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Stereoselective Synthesis of 2α-Chloropicropodophyllotoxins and Insecticidal Activity of Their Esters against Oriental Armyworm, *Mythimna separata* Walker

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Supporting Information

ABSTRACT: As part of ongoing efforts to discover new natural-product-based insecticidal agents, in the present study, an efficient method for the stereoselective α-chlorination at the C-2 position of 2′(2′,6′)-(di)halogenopodophyllotoxin derivatives was first developed. Subsequently, a series of novel esters of 2α-chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin with modified C, D, and E rings of podophyllotoxin were smoothly obtained. Finally, all of the title compounds were tested against the pre-third-instar larvae of oriental armyworm (*Mythimna separata* Walker) at 1 mg/mL. It was found that besides their 2′-halogen-substituted E ring, the stereoselective α-chlorination at the C-2 position of 2′(2′,6′)-(di)halogenopodophyllotoxins was also related to the chlorination reagents. Especially 2α-chloro-4α-(benzoyl)oxy-2′-chloropicropodophyllotoxin (**6e**) and 2α-chloro-4α-(2-chlorophenylacyl)oxy-2′-bromopicropodophyllotoxin (**8f**) showed the most potent insecticidal activities, with final mortality rates of >60%. For 4α-(alkylacyl)oxy derivatives of 2α-chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin, the effect of the length of their side chain at the C-4 position of podophyllotoxin skeleton on the insecticidal activity was not very obvious. For 4α-(arylacyl)oxy derivatives of 2α-chloro-2′-chloro/bromopicropodophyllotoxin, an electronic effect of the substituents on their phenyl ring at the C-4 position of podophyllotoxin skeleton on the insecticidal activity was observed.

KEYWORDS: *podophyllotoxin, mortality rate, insecticidal activity, chemical modification, stereoselective chlorination, natural-product-based insecticide*

INTRODUCTION

The larvae of many lepidopteran species are major insect pests in agriculture, and their outbreaks can lead to a widespread incidence and complete crop loss.[1] Therefore, many synthetic agrochemicals have been applied in agriculture to control lepidopteran pests during the past decades. Of course, these chemical pesticides have played an important role in crop protection. However, resistance in lepidopteran pest populations and environmental problems have also occurred because of the repeated and increasing use of agrochemicals for a long time.[2−6] Meanwhile, due to plant secondary metabolites originating from the interaction between the plants and the environment (life and nonlife) during the long period of plant evolution, pesticides produced from plant secondary metabolites may cause less or slower resistance development and lower environmental pollution.[7] Consequently, the discovery of new pesticides directly or indirectly from plant secondary metabolites has recently been crucial in the research and development of agrochemicals.[8−14]

Podophyllotoxin (**1**, Figure 1), extracted and isolated from the roots and rhizomes of some *Podophyllum* and *Juniperus* species, has already been used as the lead compound for chemical modifications in the field of medicinal sciences.[15] For example, some antitumor drugs originating from compound **1**, for example, etoposide (VP-16), teniposide (VM-26), and etoposide phosphate, have been successfully applied in the clinic. On the other hand, compound **1** displayed interesting insecticidal and antifungal activities.[16,17] Therefore, total synthesis[18−23] and structural modifications[24−27] of **1** and its analogues have been carried out in many research groups. Recently, we have prepared a series of 2β-chloropodophyllotoxin[28,29] (**I**, Figure 1) and 4α-acyloxy-2′(2′,6′)-(di)-halogenopodophyllotoxin derivatives[30] (**II**, Figure 1) as insecticidal agents and found that some compounds exhibited more potent insecticidal activity than toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*. More importantly, it was observed that introduction of a chlorine atom at the C-2, C-2′, or C-2′,6′ position of podophyllotoxin was important for obtaining potent derivatives. Therefore, as shown in Figure 1, we herein designed another series of 2α/β-chloro-2′(2′,6′)-(di)-halogenopodophyllotoxin derivatives (**III**) by simultaneous introduction of halogen atoms at the C-2 and C-2′ (or C-2′,6′) positions of **1**. Their insecticidal activity was tested against oriental armyworm (*Mythimna separata* Walker), a typical lepidopteran pest.

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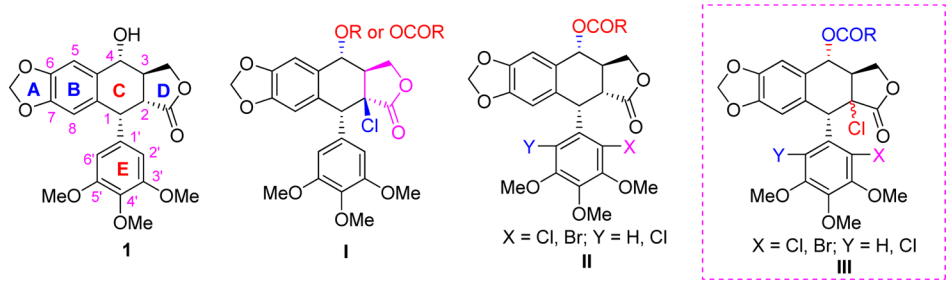

Figure 1. Chemical structures of podophyllotoxin (**1**) and its derivatives (**I**–**III**).

MATERIALS AND METHODS

General. All chemical reagents were commercially available, and solvents were purified with standard methods[31] before use. Analytical thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed with silica gel plates using silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd. Qingdao, China), and the solvent system was a mixture of ethyl acetate and petroleum ether. Melting points were carried out on an XT-4 digital melting-point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and were uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded in $CDCl_3$ on a Bruker Avance 400 or 500 MHz instrument using tetramethylsilane (TMS) as the internal standard. High-resolution mass spectra (HR-MS) were carried out with an IonSpec 4.7 T FTMS instrument. 2′-Chloropodophyllotoxin (**2a**, 85% yield), 2′,6′-dichloropodophyllotoxin (**2b**, 90% yield), and 2′-bromopodophyllotoxin (**2c**, 85% yield) were prepared in the same way as in our previous paper.[30]

Stereoselective Synthesis of 2α-Chloro-2′(2′,6′)-(di)-halogenopicropodophyllotoxin Derivatives (5a–c). Two drops of phosphorus oxychloride were added to a solution of **2a**, **2b**, or **2c** (1.8 mmol) in dihydropyran (10 mL). Then the mixture was stirred at room temperature. After 2 h, the mixture was poured into ice-cold petroleum ether (100 mL) and vigorously stirred. The resulting solids were collected and dissolved in CH_2Cl_2 (50 mL). The corresponding solution was washed by water (25 mL × 2), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography to give products **3a** (89% yield), **3b** (78% yield), or **3c** (85% yield). To a solution of lithium diisopropylamide (LDA, 0.6 mmol) in dry tetrahydrofuran (THF, 5 mL) at −78 °C under N_2 was then added dropwise a solution of **3a**, **3b**, or **3c** (0.5 mmol) in dry THF (5 mL) for 10 min. After the addition, the reaction mixture was stirred for 15 min, and a solution of hexachloroethane (C_2Cl_6, 0.7 mmol) in dry THF (5 mL) was added dropwise for 10 min. Subsequently, the solution was allowed to warm slowly from −78 °C to the ambient temperature. When the reaction was complete according to TLC analysis, the solvent was removed and the residue was diluted by CH_2Cl_2 (50 mL). The corresponding solution was washed by water (25 mL), 0.5 N aqueous HCl (25 mL), and water (25 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give the residue, which was used directly for the next step without further purification. A solution of the above residue in concentrated HCl/THF (1:9, v/v, 10 mL) was stirred for 2 h and then adjusted a pH value of 5 by using 5% aqueous Na_2CO_3. After removal of THF, the mixture was extracted with CH_2Cl_2 (40 mL × 3), and the combined organic phase was washed by 5% aqueous Na_2CO_3 (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography to give **5a** (68% yield), **5b** (62% yield), or **5c** (71% yield).

*Data for **5a**:* white solid; mp = 183–184 °C; $[\alpha]^{20}_D$ = −83 (*c* 3.1 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (s, 1H, H-5), 6.74 (s, 1H, H-8), 6.62 (s, 1H, H-6′), 5.92 (dd, *J* = 5.2, 1.2 Hz, 2H, OCH_2O), 5.48 (s, 1H, H-1), 4.90 (d, *J* = 2.8 Hz, 1H, H-4), 4.81 (dd, *J* = 9.2, 4.8 Hz, 1H, H-11), 4.47 (d, *J* = 9.2 Hz, 1H, H-11), 3.93 (s, 3H, 3′-$OCHH_3$), 3.88 (s, 3H, 5′-$OCHH_3$), 3.73 (s, 3H, 4′-$OCHH_3$), 3.14–3.16 (m, 1H, H-3), 2.47 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 152.1, 149.7, 148.7, 148.2, 142.5, 132.3, 129.7, 128.5, 121.5, 109.1, 107.4, 101.5, 73.5, 73.0, 67.0, 61.1, 61.0, 56.3, 50.7, 45.2; HRMS *m*/*z* calcd for $C_{22}H_{20}O_8Cl_2Na$ ([M + Na]$^+$) 505.0427, found 505.0428.

*Data for **5b**:* white solid; mp = 92–93 °C; $[\alpha]^{20}_D$ = −104 (*c* 3.9 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (s, 1H, H-5), 6.13 (s, 1H, H-8), 5.99 (s, 1H, H-1), 5.90 (dd, *J* = 7.2, 1.2 Hz, 2H, OCH_2O), 4.82 (dd, *J* = 8.8, 3.6 Hz, 1H, H-11), 4.62 (d, *J* = 9.2 Hz, 1H, H-11), 4.43 (d, *J* = 9.2 Hz, 1H, H-4), 3.98 (s, 3H, 3′-$OCHH_3$), 3.96 (s, 3H, 5′-$OCHH_3$), 3.82 (s, 3H, 4′-$OCHH_3$), 3.16 (dd, *J* = 9.6, 3.6 Hz, 1H, H-3), 1.58 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 149.4, 148.9, 147.7, 147.5, 147.3, 134.2, 129.6, 127.4, 127.2, 125.9, 107.6, 104.7, 101.3, 70.8, 69.0, 65.6, 61.3, 61.2, 61.1, 50.8, 44.1; HRMS *m*/*z* calcd for $C_{22}H_{19}O_8Cl_3Na$ ([M + Na]$^+$) 539.0037, found 538.0051.

*Data for **5c**:* white solid; mp = 170–172 °C; $[\alpha]^{20}_D$ = −94 (*c* 3.4 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (s, 1H, H-5), 6.74 (s, 1H, H-8), 6.64 (s, 1H, H-6′), 5.91 (dd, *J* = 5.2, 1.2 Hz, 2H, OCH_2O), 5.56 (s, 1H, H-1), 4.89 (d, *J* = 2.8 Hz, 1H, H-4), 4.81 (dd, *J* = 9.2, 4.8 Hz, 1H, H-11), 4.47 (d, *J* = 9.2 Hz, 1H, H-11), 3.92 (s, 3H, 3′-$OCHH_3$), 3.88 (s, 3H, 5′-$OCHH_3$), 3.72 (s, 3H, 4′-$OCHH_3$), 3.14 (t, *J* = 4.0 Hz, 1H, H-3), 2.53 (s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 152.8, 150.7, 148.6, 148.2, 142.4, 134.1, 129.8, 128.4, 113.1, 109.4, 108.9, 107.5, 101.5, 73.4, 73.1, 67.1, 61.0, 61.0, 56.4, 50.7, 47.8; HRMS *m*/*z* calcd for $C_{22}H_{20}O_8ClBrNa$ ([M + Na]$^+$) 548.9922, found 548.9906.

General Procedure for the Synthesis of 2α-Chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin Esters (6a–k, 7a–i, and 8a–h). A solution of compound **5a**, **5b**, or **5c** (0.15 mmol), the corresponding carboxylic acids RCO_2H (0.18 mmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 0.18 mmol), and 4-*N*,*N*-dimethylaminopyridine (DMAP, 0.03 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature. When the reaction was complete as checked by TLC analysis, the solution of the mixture was diluted with CH_2Cl_2 (40 mL). Subsequently, the solution was washed by water (15 mL), 0.1 N aqueous HCl (25 mL), 5% aqueous Na_2CO_3 (25 mL), and brine (25 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by PTLC to give the target products **6a–k**, **7a–i**, and **8a–h**. The example data of **6a–c**, **7a–c**, and **8a–c** are shown as follows, whereas data of **6d–k**, **7d–i**, and **8d–h** can be found in the Supporting Information.

*Data for **6a**:* yield 82%; white solid; mp = 150–151 °C; $[\alpha]^{20}_D$ = −78 (*c* 3.5 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.68 (s, 1H, H-5), 6.64 (s, 1H, H-8), 6.58 (s, 1H, H-6′), 5.93–5.95 (m, 3H, H-4, OCH_2O), 5.52 (s, 1H, H-1), 4.80–4.81 (m, 2H, H-11), 3.94 (s, 3H, 3′−$OCHH_3$), 3.88 (s, 3H, 5′-$OCHH_3$), 3.76 (s, 3H, 4′-$OCHH_3$), 2.97–2.98 (m, 1H, H-3), 2.16 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 170.6, 152.0, 149.9, 149.2, 148.2, 142.6, 132.1, 130.8, 123.9, 122.0, 108.8, 108.6, 108.3, 101.7, 75.1, 73.2, 66.8, 61.1, 61.1, 56.1, 49.3, 44.7, 21.1; HRMS *m*/*z* calcd for $C_{24}H_{22}O_9Cl_2Na$ ([M + Na]$^+$) 547.0533, found 547.0529.

*Data for **6b**:* yield 78%; white solid; mp = 179–180 °C; $[\alpha]^{20}_D$ = −80 (*c* 2.9 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (s, 1H, H-5), 6.62 (s, 1H, H-8), 6.57 (s, 1H, H-6′), 5.93–5.94 (m, 3H, OCH_2O, H-4), 5.51 (s, 1H, H-1), 4.80–4.81 (m, 2H, H-11), 3.93 (s, 3H, 3′-$OCHH_3$), 3.88 (s, 3H, 5′-$OCHH_3$), 3.75 (s, 3H, 4′-$OCHH_3$),

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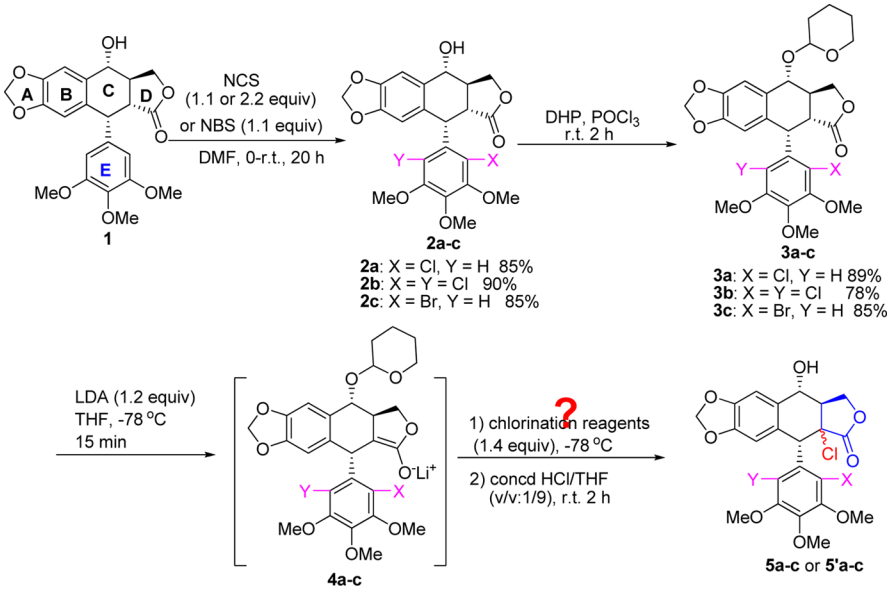
Scheme 1. Synthetic Approach for 2α-Chloropicropodophyllotoxin Derivatives (5a−c) and 2β-Chloropodophyllotoxin Derivatives (5a′−c′)



2.96−2.97 (m, 1H, H-3), 2.35−2.42 (m, 2H, CH_3CH_2), 1.20 (t, J = 7.6 Hz, 3H, CH_3CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 171.9, 152.1, 150.0, 149.2, 148.2, 142.8, 132.2, 130.7, 124.2, 122.1, 108.8, 108.6, 108.5, 101.7, 74.9, 73.1, 66.8, 61.1, 61.1, 56.2, 49.4, 44.7, 27.5, 9.0; HRMS m/z calcd for $C_{25}H_{24}O_9Cl_2Na$ ([M + Na]$^+$) 561.0689, found 561.0691.

Data for ***6c:*** yield 77%; white solid; mp = 79−80 °C; $[\alpha]^{19}_D$ = −66 (c 3.6 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27−7.39 (m, 5H), 6.67 (s, 1H, H-5), 6.57 (s, 1H, H-8), 6.51 (s, 1H, H-6′), 5.93 (d, J = 1.2 Hz, 2H, OCH_2O), 5.89 (d, J = 3.2 Hz, 1H, H-4), 5.50 (s, 1H, H-1), 4.77−4.78 (m, 2H, H-11), 3.93 (s, 3H, 3′-$OCHH_3$), 3.89 (s, 3H, 5′-$OCHH_3$), 3.75 (s, 3H, 4′-$OCHH_3$), 3.67 (d, J = 6.8 Hz, 2H, $PhCH_2$), 2.99−3.00 (m, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 171.6, 152.0, 150.0, 149.2, 148.2, 142.8, 132.6, 132.1, 130.6, 129.0, 128.9, 127.7, 123.9, 122.1, 108.7, 108.6, 108.4, 101.7, 75.5, 72.9, 66.7, 61.1, 61.1, 56.3, 49.2, 44.6, 41.1; HRMS m/z calcd for $C_{30}H_{26}O_9Cl_2Na$ ([M + Na]$^+$) 623.0846, found 623.0849.

Data for ***7a:*** yield 90%; white solid; mp = 92−93 °C; $[\alpha]^{20}_D$ = −86 (c 3.3 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.51 (s, 1H, H-5), 6.23 (s, 1H, H-8), 6.07 (s, 1H, H-1), 5.92−5.93 (m, 3H, OCH_2O, H-4), 4.76−4.78 (m, 1H, H-11), 4.57−4.59 (m, 1H, H-11), 3.98 (s, 3H, 3′-$OCHH_3$), 3.96 (s, 3H, 5′-$OCHH_3$), 3.83 (s, 3H, 4′-$OCHH_3$), 3.39−3.41 (m, 1H, H-3), 2.24 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 171.1, 149.3, 148.9, 148.1, 147.6, 147.4, 133.3, 127.5, 125.9, 125.5, 107.8, 105.7, 101.5, 71.2, 70.9, 64.9, 61.3, 61.3, 61.2, 48.9, 44.1, 21.1; HRMS m/z calcd for $C_{24}H_{21}O_9Cl_3Na$ ([M + Na]$^+$) 581.0143, found 581.0150.

Data for ***7b:*** yield 61%; white solid; mp = 84−86 °C; $[\alpha]^{20}_D$ = −107 (c 2.8 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.49 (s, 1H, H-5), 6.23 (s, 1H, H-8), 6.07 (s, 1H, H-1), 5.90−5.93 (m, 3H, OCH_2O, H-4), 4.75 (dd, J = 7.2, 3.2 Hz, 1H, H-11), 4.58 (d, J = 7.6 Hz, 1H, H-11), 3.98 (s, 3H, 3′-$OCHH_3$), 3.96 (s, 3H, 5′-$OCHH_3$), 3.83 (s, 3H, 4′-$OCHH_3$), 3.38 (dd, J = 5.6, 2.8 Hz, 1H, H-3), 2.49−2.52 (m, 2H, $COCH_2CH_3$), 1.23 (t, J = 6.0 Hz, 3H, $COCH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 172.8, 149.3, 149.0, 148.1, 147.6, 147.4, 133.3, 127.5, 126.1, 125.5, 107.8, 105.8, 101.4, 71.3, 70.9, 65.0, 61.3, 61.2, 61.1, 49.1, 44.1, 27.7, 9.1; HRMS m/z calcd for $C_{25}H_{23}O_9Cl_3Na$ ([M + Na]$^+$) 595.0299, found 595.0300.

Data for ***7c:*** yield 71%; white solid; mp = 78−80 °C; $[\alpha]^{20}_D$ = −95 (c 2.8 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.38 (m, 5H), 6.34 (s, 1H, H-5), 6.21 (s, 1H, H-8), 6.05 (s, 1H, H-1), 5.88 (dd, J = 10.0, 1.2 Hz, 2H, OCH_2O), 5.86 (d, J = 7.2 Hz, 1H, H-4), 4.65 (dd, J = 9.2, 4.0 Hz, 1H, H-11), 4.46 (d, J = 9.6 Hz, 1H, H-11), 3.97 (s, 3H, 3′-$OCHH_3$), 3.95 (s, 3H, 5′-$OCHH_3$), 3.83 (s, 3H, 4′-$OCHH_3$), 3.76 (s, 2H, $PhCH_2$), 3.31 (dd, J = 7.2, 3.6 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 171.6, 149.2, 148.9, 148.1, 147.6, 147.3, 133.2, 133.1, 129.2, 128.8, 127.6, 127.5, 127.5, 125.8, 125.5, 107.7, 105.8, 101.4, 71.6, 71.1, 64.9, 61.3, 61.2, 61.1, 49.0, 44.1, 41.5; HRMS m/z calcd for $C_{30}H_{25}O_9Cl_3Na$ ([M + Na]$^+$) 657.0456, found 657.0424.

Data for ***8a:*** yield 95%; white solid; mp = 176−178 °C; $[\alpha]^{20}_D$ = −94 (c 3.4 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (s, 1H, H-5), 6.63 (s, 1H, H-8), 6.60 (s, 1H, H-6′), 5.93−5.95 (m, 3H, OCH_2O, H-4), 5.59 (s, 1H, H-1), 4.80−4.81 (m, 2H, H-11), 3.92 (s, 3H, 3′-$OCHH_3$), 3.88 (s, 3H, 5′-$OCHH_3$), 3.76 (s, 3H, 4′-$OCHH_3$), 2.97 (dd, J = 5.2, 2.8 Hz, 1H, H-3), 2.15 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 170.6, 152.7, 150.9, 149.2, 148.2, 142.6, 133.9, 130.9, 123.9, 113.7, 108.8, 108.7, 108.6, 101.7, 75.1, 73.1, 66.8, 61.1, 61.0, 56.2, 49.4, 47.3, 21.0; HRMS m/z calcd for $C_{24}H_{22}O_9ClBrNa$ ([M + Na]$^+$) 591.0028, found 591.0028.

Data for ***8b:*** yield 93%; white solid; mp = 169−170 °C; $[\alpha]^{20}_D$ = −83 (c 3.2 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (s, 1H, H-5), 6.62 (s, 1H, H-8), 6.60 (s, 1H, H-6′), 5.93−5.94 (m, 3H, OCH_2O, H-4), 5.59 (s, 1H, H-1), 4.80−4.81 (m, 2H, H-11), 3.92 (s, 3H, 3′-$OCHH_3$), 3.88 (s, 3H, 5′-$OCHH_3$), 3.75 (s, 3H, 4′-$OCHH_3$), 2.96−2.99 (m, 1H, H-3), 2.35 (q, J = 7.6 Hz, 2H, $COCH_2CH_3$), 1.20 (t, J = 7.6 Hz, 3H, $COCH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 171.9, 152.7, 150.9, 149.2, 148.2, 142.7, 134.0, 130.8, 124.1, 113.7, 108.9, 108.7, 108.5, 101.7, 74.8, 73.1, 66.8, 61.1, 61.0, 56.2, 49.4, 47.2, 27.5, 9.0; HRMS m/z calcd for $C_{25}H_{24}O_9ClBrNa$ ([M + Na]$^+$) 605.0184, found 605.0169.

Data for ***8c:*** yield 98%; white solid; mp = 88−90 °C; $[\alpha]^{20}_D$ = −68 (c 3.6 mg/mL, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28−7.39 (m, 5H), 6.70 (s, 1H, H-5), 6.59 (s, 1H, H-8), 6.51 (s, 1H, H-6′), 5.94 (d, J = 0.8 Hz, 2H, OCH_2O), 5.88 (d, J = 3.2 Hz, 1H, H-4), 5.58 (s, 1H, H-1), 4.78−4.79 (m, 2H, H-11), 3.92 (s, 3H, 3′-$OCHH_3$), 3.88 (s, 3H, 5′-$OCHH_3$), 3.75 (s, 3H, 4′-$OCHH_3$), 3.67 (d, J = 7.2 Hz, 2H, $PhCH_2$), 2.99 (dd, J = 5.2, 3.2 Hz, 1H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 171.6, 152.7, 151.0, 149.2, 148.2, 142.7, 133.9, 132.6, 130.8, 129.0, 128.9, 127.7, 123.8, 113.8, 108.9, 108.6, 108.3, 101.7, 75.4, 72.9, 66.8, 61.09, 61.01, 56.3, 49.3, 47.2, 41.1; HRMS m/z calcd for $C_{30}H_{26}O_9ClBrNa$ ([M + Na]$^+$) 667.0341, found 667.0320.

Biological Assay. On the basis of the leaf-dipping method,[30] the biological activity of 2α-chloro-2′(2′,6′)-(di)-halogenopicropodophyllotoxin esters (**6a−k**, **7a−i**, and **8a−h**) was evaluated as the mortality rate values against *Mythimna separata* Walker. For each compound, 30 pre-third-instar larvae (10 larvae per

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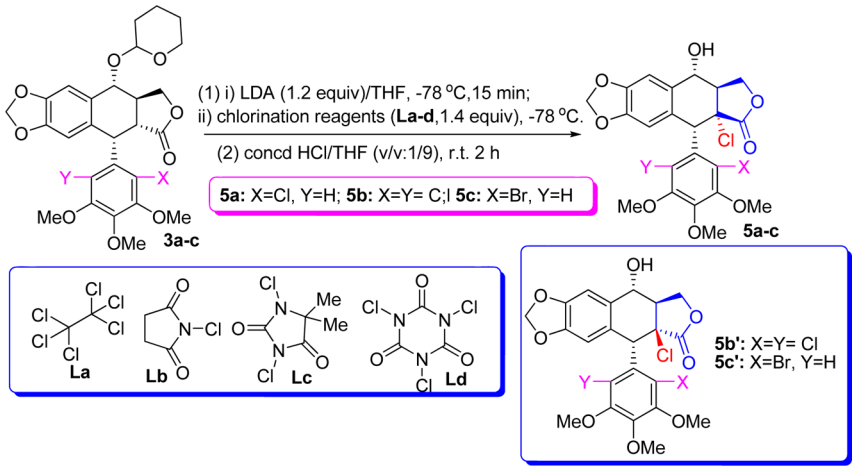
Table 1. Investigation of 3a−c Reacting with Different Chlorination Reagents (La−d)



entry	compd	chlorination reagent	T (°C)	t (h)	isolated yield (%) of **5**
1	**3a**	**La**	−78 to 23	16	**5a** (68)
2	**3a**	**Lb**	−78 to −18	3.5	**5a** (56)
3	**3a**	**Lc**	−78 to −39	2	**5a** (66)
4	**3a**	**Ld**	−78 to 27	24	**5a** (0)
5	**3b**	**La**	−78 to 26	18	**5b** (62)
6	**3b**	**Lb**	−78 to −23	3	**5b** (45) + **5b′** (14)[a]
7	**3b**	**Lc**	−78 to −40	2	**5b** (49) + **5b′** (21)[a]
8	**3b**	**Ld**	−78 to 27	24	**5b** (0)
9	**3c**	**La**	−78 to 17	12	**5c** (71)
10	**3c**	**Lb**	−78 to −21	3	**5c** (13) + **5c′** (44)[a]
11	**3c**	**Lc**	−78 to −33	2.5	**5c** (45) + **5c′** (20)[a]
12	**3c**	**Ld**	−78 to 27	24	**5c** (0)

[a]Determined by ^{1}H NMR spectra.

group) were used. Acetone solutions of compounds **6a−k**, **7a−i**, and **8a−h** were prepared at the concentration of 1 mg/mL. Toosendanin was used as the positive control at 1 mg/mL. Fresh wheat leaves were dipped into the corresponding solution for 3 s, then taken out, and dried in a room. Leaves treated with acetone alone were used as a blank control group. Several treated leaves were kept in each dish (10 larvae were raised in each dish, and three dishes were used per treatment), which was then placed in a conditioned room (25 ± 2 °C, 65−80% relative humidity (RH), 12 h/12 h (light/dark) photoperiod). If the treated leaves were consumed, additional treated leaves were added to the dish. Forty-eight hours later, untreated fresh leaves were added to all dishes until adult emergence. The corrected mortality rate was assessed by the formula

$$\text{corrected mortality rate (\%)} = (T - C) \times 100/(100\% - C)$$

where T is the mortality rate in the tested compounds group and C is the mortality rate in the blank control group (T and C were expressed as percentages).

RESULTS AND DISCUSSION

Synthesis. As shown in Scheme 1, first, three halogenation products of **1** such as 2′-chloropodophyllotoxin (**2a**), 2′,6′-dichloropodophyllotoxin (**2b**), and 2′-bromopodophyllotoxin (**2c**) were smoothly prepared by the reaction of **1** with *N*-chlorosuccinimide (NCS) or *N*-bromosuccinimide (NBS).[30] Second, the 4-hydroxy group of **2a−c** was protected by a tetrahydropyranyl (THP) group in the presence of phosphorus oxychloride ($POCl_3$) and dihydropyran (DHP) to produce **3a−c**. Subsequently, as described in Table 1, compounds **3a−c** reacted with lithium diisopropylamide (LDA) at −78 °C via the intermediates **4a−c** (lithium enolates), followed by reaction with four different chlorination reagents, such as hexachloroethane (C_2Cl_6, **La**), NCS (**Lb**), 1,3-dichloro-5,5-dimethylhydantoin (**Lc**), and 1,3,5-trichloroisocyanuric acid (**Ld**), were investigated. The chlorination of **3a−c** with **La** stereoselectively afforded 2α-chloropicropodophyllotoxins (**5a−c**) in 62−71% yields (entries 1, 5, and 9), and the chlorination of **3a** with **La−c** stereoselectively gave **5a** in 56−68% yields (entries 1−3). Although chlorination of **3a** with **Lb** or **Lc** stereoselectively produced **5a**, chlorination of **3b** and **3c** with **Lb** or **Lc** gave mixtures of two isomers, **5b**/**5b′** (2β-chloro-2′,6′-dichloropodophyllotoxin) and **5c**/**5c′** (2β-chloro-2′-bromopodophyllotoxin) (entries 6, 7, 10, and 11), respectively. The ratio of **5b**/**5b′** or **5c**/**5c′** was determined by the corresponding ^{1}H NMR spectra (see the Supporting Information). However, chlorination of **3a−c** with **Ld** did not afford any products except the starting materials, even if the reaction time was prolonged to 24 h (entries 4, 8, and 12). Obviously, chlorination reagent **La** could be used to stereoselectively prepare **5a−c**. In our previous paper, we found when **1** reacted with **La** in the presence of LDA, 2β-chloropodophyllotoxin was stereoselectively obtained.[28,29] According to the above results, this suggested that besides their 2′-halogen-substituted E ring, stereoselective α-chlorination at the C-2 position of **3a−c** was also related to the chlorination reagents. The structures of **5a−c** were well characterized by ^{1}H NMR, ^{13}C NMR, HRMS, optical rotation, and mp. Moreover, the precise three-dimensional steric structures of **5b** and **5c** were further ascertained by single-crystal X-ray diffraction (Figures 2 and 3). This obviously demonstrated that the C-2 chlorine atoms of **5b** and **5c** were clearly present in α configuration. The configuration of **5a** was

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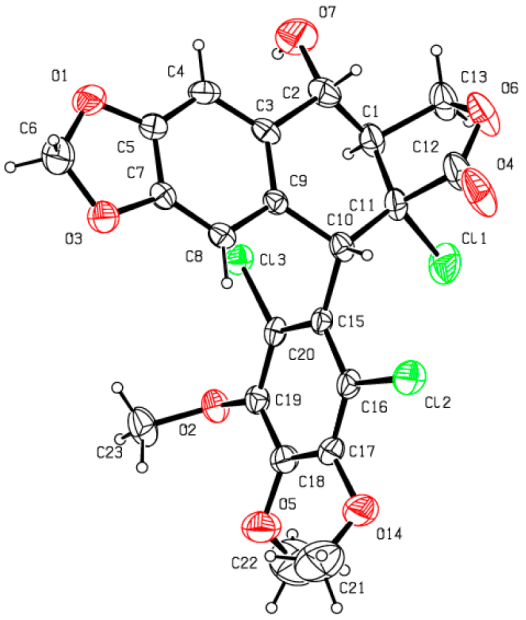


Figure 2. X-ray crystal structure of **5b**.

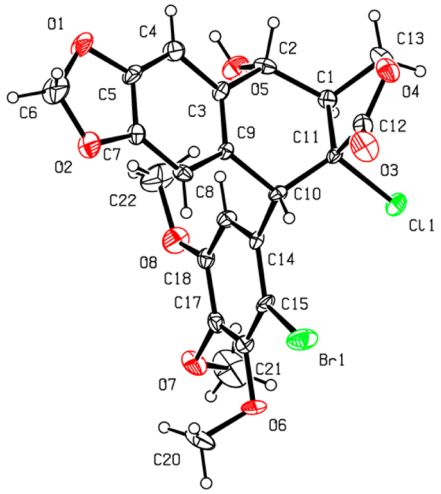


Figure 3. X-ray crystal structure of **5c**.

confirmed on the basis of the X-ray crystallography structure of its ester **6a** (see Figure 4), and the C-2 chlorine atom of **6a** adopted an α configuration. Therefore, the C-2 chlorine atom of **5a** should also adopt an α configuration. Finally, 2α-chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin esters (**6a**−**k**, **7a**−**i**, and **8a**−**h**) were afforded by reaction of **5a**, **5b**, or **5c** with the corresponding carboxylic acids (**9**) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-*N*,*N*-dimethylaminopyridine (DMAP) (Scheme 2). The structures of the title compounds were identified by ^{1}H NMR, ^{13}C NMR, HRMS, optical rotation, and mp. Crystallographic data (excluding structure factors) for the structures of three compounds, **5b**, **5c**, and **6a**, have been deposited at the Cambridge Crystallographic Data Centre with Data CCDC 918889, 918888, and 918696, respectively. These data can be obtained free of charge from the CCDC [fax +44 (0)1223 336033 or e-mail deposit@ccdc.cam.ac.uk].

Insecticidal Activity. The insecticidal activity of novel 2α-chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin esters (**6a**−**k**, **7a**−**i**, and **8a**−**h**) against the pre-third-instar larvae of *M.*

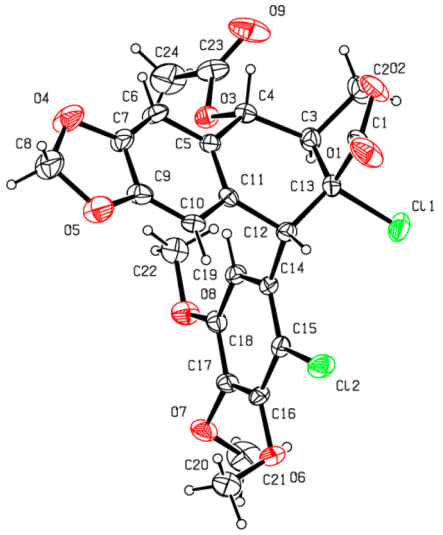
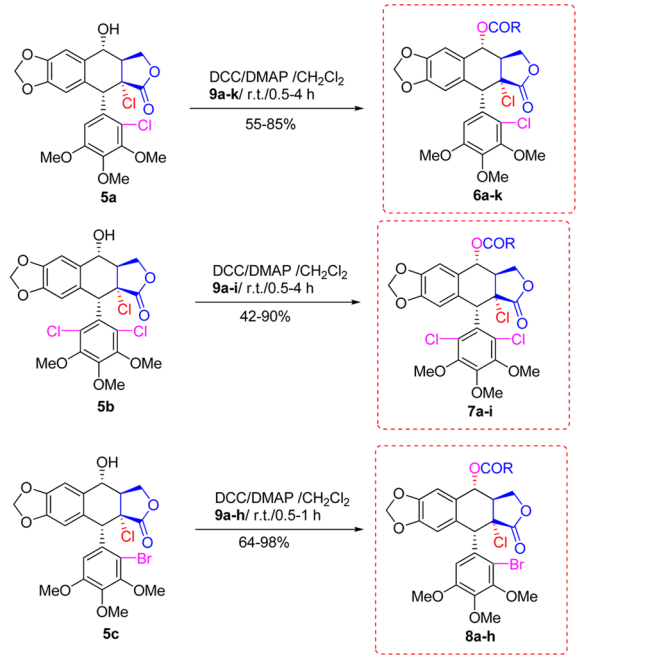


Figure 4. X-ray crystal structure of **6a**.

Scheme 2. Route for Synthesis of 2α-Chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin Esters (6a−k, 7a−i, and 8a−h)



separata was tested by the leaf-dipping method at 1 mg/mL. Toosendanin was used as the positive control at the concentration of 1 mg/mL. In general, we found that the mortality rates of *M. separata* caused by these derivatives after 35 days were higher than those after 10 and 20 days in the same way as in our previous studies,[28−30,32] As described in Table 2, foe **6e**, for instance, the corrected mortality rates against *M. separata* after 10 and 20 days were 10 and 43.3%, respectively, but after 35 days, the mortality rate was increased to 66.7%, which was >6 times that after 10 days. Meanwhile, the

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Table 2. Insecticidal Activity of 2α-Chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin Esters (6a−k, 7a−i, and 8a−h) against the Pre-third-Instar Larvae of *M. separata* on Leaves Treated with a Concentration of 1 mg/mL

	corrected mortality rate (%)		
compd	10 days	20 days	35 days
1	6.7 ± 3.3	30.0 ± 0	37.0 ± 3.3
2a	10.0 ± 0	36.7 ± 3.3	51.9 ± 0
2b	10.0 ± 5.8	36.7 ± 3.3	55.6 ± 0
2c	0 ± 0	20.0 ± 5.8	48.1 ± 3.3
5a	16.7 ± 3.3	33.3 ± 3.3	48.1 ± 3.3
5b	10.0 ± 0	23.3 ± 3.3	33.3 ± 5.8
5c	10.0 ± 5.8	23.3 ± 3.3	51.9 ± 3.3
6a	6.7 ± 3.3	13.3 ± 3.3	37.0 ± 3.3
6b	10.0 ± 5.8	33.3 ± 6.7	40.7 ± 3.3
6c	13.3 ± 3.3	23.3 ± 3.3	25.9 ± 3.3
6d	16.7 ± 3.3	30.0 ± 0	44.4 ± 5.8
6e	10.0 ± 5.8	43.3 ± 3.3	66.7 ± 5.8
6f	3.3 ± 4.7	40.0 ± 0	55.6 ± 0
6g	3.3 ± 3.3	20.0 ± 0	48.1 ± 3.3
6h	6.7 ± 3.3	26.7 ± 3.3	37.0 ± 3.3
6i	10.0 ± 5.8	30.0 ± 0	44.4 ± 5.8
6j	13.3 ± 3.3	40.0 ± 5.8	48.1 ± 3.3
6k	0 ± 0	26.7 ± 3.3	40.7 ± 3.3
7a	6.7 ± 3.3	20.0 ± 0	33.3 ± 5.8
7b	0 ± 0	20.0 ± 0	37.0 ± 3.3
7c	10.0 ± 5.8	36.7 ± 3.3	55.6 ± 5.8
7d	10.0 ± 8.2	26.7 ± 3.3	37.0 ± 3.3
7e	10.0 ± 5.8	33.3 ± 3.3	44.4 ± 5.8
7f	6.7 ± 6.7	23.3 ± 3.3	44.4 ± 0
7g	0 ± 0	36.7 ± 3.3	51.9 ± 3.3
7h	16.7 ± 3.3	33.3 ± 3.3	40.7 ± 3.3
7i	6.7 ± 3.3	36.7 ± 3.3	40.7 ± 3.3
8a	10.0 ± 0	40.0 ± 5.8	44.4 ± 0
8b	6.7 ± 3.3	26.7 ± 3.3	44.4 ± 0
8c	10.0 ± 0	26.7 ± 3.3	33.3 ± 5.8
8d	13.3 ± 6.7	30.0 ± 0	40.7 ± 3.3
8e	3.3 ± 3.3	40.0 ± 0	44.4 ± 5.8
8f	6.7 ± 3.3	53.3 ± 3.3	63.0 ± 3.3
8g	13.3 ± 3.3	30.0 ± 0	48.1 ± 3.3
8h	6.7 ± 3.3	23.3 ± 3.3	51.9 ± 3.3
toosendanin[a]	6.7 ± 3.3	36.7 ± 3.3	51.9 ± 3.3

[a]Toosendanin was used as a positive control at 1 mg/mL.

symptoms of *M. separata* in the treated groups were characterized; for example, at the larval stage some larvae with slim and wrinkled bodies were dying due to feeding too much on treated leaves during the first 48 h (Figure 5), some larvae were molting to malformed pupae or dying during the pupation period (Figure 6), and malformed moths were appearing with imperfect wings (Figure 7).[28−30,32] According to the above symptoms, the tested compounds probably exhibited the antimolting hormone effect. As shown in Table 2, nine compounds, **2a**, **2b**, **5c**, **6e**, **6f**, **7c**, **7g**, **8f**, and **8h**, displayed insecticidal activity equal to or higher than that of toosendanin. Among all of the derivatives, especially compounds **6e** and **8f** exhibited the most potent insecticidal activity with final mortality rates >60%. As in our previous studies,[29,30] introduction of a chlorine or bromine atom at the C-2′ or C-2′ and C-6′ position on the E ring or at the C-2 position of podophyllotoxin could lead to the more potent compounds **2a−c**, **5a**, and **5c** than their precursor podophyllotoxin, except

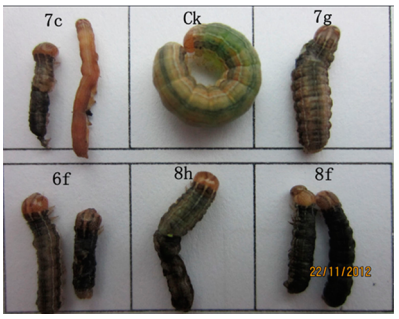

Figure 5. Representative abnormal larvae pictures of **6f**, **7c**, **7g**, **8f**, and **8h** during the larval period (CK, blank control group).

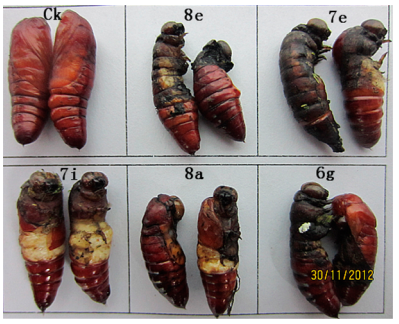

Figure 6. Representative malformed pupae pictures of **6g**, **7e**, **7i**, **8a**, and **8e** during the pupation period (CK, blank control group).

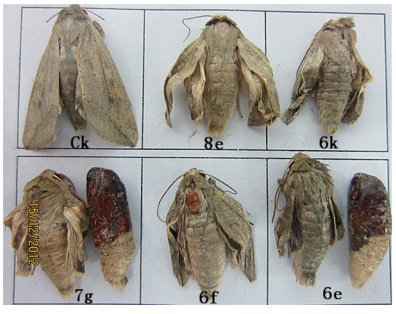

Figure 7. Representative malformed moth pictures of **6e**,**6f**,**6k**, **7g**, and **8e** during the emergence period (CK, blank control group).

5b. For 4α-(alkylacyl)oxy derivatives of **5a−c**, the effect of the length of their side chain at the C-4 position of the podophyllotoxin skeleton on the insecticidal activity was not very obvious (e.g., 37% for **6a**, 40.7% for **6b**, and 40.7% for **6k**; 33.3% for **7a** and 37% for **7b**; 44.4% for **8a** and 44.4% for **8b**). However, for 4α-(arylacyl)oxy derivatives of **5a**, introduction of the electron-withdrawing groups on their phenyl ring at the C-4 position of podophyllotoxin skeleton could lead to the potent compounds. For example, the final mortality rates of **6e** (R = Ph), **6f** (R = 2-ClPh), and **6g** (R = 3-ClPh) were 66.7, 55.6, and 48.1%, respectively, whereas the final mortality rate of **6h** (R = 3-MePh) was only 37%. For 4α-(arylacyl)oxy derivatives of **5c**, introduction of the electron-withdrawing or electron-denoting substituents on their phenyl ring at the C-4 position of podophyllotoxin skeleton could lead to the more potent compounds. For example, the final mortality rates of **8f** (R = 2-ClPh), **8g** (R = 3-ClPh), and **8h** (R = 3-MePh) were 63, 48.1, and 51.9%, respectively, whereas the final mortality rate of **8e** (R = Ph) was 44.4%. However, introduction of the (heterocyclylacyl)oxy group at the C-4 position of **5a** or **5b**

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could not afford the more potent compounds (e.g., 44.4% for **6i** and 48.1% for **6j**; 40.7% for **7i**). In our previous paper, we found that introduction of a (1-naphthylacetyl)oxy group at the C-4 position of the corresponding podophyllotoxin derivatives led to the more potent compounds,[29,30] but herein introduction of a (1-naphthylacetyl)oxy group at the C-4 position of **5a**−**c** did not result in the potent compounds. For example, the final mortality rates of **6d**, **7d**, and **8d** were only 44.4, 37, and 40.7%, respectively, so the insecticidal activity of **6d**, **7d**, and **8d** was also related to the chemical structures of their precursors.

In summary, a series of novel esters of 2α-chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin with modified C, D, and E rings of podophyllotoxin were prepared. In the meantime, an efficient method for the stereoselective α-chlorination at the C-2 position of 2′(2′,6′)-(di)halogenopodophyllotoxin derivatives in the presence of hexachloroethane (C_2Cl_6) was developed. Three steric structures of compounds **5b**, **5c**, and **6a** were determined by single-crystal X-ray diffraction. Their insecticidal activity was evaluated against pre-third-instar larvae of the oriental armyworm, *M. separata* (Walker), in vivo at a concentration of 1 mg/mL. This demonstrated that besides their 2′-halogen-substituted E ring, the stereoselective α-chlorination at the C-2 position of 2′(2′,6′)-(di)-halogenopodophyllotoxins was also related to the chlorination reagents. Among all of the derivatives, 2α-chloro-4α-(benzoyl)-oxy-2′-chloropicropodophyllotoxin (**6e**) and 2α-chloro-4α-(2-chlorophenylacyl)oxy-2′-bromopicropodophyllotoxin (**8f**) showed the most potent insecticidal activities with final mortality rates of >60%. For 4α-(alkylacyl)oxy derivatives of 2α-chloro-2′(2′,6′)-(di)halogenopicropodophyllotoxin, the effect of the length of their side chain at the C-4 position of podophyllotoxin skeleton on the insecticidal activity was not very obvious. For 4α-(arylacyl)oxy derivatives of 2α-chloro-2′-chloro/bromopicropodophyllotoxin, the electronic effect of the substituents on their phenyl ring at the C-4 position of podophyllotoxin skeleton on the insecticidal activity was observed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Data on ^{1}H NMR, ^{13}C NMR, optical rotation, and melting point for the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

‖L.F. and Y.G. contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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