\text{C}_{17}\text{H}_{29}\text{O}_4$, 297.2066). The next the olefin metathesis reaction of diastereomeric mixture **7c** carried out promptly. The *ent*-**7c** was also prepared same reaction procedure as above (60%).

(–)-(*5S*,7*R*)-7-Hydroxy-9-oxopentadec-2-en-5-olide (**5**) and (–)-(*5S*,7*S*)-7-Hydroxy-9-oxopentadec-2-en-5-olide (**6**)

A solution of aldol adduct **7c** (57.6mg, 0.19mmol) in dry CH_2Cl_2 (1.5mL) was combined with the 2nd generation Grubbs catalyst (16.5mg, 0.019mmol), and the mixture was refluxed for 17h 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 (–)-**5** and (–)-**6** in 60% yield (34.4mg), as a brown solid. Each diastereomer was isolated by HPLC with a chiral-phase column [CHIRALPACK AD, 20 $\times$ 250mm, *n*-hexane–2-propanol (9 : 1), 8mL/min], with a UV (220nm) detector. The retention times of (–)-**5** and (–)-**6** were 74.4 and 62.5min, respectively.

(–)-(*5S*,7*R*)-7-Hydroxy-9-oxopentadec-2-en-5-olide ((–)-**5**): colorless solid; mp 46–49°C; $[\alpha]_{\text{D}}^{17}$ –57.6 (*c* 1.02, CHCl_3).

$^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 6.89 (1H, ddd, $J=9.8, 6.0, 2.6$ Hz), 6.03 (1H, ddd, $J=9.8, 2.6, 0.9$ Hz), 4.74 (1H, m), 4.39 (1H, brt, $J=9.5$ Hz), 3.40 (1H, s), 2.66 (1H, dd, $J=17.8, 2.7$ Hz), 2.53 (1H, dd, $J=17.8, 9.2$ Hz), 2.31–2.45 (4H, m), 1.82 (1H, ddd, $J=14.5, 9.0, 3.0$ Hz), 1.75 (1H, ddd, $J=14.5, 9.8, 3.2$ Hz), 1.57 (2H, quint, $J=7.3$ Hz), 1.26–1.31 (6H, m), 0.89 (3H, t, $J=7.0$ Hz); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 212.2, 164.2, 145.2, 121.4, 74.8, 63.7, 48.8, 43.5, 41.5, 31.5, 29.9, 28.8, 23.5, 22.4, 14.0; IR (ATR) cm^{-1} : 3424, 2926, 1710; CI-MS m/z : 269 ($[\text{M} + \text{H}]^+$), 251, 198; CI-HRMS m/z : 269.1754 ($[\text{M} + \text{H}]^+$) (Calcd for $\text{C}_{15}\text{H}_{25}\text{O}_4$ 269.1753).

(–)-(5*S*,7*S*)-7-Hydroxy-9-oxopentadec-2-en-5-olide ((–)-6): colorless solid; mp 31–32.5°C; $[\alpha]_{\text{D}}^{17} -46.3$ (c 1.00, CHCl_3). $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ : 6.90 (1H, dt, $J=9.8, 4.5$ Hz), 6.03 (1H, dt, $J=9.8, 1.8$ Hz), 4.72 (1H, m), 4.30 (1H, m), 3.35 (1H, s), 2.69 (1H, dd, $J=17.8, 4.1$ Hz), 2.64 (1H, dd, $J=17.8, 7.8$ Hz), 2.42–2.46 (4H, m), 2.02 (1H, ddd, $J=14.5, 8.1, 6.4$ Hz), 1.81 (1H, ddd, $J=14.5, 6.1, 3.9$ Hz), 1.58 (2H, brs), 1.25–1.31 (6H, m), 0.88 (3H, brt, $J=7.0$ Hz); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 212.4, 164.2, 145.3, 121.3, 75.4, 64.3, 48.5, 43.6, 40.5, 31.5, 29.1, 28.8, 23.5, 22.5, 14.0; IR (ATR) cm^{-1} : 3448, 2925, 1704; CI-MS m/z : 269 ($[\text{M} + \text{H}]^+$), 251, 198; CI-HRMS m/z : 269.1755 ($[\text{M} + \text{H}]^+$) (Calcd for $\text{C}_{15}\text{H}_{25}\text{O}_4$ 269.1753).

(+)-(5*R*,7*S*)-7-Hydroxy-9-oxopentadec-2-en-5-olide ((+)-5) and (+)-(5*R*,7*R*)-7-Hydroxy-9-oxopentadec-2-en-5-olide ((+)-6)

The antipodes of (–)-5 and (–)-6 were synthesized according to above experimental procedure.

(+)-(5*R*,7*S*)-7-Hydroxy-9-oxopentadec-2-en-5-olide ((+)-5): colorless solid; mp 46–48°C; $[\alpha]_{\text{D}}^{15} +55.6$ (c 1.00, CHCl_3). IR (ATR) cm^{-1} : 3432, 2925, 1707. The NMR spectral data (^1H - and ^{13}C -NMR) of (+)-5 were in agreement with (–)-5.

(+)-(5*R*,7*R*)-7-Hydroxy-9-oxopentadec-2-en-5-olide ((+)-6): colorless solid; mp 29–31°C; $[\alpha]_{\text{D}}^{13} +46.1$ (c 1.00, CHCl_3). IR (ATR) cm^{-1} : 3448, 2925, 1707. The NMR spectral data (^1H - and ^{13}C -NMR) of (+)-6 were in agreement with (–)-6.

MTT Assay for Cytotoxic Activity Human promyelocytic leukemia (HL-60) cells were grown in suspension culture in RPMI-1640 medium supplemented with 10% FBS and glutamine (2 mM) (standard medium). The cytotoxicities of **1–6** and their antipode (*ent*-**1–6**) for HL-60 cells were analyzed by the colorimetric MTT assay, with some modifications.²⁰ HL-60 cells (1×10^4) were plated in 96-well plates with 90 μL of standard medium at each well, mixed with 10 μL of serially-diluted test compound solutions of **1–6** and their antipode (*ent*-**1–6**), and then, incubated at 37°C in 5% CO_2 /95% air for 24 h. The final concentrations of **1–6** and their antipode (*ent*-**1–6**) in the sample wells ranged from 0.63–100 μM . After the 24 h incubation, the cells were mixed with 10 μL of MTT stock solution (5 mg/mL) and incubated for an additional 4 h at 37°C. Next, the cells were mixed with 100 μL of 20% sodium dodecyl sulfate in 0.01 N HCl and incubated for 12 h at room temperature 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% dimethyl sulfoxide (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 .²¹⁾

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Conflict of Interest The authors declare no conflict of interest.

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