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 (yellow and red pigments), viridaphin A₁ (green pigment), furanaphin (yellow pigment having fluorescence) and megouraphin glucosides (yellow pigments having fluorescence). These pigments exhibit interesting biological activities, such as cytotoxicity and antibacterial activity. We also accomplished total syntheses of two pigments, furanaphin and xanthouroleuconaphin.

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: yabusanazashi), and we obtained four colorless polyketides, cryptolactones A₁ (1), A₂ (2), B₁ (3), and B₂ (4) (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. 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.

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-
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. Since elongating the side chains with various lengths could be suitable for Mukaiyama aldol reaction with silyl enol ether,\textsuperscript{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.\textsuperscript{14–16}

Our synthesis commenced with preparation of common intermediate (S)-8 from (R)-9 as previously reported.\textsuperscript{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 BF\textsubscript{3}·OEt\textsubscript{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 chain\textsuperscript{17} (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 (7R) configuration were more cytotoxic than compounds 2, 4, and 6 with (7S) configuration, respectively. Thus, the cytotoxicity of compounds with anti-1,3-dioxo 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\textsubscript{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.\textsuperscript{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. \textsuperscript{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\textsubscript{3}. \textsuperscript{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\textsubscript{3}; δ\textsubscript{c} 77.0 ppm). Signal multiplicities were estab-

| Compounds | HL-60 (IC\textsubscript{50}, µM) |
|-----------|-----------------|
| 1         | 2.7             |
| 2         | 8.6             |
| 3         | 2.1             |
| 4         | 9.4             |
| 5         | 16.2            |
| 6         | 38.5            |
| ent-1     | 4.7             |
| ent-2     | 5.5             |
| ent-3     | 2.2             |
| ent-4     | 4.0             |
| ent-5     | 15.7            |
| ent-6     | 41.6            |

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| ent-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 chromatog-
raphy was conducted with silica gel 60N (Kanto Chemical
Co. Inc., Japan, 63–200 mm). Preparative TLC was performed
with a Merck pre-coated silica gel (60 RP-18 WF254S). Pre-
parative 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.

Triethylamine was purchased from Nacalai Tesque Inc.
(Japan). Dimethyl sulfoxide (DMSO), tetrahydrofuran (THF, dehydrated stabilizer free: super plus), and CH2Cl2 (dehydrated: super) were purchased from Kanto Chemical Co., Inc. Acryloyl chloride was purchased from Wako Pure Chemical
Corporation (Japan). Boron trifluoride etherate (BF3·Et2O) 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 re-
agents were used without further purification. Silyl enol ethers
10a–c (8,19) 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 Cryptomy-
zus 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, B2, and Their Antipodes Title compounds and their
antipodes were synthesized according to our previous syn-
thetic strategy in Chart 1.10

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

(−)-Cryptolactone A1, (−)-I: colorless solid; mp 41–45°C;
[α]20D −55.6 (c 0.63, CHCl3) (lit.10)[α]20D −53.5 (c 2.22, CHCl3);
All spectral data (IR, Mass, HRMS, 1H- and 13C-NMR,) are in
agreement with natural product (−)-I.

(−)-Cryptolactone A1, (−)-I: colorless solid; mp 43–45°C;
[α]20D +52.9 (c 0.99, CHCl3). The NMR spectral data (1H-NMR)
of (−)-I were in agreement with (−)-I.

(−)-Cryptolactone A2, (−)-II: colorless solid; mp 44–51°C;
[α]20D −44.4 (c 0.62, CHCl3) (lit.10)[α]20D −44.1 (c 0.42, CHCl3);
All spectral data (IR, Mass, HRMS, 1H- and 13C-NMR,) are in
agreement with natural product (−)-II.

(−)-Cryptolactone B1, (−)-I: colorless solid; mp 48–51°C;
[α]20D +42.4 (c 1.00, CHCl3). The NMR spectral data (1H-NMR)
of (−)-I of (−)-2 were in agreement with (−)-2.

(−)-Cryptolactone B1, (−)-I: colorless solid; mp 50–55°C;
[α]20D −48.4 (c 0.89, CHCl3) (lit.10)[α]20D −46.1 (c 0.71, CHCl3);
All spectral data (IR, Mass, HRMS, 1H- and 13C-NMR,) are in
agreement with natural product (−)-I.

(−)-Cryptolactone B1, (+)-3: colorless solid; mp 55–57°C;
[α]20D +49.0 (c 0.99, CHCl3). The NMR spectral data (1H-NMR)
of (−)-3 were in agreement with (−)-3.

(−)-Cryptolactone B2, (−)-I: colorless solid; mp 53–58°C;
[α]20D −33.9 (c 0.85, CHCl3); 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;
[α]20D +37.0 (c 1.00, CHCl3). The NMR spectral data (1H-NMR)
of (−)-4 were in agreement with (−)-4.

Synthesis and Analytic Data for New Compounds (5 and 6)

(1S,3RS)-3-Hydroxy-5-oxo-1-(prop-2-ynyl)undecanoyl Acrylate
(7e)

A solution of silyl enol ether 10e (144 mg, 0.72 mmol) in
dry CH2Cl2 (1.5 mL) was mixed with BF3·Et2O (67.0 µL,
0.53 mmol) at −78°C; then, a solution of aldehyde (5)-8 (81 mg,
0.48 mmol) in dry CH2Cl2 (1.6 mL) was added dropwise to the
mixture over 15 min at −78°C. After adding a solution of
pyridine (90 µL, 0.98 mmol) and pyridinium p-toluenesulfo-
nate (PPTS) (242 mg, 0.96 mmol) in dry CH2Cl2 (1.0 mL), the
mixture was stirred for 5 min at −78°C, then treated with
saturated aqueous NaHCO3 solution (1.5 mL, then 30 mL).
The resulting mixture was extracted with CH2Cl2 (3 × 20 mL),
and the combined organic extracts were dried over MgSO4,
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
7e (ratio approx. 1:1.2) in 40% yield (57.6 mg), 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
C19H25O3, 297.2066). The next the olefin metathesis reaction of
diastereomeric mixture 7e carried out promptly. The ent-7e
was also prepared same reaction procedure as above (60%).

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

A solution of aldol adduct 7e (57.6 mg, 0.19 mmol) in dry
CH2Cl2 (1.5 mL) was combined with the 2nd generation
Grubbs’ catalyst (16.5 mg, 0.019 mmol), and the mixture was
refluxed for 17 h under an Ar atmosphere. The resulting mixture
was concentrated and purified by silica gel column chromato-
graphy (n-hexane–EtOAc = 10:1, then 5:1, then 1:1) to
give a diastereomeric mixture of (−)-5 and (−)-6 in 60% yield
(34.4 mg), as a brown 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 (−)-5 and (−)-6
were 74.4 and 62.5 min, respectively.

(−)-(5S,7R)-7-Hydroxy-9-oxopentadec-2-en-5-olide (−)-5):
colorless solid; mp 46–49°C; [α]20D −57.6 (c 1.02, CHCl3).

(−)-(5S,7S)-7-Hydroxy-9-oxopentadec-2-en-5-olide (−)-5):
colorless solid; mp 46–49°C; [α]20D −57.6 (c 1.02, CHCl3).
$^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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, dd, $J = 14.5$, 9.8, 3.2 Hz), 1.57 (2H, quint, $J = 7.3$ Hz), 1.26–1.31 (6H, m), 0.89 (3H, t, $J = 7.3$ Hz), 1.81 (1H, ddd, $J = 13.2$, 9.9, 4.5 Hz), 2.28–2.33 (4H, m), 1.81 (1H, ddd, $J = 14.5$, 9.0, 2.6 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); $^1$C-NMR (125 MHz, CDCl$_3$) $\delta$: 212.2, 164.2, 145.2, 121.4, 75.4, 64.3, 48.5, 43.6, 40.5, 31.5, 29.1, 28.6, 23.5, 22.5, 14.0; IR (ATR) cm$^{-1}$: 3448, 2925, 1707; CI-MS m/z: 269 (M + H$^+$), 251, 198; CI-CHRMS m/z: 269.1754 ((M + H$^+$) $^2$)
(Calcld for C$_{15}$H$_{25}$O$_4$ 269.1753).

(−)-(5S,7S)-7-Hydroxy-9-oxopentadec-2-en-5-olide (−)−6: colorless solid; mp 31–32.5°C; [α]$_D^{20}$ = −46.3 (c 1.00, CHCl$_3$). $^1$H-NMR (500 MHz, CDCl$_3$) $\delta$: 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, ddd, $J = 17.8$, 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); $^1$C-NMR (125 MHz, CDCl$_3$) $\delta$: 212.2, 164.2, 145.3, 121.3, 75.4, 64.3, 48.5, 43.6, 40.5, 31.5, 29.1, 28.8, 23.5, 22.4, 14.0; IR (ATR) cm$^{-1}$: 3448, 2925, 1704; CI-MS m/z: 269 (M + H$^+$), 251, 198; CI-CHRMS m/z: 269.1755 ((M + H$^+$) $^2$) (Calcld for C$_{15}$H$_{25}$O$_4$ 269.1753).

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

(−)-(5R,7R)-7-Hydroxy-9-oxopentadec-2-en-5-olide (−)−5: colorless solid; mp 46–48°C; [α]$_D^{20}$ $^5$ = +55.6 (c 1.00, CHCl$_3$). IR (ATR) cm$^{-1}$: 3432, 2925, 1707. The NMR spectral data (1H and 13C-NMR) of (−)−5 were in agreement with (−)−5.

(−)-(5R,7R)-7-Hydroxy-9-oxopentadec-2-en-5-olide (−)−6: colorless solid; mp 29–31°C; [α]$_D^{20}$ $^6$ = +46.1 (c 1.00, CHCl$_3$). IR (ATR) cm$^{-1}$: 3448, 2925, 1707. The NMR spectral data (1H and 13C-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 following MTT assay, with some modifications. HL-60 cells (1 $\times$ 10$^5$) were plated in 96-well plates with 90 L of standard medium at each well, mixed with 10 $\mu$L 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 $\mu$L. After the 24 h incubation, the cells were mixed with 10 $\mu$L of MTT stock solution (5 mg/mL) and incubated for an additional 4 h at 37°C. Next, the cells were mixed with 100 $\mu$L 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 $\mu$M.$^{23}$