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Synthesis of Novel 4α-(Acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin Derivatives as Insecticidal Agents

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Supporting Information

ABSTRACT: In continuation of our program aimed at the discovery and development of natural-product-based insecticidal agents, we have prepared three series of novel 4α-(acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin derivatives modified in the C and E rings of podophyllotoxin, which is a naturally occurring aryltetralin lignan isolated from the roots and rhizomes of *Podophyllum hexandrum*. Their structures were well characterized by ^{1}H NMR, HRMS, ESI-MS, optical rotation, and mp. The stereochemical configurations of compounds **5s**, **6b**, **6d**, and **7q** were unambiguously confirmed by single-crystal X-ray diffraction. Their insecticidal activity was evaluated against the pre-third-instar larvae of oriental armyworm, *Mythimna separata* Walker, in vivo at a concentration of 1 mg/mL. These derivatives likely displayed the antimolting hormone effect. Among all the derivatives, especially compounds **5a**, **5n**, **7f**, **7n**, and **7w** exhibited the most potent insecticidal activity with final mortality rates of 70% or so. This suggested that a chlorine or bromine atom introduced at the C2′ or C2′ and C6′ positions on the E ring of podophyllotoxin was necessary for obtaining the potent compounds. This will pave the way for further design, structural modification, and development of podophyllotoxin derivatives as insecticidal agents.

KEYWORDS: *podophyllotoxin, acyloxy, halogenation, botanical insecticide, insecticidal activity, Mythimna separata Walker*

INTRODUCTION

Lepidoptera is one of the most widespread and widely recognizable insect orders in the world. The larvae of many lepidopteran species are major pests in agriculture and can cause extensive damage to certain crops, especially new plants.[1] For example, the oriental armyworm (*Mythimna separata* Walker), a typical lepidopteran pest, is widely distributed in China, Japan, Southeast Asia, India, eastern Australia, New Zealand, and some Pacific Islands, and sometimes its outbreaks result in widespread incidence and complete crop loss.[2] Although a wide variety of synthetic insecticides were used to control lepidopteran pests, repeat application of those agrochemicals over years has led to the development of resistance in lepidopteran pest populations and environmental problems.[3−6] On the other hand, plant secondary metabolites result from the interaction between plants and the environment (life and nonlife) during the long period of evolution in plants, and pesticides produced from plant secondary metabolites may result in less or slower resistance development in pest populations and lower environmental pollution.[7] Consequently, the discovery and development of new insecticidal compounds directly from plant secondary metabolites, or by using them as lead compounds for further structural modifications, have recently been an important area of research and development of new pesticides.[8−11] Nowadays, some botanical insecticides, such as nicotine, pyrethrum, and neem extracts, are made by plants as defenses against insect pests.[12]

Podophyllotoxin (**1**; Figure 1), a naturally occurring aryltetralin lignan, is isolated from the roots and rhizomes of *Podophyllum hexandrum* and some *Juniperus* species.[13] Besides its use as the lead compound for the preparation of potent anticancer drugs such as etoposide, teniposide, and etopophos,[14−17] compound **1** has also received much research attention for its interesting insecticidal and antifungal activities.[18−23] More recently, we have investigated the insecticidal activity of a series of 2β-chloropodophyllotoxin (**I**; Figure 1) and 2α/β-bromopodophyllotoxin (**II** and **III**; Figure 1) derivatives with modified C and D rings of **1**[24−28] and 4-(acyloxy)podophyllotoxin derivatives (**IV**; Figure 1) with modified A and C rings of **1**.[29] Some compounds showed more potent insecticidal activity than toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*. Encouraged by the above-mentioned interesting results, and to find novel natural-product-based insecticidal agents to control the lepidopteran pests, we herein designed and synthesized three series of novel 4α-(acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (**V**; Figure 1) by introduction of the halogen atom at the C2′ or C2′ and C6′ positions on the E ring as insecticidal agents against the pre-third-instar larvae of oriental armyworm, *M. separata* Walker, in vivo. Additionally, their structure−activity relationship (SAR) studies were also described.

MATERIALS AND METHODS

General Procedures. Podophyllotoxin was purchased from Gansu Gerui Medicinal Materials Co., Ltd. All reagents and solvents were of reagent grade or purified according to standard methods 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.). Silica gel

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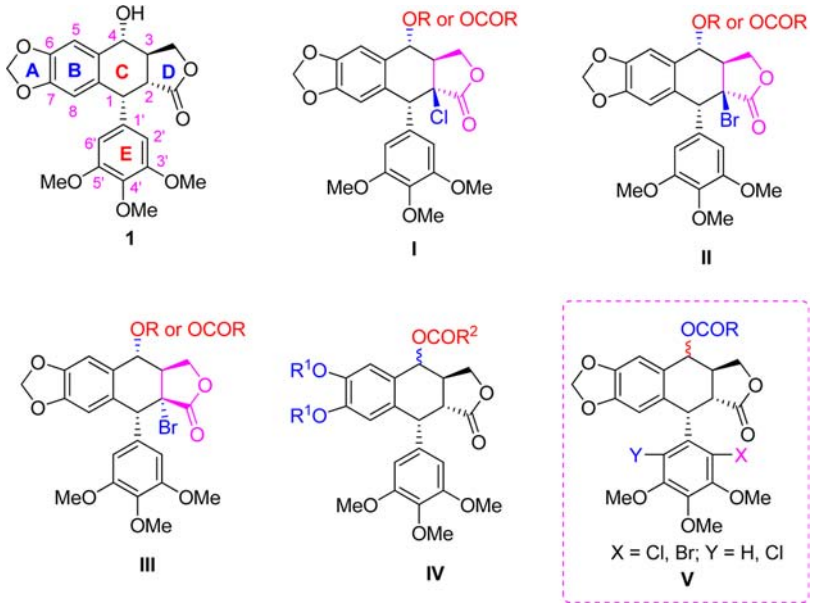

Figure 1. Chemical structures of podophyllotoxin (**1**) and its derivatives (**I**–**V**).

column chromatography was performed with silica gel 200–300 mesh (Qingdao Haiyang Chemical Co., Ltd.). Melting points were determined on a digital melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Bruker Avance III 500 MHz instrument in $CDCl_3$ using tetramethylsilane (TMS) as the internal standard. High-resolution mass spectrometry (HR-MS) and electrospray ion trap mass spectrometry (ESI-MS) were carried out with an IonSpec 4.7 T FTMS instrument and a Bruker ESI-TRAP Esquire 6000 plus mass spectrometry instrument, respectively.

Synthesis of 2′-Chloropodophyllotoxin (2). To a solution of **1** (0.4 mmol) in dry DMF (4 mL) at 0 °C was added dropwise for 10 min a solution of *N*-chlorosuccinimide (NCS; 62.6 mg, 0.46 mmol) in dry DMF (4 mL). During 1 h, the solution was allowed to warm slowly from 0 to 28 °C. When the reaction was complete, checked by TLC analysis, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (30 mL × 3). Subsequently, the combined organic phase was washed by saturated aqueous Na_2CO_3 (30 mL × 3) and brine (30 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (1:2, v/v) to afford **2**: white solid; yield 79%; mp 116–117 °C; $[\alpha]_D^{20}$ = −63 (*c* 3.8 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (s, 1H, H-5), 6.37 (s, 1H, H-8), 6.19 (s, 1H, H-6′), 5.94 (s, 2H, OCH_2O), 5.20 (d, *J* = 4.0 Hz, 1H, H-1), 4.79 (d, *J* = 8.5 Hz, 1H, H-4), 4.66 (dd, *J* = 8.5, 6.5 Hz, 1H, H-11), 4.10 (t, *J* = 9.0 Hz, 1H, H-11), 3.91 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 2.93–2.96 (m, 2H, H-2, 3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 151.6, 150.1, 147.9, 147.6, 142.8, 133.6, 132.6, 131.4, 110.6, 109.7, 105.6, 101.5, 72.0, 70.9, 61.1, 61.1, 56.5, 44.2, 41.9; MS (ESI) *m*/*z* (rel intens) 466 ([M + NH_4]$^+$, 100); HRMS *m*/*z* calcd for $C_{22}H_{25}NO_8Cl$ ([M + NH_4]$^+$) 466.1263, found 466.1270.

Synthesis of 2′,6′-Dichloropodophyllotoxin (3). To a solution of **1** (0.4 mmol) in dry DMF (4 mL) at 0 °C was added dropwise for 10 min a solution of NCS (109 mg, 0.8 mmol) in dry DMF (4 mL). During 1 h, the solution was allowed to warm slowly from 0 to 28 °C. When the reaction was complete, checked by TLC analysis, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (30 mL × 3). Subsequently, the combined organic phase was washed by saturated aqueous Na_2CO_3 (30 mL × 3) and brine (30 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (1:2, v/v) to afford **3**: white solid; yield 90%; mp 159–160 °C; $[\alpha]_D^{20}$ = −30 (*c* 3.3 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.15 (s, 1H, H-5), 6.31 (s, 1H, H-8), 5.94 (d, *J* = 0.5 Hz, 2H, OCH_2O), 5.52 (d, *J* = 8.5 Hz, 1H, H-1), 4.77 (d, *J* = 10.0 Hz, 1H, H-4), 4.66 (dd, *J* = 8.5 Hz, 7.0 Hz, 1H, H-11), 4.08 (dd, *J* = 10.5, 9.0 Hz, 1H, H-11), 3.95 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.49–3.53 (m, 1H, H-3), 3.01 (dd, *J* = 15, 8.5 Hz, 1H, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8, 149.4, 149.0, 147.8, 147.4, 147.3, 132.6, 130.9, 130.2, 129.1, 124.2, 107.8, 105.8, 101.4, 72.5, 70.9, 61.3, 61.2, 61.1, 43.9, 43.1, 39.3; MS (ESI) *m*/*z* (rel intens) 505 ([M + Na]$^+$, 100), 507 ([M + Na]$^+$, 73), 509 ([M + Na]$^+$, 22); HRMS *m*/*z* calcd for $C_{22}H_{24}NO_8Cl_2$ ([M + NH_4]$^+$) 500.0873, found 500.0868.

Synthesis of 2′-Bromopodophyllotoxin (4). To a solution of **1** (83 mg, 0.2 mmol) in dry DMF (2 mL) at 0 °C was added dropwise for 10 min a solution of *N*-bromosuccinimide (NBS) (41.6 mg, 0.23 mmol) in dry DMF (2 mL). During 1 h, the solution was allowed to warm slowly from 0 to 16 °C. When the reaction was complete, checked by TLC analysis, the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (30 mL × 3). Subsequently, the combined organic phase was washed by saturated aqueous Na_2CO_3 (30 mL × 3) and brine (30 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (1:1, v/v) to afford **4**: CAS no. 40456-15-3; white solid; yield 88%; mp 140–141 °C (lit.[30] mp not reported); $[\alpha]_D^{20}$ = −42 (*c* 3.5 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (s, 1H, H-5), 6.36 (s, 1H, H-8), 6.20 (s, 1H, H-6′), 5.94 (s, 2H, OCH_2O), 5.27 (s, 1H, H-1), 4.78 (d, *J* = 9.0 Hz, 1H, H-4), 4.63–4.66 (m, 1H, H-11), 4.10 (t, *J* = 9.0 Hz, 1H, H-11), 3.91 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 2.91–2.99 (m, 2H, H-2, 3), 2.52 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 152.2, 150.9, 147.9, 147.6, 142.6, 135.6, 132.4, 131.5, 114.5, 110.5, 109.6, 105.6, 101.5, 71.8, 70.9, 61.0, 56.4, 44.1, 41.8, 41.7; MS (ESI) *m*/*z* (rel intens) 515 ([M + Na]$^+$, 100), 517 ([M + Na]$^+$, 98).

General Procedure for the Synthesis of 5a–o,s,w,a′,b′, 6b,d–g,i,k,l, 7a–d,f,j–w,a′, and 8b,d,k,l. A mixture of **2**, **3**, **4**, or **1** (0.2 mmol), the corresponding acids RCO_2H (0.24 mmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC; 0.24 mmol), and 4-(*N*,*N*-dimethylamino)pyridine (DMAP; 0.04 mmol) in dry CH_2Cl_2 (10 mL) was stirred at 28 °C. When the reaction was complete according to TLC analysis, the mixture was filtered, and the filtrate was collected and diluted by CH_2Cl_2 (40 mL). Subsequently, the mixture was washed by 0.1 N HCl (25 mL), saturated aqueous $NaHCO_3$ (25 mL), and brine (25 mL), dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by PTLC to give the pure target products **5a–o,s,w,a′,b′**, **6b,d–g,i,k,l**, **7a–d,f,j–w,a′**, and **8b,d,k,l**. The example data

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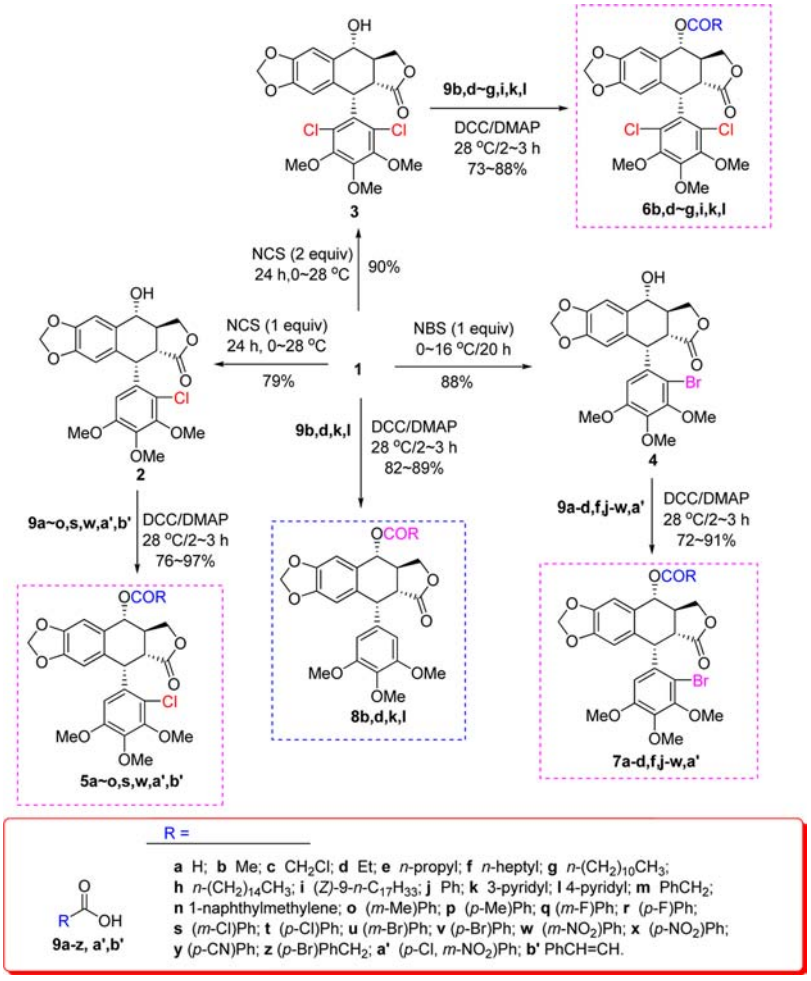
Scheme 1. Route for the Synthesis of 4α-(Acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin Derivatives 5a−o,s,w,a′,b, 6b,d-g,i,k,l, and 7a−d,f,j-w,a′



for **5a,b**, **6b,d**, **7a,b**, and **8b,d** are shown as follows, whereas data for **5c−o,s,w,a′,b′**, **6e−g,i,k,l**, **7c,d,f,j−w,a′**, and **8k,l** can be found in the Supporting Information.

Data for ***5a:*** yield 85%; white solid; mp 94−95 °C; $[\alpha]_D^{20}$ = +48 (*c* 3.3 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H, CHO), 6.77 (s, 1H, H-5), 6.42 (s, 1H, H-8), 6.21 (s, 1H, H-6′), 6.06 (d, *J* = 9.5 Hz, 1H, H-4), 5.96 (s, 2H, OCH_2O), 5.25 (d, *J* = 5.0 Hz, 1H, H-1), 4.46 (dd, *J* = 9.0, 7.0 Hz, 1H, H-11), 4.18−4.22 (m, 1H, H-11), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.13−3.16 (m, 1H, H-3), 3.01−3.05 (m, 1H, H-2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 160.9, 151.5, 150.2, 148.4, 147.6, 142.8, 132.8, 132.5, 126.9, 122.7, 110.4, 109.7, 106.2, 101.6, 72.7, 70.6, 61.1, 61.0, 56.2, 44.3, 39.3; MS (ESI-TRAP) *m*/*z* (rel intens) 499 ($[M + Na]^+$, 100), 501 ($[M + Na]^+$, 43); HRMS *m*/*z* calcd for $C_{23}H_{25}NO_9Cl$ ($[M + NH_4]^+$) 494.1212, found 494.1222.

Data for ***5b:*** yield 89%; white solid; mp 246−247 °C; $[\alpha]_D^{20}$ = +59 (*c* 3.3 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.74 (s, 1H, H-5), 6.41 (s, 1H, H-8), 6.21 (s, 1H, H-6′), 5.95 (d, *J* = 2.5 Hz, 2H, OCH_2O), 5.92 (d, *J* = 9.5 Hz, 1H, H-4), 5.23 (d, *J* = 4.5 Hz, 1H, H-1), 4.44 (dd, *J* = 9.0, 6.5 Hz, 1H, H-11), 4.17−4.21 (m, 1H, H-11), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 2.99−3.07 (m, 2H, H-2, 3), 2.23 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 171.2, 151.4, 150.1, 148.2, 147.5, 142.8, 133.0, 132.3, 127.7, 122.7, 110.5, 109.7, 106.2, 101.6, 73.0, 70.7, 61.1, 61.0, 56.3, 44.4, 39.5, 21.1; HRMS *m*/*z* calcd for $C_{24}H_{27}NO_9Cl$ ($[M + NH_4]^+$) 508.1369, found 508.1361.

Data for ***6b:*** yield 88%; white solid; mp 191−192 °C; $[\alpha]_D^{20}$ = −26 (*c* 4.5 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (s, 1H, H-5), 6.34 (s, 1H, H-8), 5.94−5.96 (m, 3H, H-4 and OCH_2O), 5.54 (d, *J* = 8.5 Hz, 1H, H-1), 4.41 (t, *J* = 8.0 Hz, 1H, H-11), 4.13−4.17 (m, 1H, H-11), 3.95 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.60−3.64 (m, 1H, H-3), 3.11 (dd, *J* = 15.0, 8.5 Hz, 1H, H-2), 2.23 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 171.5, 149.4, 149.0, 148.0, 147.4, 147.1, 130.8, 130.7, 129.1, 127.9, 124.2, 107.8, 106.1, 101.4, 73.0, 70.6, 61.2, 61.1, 61.0, 43.0, 41.6, 39.0, 21.1; HRMS *m*/*z* calcd for $C_{24}H_{26}NO_9Cl_2$ ($[M + NH_4]^+$) 542.0979, found 542.0971.

Data for ***6d:*** yield 78%; white solid; mp 190−191 °C; $[\alpha]_D^{20}$ = −27 (*c* 3.3 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.74 (s, 1H, H-5), 6.34 (s, 1H, H-8), 5.94−5.97 (m, 3H, H-4 and OCH_2O), 5.54 (d, *J* = 8.0 Hz, 1H, H-1), 4.36−4.39 (m, 1H, H-11), 4.17 (t, *J* = 10.0 Hz, 1H, H-11), 3.95 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.59−3.63 (m, 1H, H-3), 3.11 (dd, *J* = 15.0, 8.5 Hz, 1H, H-2), 2.47−2.53 (m, 2H, CH_2CH_3), 1.26 (t, *J* = 7.5 Hz, 3H, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.9, 172.2, 149.4, 149.0, 147.9, 147.4, 147.0, 130.8, 130.7, 129.1, 128.0, 124.2, 107.8, 106.1, 101.4, 72.8, 70.7, 61.2, 61.1, 61.0, 43.1, 41.6, 39.0, 27.7, 9.2; HRMS *m*/*z* calcd for $C_{25}H_{28}NO_9Cl_2$ ($[M + NH_4]^+$) 556.1136, found 556.1130.

Data for ***7a:*** yield 78%; white solid; mp 98−99 °C; $[\alpha]_D^{20}$ = −52 (*c* 4.1 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H, CHO), 6.77 (s, 1H, H-5), 6.42 (s, 1H, H-8), 6.23 (s, 1H, H-6′), 6.05 (d, *J* = 9.5 Hz, 1H, H-4), 5.96 (s, 2H, OCH_2O), 5.32 (s, 1H, H-1), 4.46 (dd, *J* = 9.0, 7.0 Hz, 1H, H-11), 4.21 (t, *J* = 9.5 Hz, 1H, H-11), 3.91 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.14−3.18 (m, 1H, H-3), 3.05 (dd, *J* = 15.0, 6.0 Hz, 1H, H-2); HRMS *m*/*z* calcd for $C_{23}H_{21}O_9Br$ ($[M]^+$) 520.0363, found 520.0366.

Data for ***7b:*** yield 73%; white solid; mp 234−235 °C; $[\alpha]_D^{20}$ = −48 (*c* 3.6 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.74 (s, 1H, H-5),

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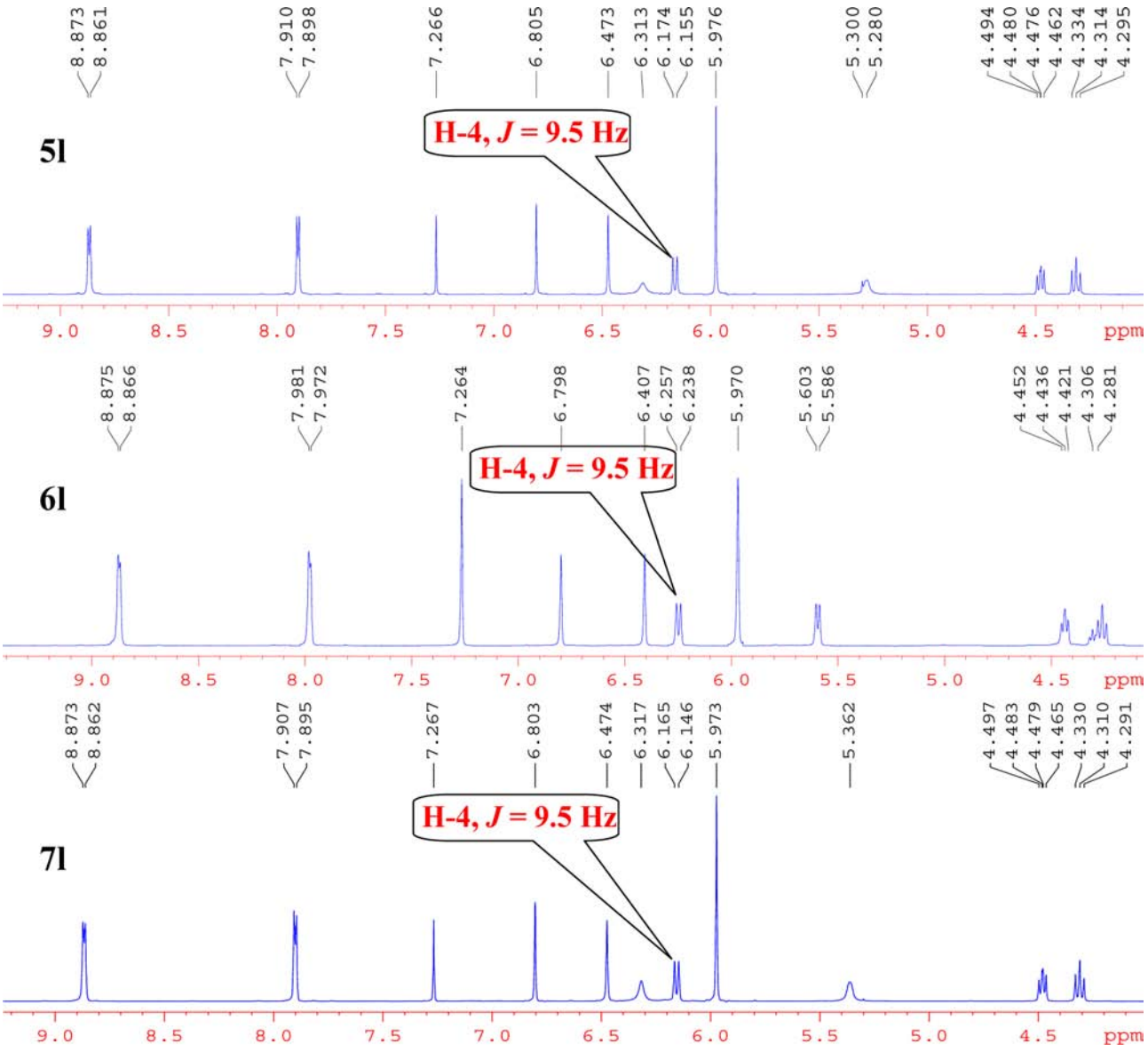

Figure 2. Partial ^{1}H NMR spectra of **5l**, **6l**, and **7l**.

6.41 (s, 1H, H-8), 6.23 (s, 1H, H-6′), 5.95 (d, J = 2.5 Hz, 2H, OCH_2O), 5.91 (d, J = 9.5 Hz, 1H, H-4), 5.30 (d, J = 4.0 Hz, 1H, H-1), 4.44 (dd, J = 9.0, 7.0 Hz, 1H, H-11), 4.20 (t, J = 10.0 Hz, 1H, H-11), 3.91 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.07−3.12 (m, 1H, H-3), 3.03 (dd, J = 15.0, 6.0 Hz, 1H, H-2), 2.23 (s, 3H, CH_3); HRMS m/z calcd for $C_{24}H_{23}O_9Br$ ($[M]^+$) 534.0520, found 534.0523.

Data for ***8b****:* yield 89%; white solid; mp 210−212 °C (lit.[31] mp 210−211 °C); $[\alpha]_D^{20}$ = −149 (c 4.4 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.77 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.39 (s, 2H, H-2′, 6′), 5.99 (d, J = 5.5 Hz, 2H, OCH_2O), 5.89 (d, J = 9.0 Hz, 1H, H-4), 4.60 (d, J = 4.0 Hz, 1H, H-1), 4.36−4.40 (m, 1H, H-11), 4.18−4.22 (m, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.76 (s, 6H, 3′, 5′-OCH_3), 2.94 (dd, J = 14.5, 4.5 Hz, 1H, H-2), 2.80−2.87 (m, 1H, H-3), 2.18 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6, 171.3, 152.6, 148.1, 147.6, 137.2, 134.8, 132.3, 128.3, 109.7, 108.1, 107.0, 101.6, 73.6, 71.3, 60.7, 56.1, 45.6, 43.7, 38.7, 21.0; MS (ESI) m/z (rel intens) 479 ($[M + Na]^+$, 100).

Data for ***8d****:* yield 82%; white solid; mp 137−138 °C (lit.[31] mp 136−138 °C);[31] $[\alpha]_D^{20}$ = −138 (c 3.6 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.39 (s, 2H, H-2′, 6′), 5.99 (d, J = 5.5 Hz, 2H, OCH_2O), 5.90 (d, J = 9.0 Hz, 1H, H-4), 4.61 (d, J = 4.0 Hz, 1H, H-1), 4.35−4.39 (m, 1H, H-11), 4.22 (t, J = 10.0 Hz, 1H, H-11), 3.81 (s, 3H, 4′-OCH_3), 3.76 (s, 6H, 3′, 5′-OCH_3), 2.94 (dd, J = 14.5, 4.0 Hz, 1H, H-2), 2.81−2.84 (m, 1H, H-3), 2.44−2.49 (m, 2H, CH_2CH_3), 1.21 (t, J = 7.5 Hz, 3H, CH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.8, 173.6, 152.6, 148.1, 147.6, 137.2, 134.8, 132.3, 128.4, 109.7, 108.1, 106.9, 101.5, 73.4, 71.3, 60.7, 56.1, 45.6, 43.7, 38.7, 27.7, 9.1; MS (ESI) m/z (rel intens) 488 ($[M + NH_4]^+$, 100).

Biological Assay. The insecticidal activity of 4α-(acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin derivatives **5a−o,s,w,a′,b′**, **6b,d−g,i,k,l**, and **7a−d,f,j−w,a′** against the pre-third-instar larvae of *M. separata* was assessed by the leaf-dipping method as described previously.[32,33] For each compound, 30 pre-third-instar larvae (10 larvae per group) were used. Acetone solutions of compounds **5a−o,s,w,a′,b′**, **6b,d−g,i,k,l**, and **7a−d,f,j−w,a′** were prepared at a concentration of 1 mg/mL. Compounds **8b,d,k,l** and toosendanin were used as the positive control at a concentration of 1 mg/mL. Fresh wheat leaves were dipped into the corresponding solution for 3 s and 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, in which 10 larvae were raised. If the treated leaves were consumed, additional treated leaves were added to the dish. After 48 h, untreated fresh leaves were added to all dishes until adult emergence. The experiment was carried out at 25 ± 2 °C

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and a relative humidity (RH) of 65−80% and in a 12 h/12 h (light/dark) photoperiod. The insecticidal activity of the tested compounds against the pre-third-instar larvae of *M. separata* was calculated by the formula

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

where T is the mortality rate in the treated group expressed as a percentage and C is the mortality rate in the untreated group expressed as a percentage.

RESULTS AND DISCUSSION

Synthesis. Three series of novel 4α-(acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (**5a−o,s,w,a′,b′**, **6b,d−g,i,k,l**, and **7a−d,f,j−w,a′**) modified in the C and E rings were prepared as shown in Scheme 1. First, three intermediates (**2−4**) were smoothly synthesized by reaction of **1** with NCS or NBS. Then compounds **5a−o,s,w,a′,b′**, **6b,d−g,i,k,l**, **7a−d,f,j−w,a′**, and **8b,d,k,l** (used as the control for the next bioassay) were obtained by reaction of **2**, **3**, **4**, or **1** with the corresponding carboxylic acids **9** in the presence of DCC and DMAP. The structures of the target compounds were well characterized by ^{1}H NMR, HRMS, ESI-MS, optical rotation, and mp. The assignment of the configuration at the C-4 position of the above derivatives was based on $J_{3,4}$ coupling constants: The C-4β-substituted compounds have a $J_{3,4} \approx$ 4.0 Hz due to a *cis* relationship between H-3 and H-4. $J_{3,4} \geq$ 10.0 Hz indicates that H-3 and H-4 are in a *trans* relationship, and the substituent at the C-4 position of podophyllotoxin is in the α configuration.[34] For example, as described in Figure 2, the $J_{3,4}$ values of H-4 of **5l**, **6l**, and **7l** were all 9.5 Hz; therefore, the 4-pyridyloyloxy groups at the C-4 position of **5l**, **6l**, and **7l** were all in the α configuration. The precise three-dimensional structural information on **5s**, **6b**, **6d**, and **7q** was further determined by single-crystal X-ray diffraction as illustrated in Figures 3−6. It obviously suggested that the substituents on the C-4 position of **5s**, **6b**, **6d**, and **7q** were all in the α configuration, and the chlorine atom on the E ring of **5s** and the bromine atom on the E ring of **7q** were at the C-2′ position. Crystallographic data (excluding structure factors) for the structures of **5s**, **6b**, **6d**, and **7q** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 894364, 894557, 894575, and 941537, respectively. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Rd., Cambridge CB2 1EZ, U.K. [fax +44 (0)1223 336033 or e-mail deposit@ccdc.cam.ac.uk].

Insecticidal Activity. The insecticidal activity of novel 4α-(acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin derivatives **5a−o,s,w,a′,b′**, **6b,d−g,i,k,l**, and **7a−d,f,j−w,a′**) against the pre-third-instar larvae of *M. separata* was tested by the leaf-dipping method at a concentration of 1 mg/mL. Compounds **8b,d,k,l** and toosendanin were used as the positive control at a concentration of 1 mg/mL. As shown in Table 1, the corresponding mortality rates caused by these compounds after 30 days were generally higher than those after 10 and 20 days. For example, the corrected mortality rate of **7f** against *M. separata* after 10 days was only 6.7%, and after 20 days, the corresponding mortality rate was increased to 48.3%. However, after 30 days, the corresponding mortality rate was sharply increased to 74.1%, which was nearly 11-fold greater than that after 10 days. That is, these compounds, in a time-dependent manner, different from other conventional neurotoxic insecticides such as organophosphates, carbamates, and pyrethroids, showed delayed insecticidal activity. On the other hand, the symptoms of the tested *M. separata* were also characterized in the same way as in our previous reports:[23,29,32]

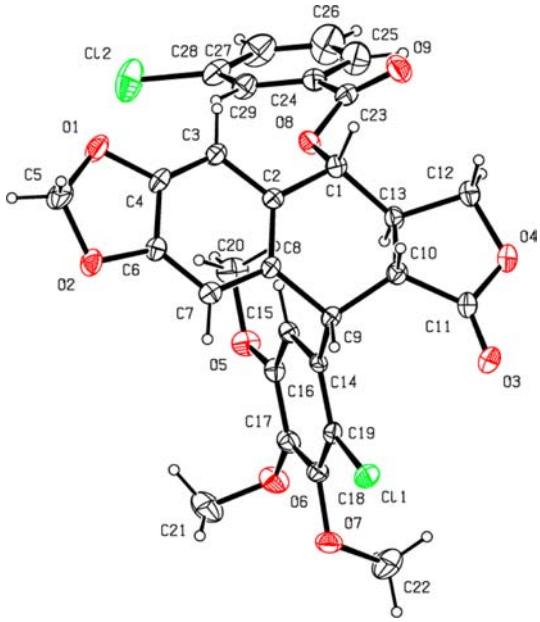

Figure 3. X-ray crystal structure of compound **5s**.

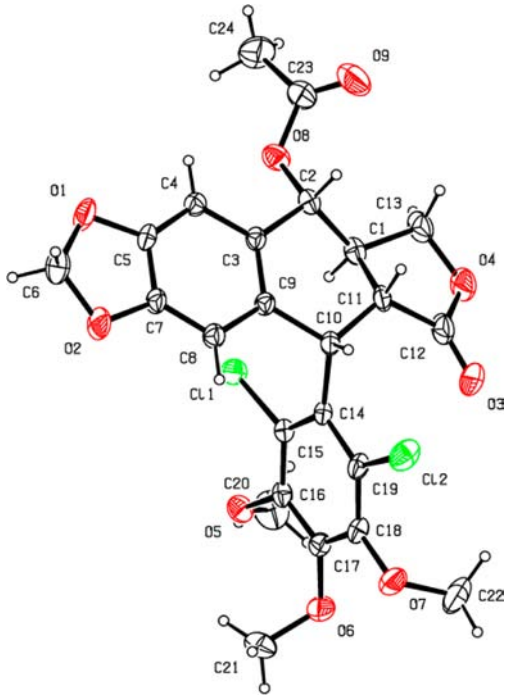

Figure 4. X-ray crystal structure of compound **6b**.

(a) due to feeding too many treated leaves during the first 48 h, some larvae died slowly with slim and wrinkled bodies during the larval period (Figure 7); (b) many larvae of the treated groups molted to malformed pupae and died during the pupation stage (Figure 8); (c) malformed moths with imperfect wings also appeared in the treated groups (Figure 9). According to the above-mentioned symptoms, the prepared derivatives likely exhibited the antimolting hormone effect. As described in Table 1, many compounds exhibited insecticidal activity equal to or higher than that of toosendanin. Among all the derivatives, the final mortality rates of compounds **2**, **3**, **5a**, **5f**, **5j**, **5m−o**, **6g**, **7f**, **7n**, **7w**, and **7z** were all greater than 60%. Especially compounds **5a**, **5n**, **7f**, **7n**, and **7w** exhibited the most potent insecticidal activity with final

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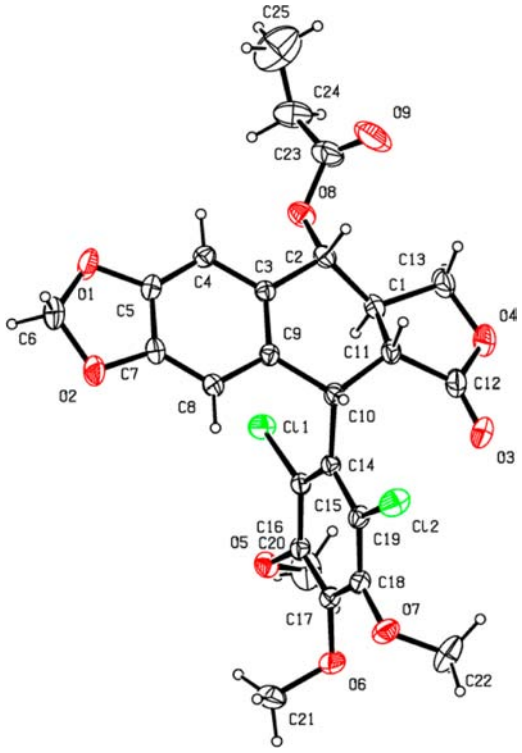

Figure 5. X-ray crystal structure of compound **6d**.

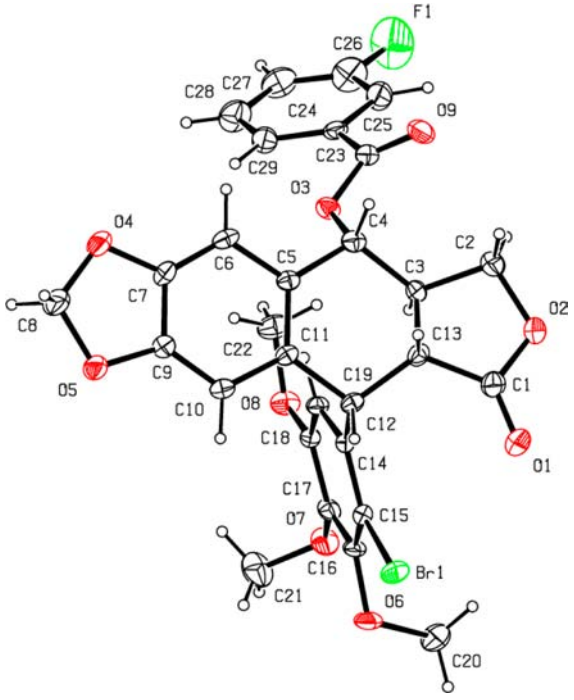

Figure 6. X-ray crystal structure of compound **7q**.

mortality rates of approximately 70%. It is noteworthy that introduction of a chlorine or bromine atom at the C2′ or C2′ and C6′ positions on the E ring of podophyllotoxin could result in more potent compounds compared to their precursor podophyllotoxin. For example, the final mortality rate of compound **1** was only 37%, whereas the final mortality rates of the E ring halogenation products of podophyllotoxin (**2**−**4**) were 65.4%, 61.5%, and 55.6%, respectively. In addition, the insecticidal activity of some 4α-(acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin derivatives (e.g., **5b**,**d**,**k**,**l**, **6b**,**d**,**k**,**l**, and **7b**,**d**,**k**,**l**) was compared with that of the corresponding 4α-(acyloxy)podophyllotoxin derivatives (e.g., **8b**,**d**,**k**,**l**). This further demonstrated that a chlorine or bromine atom introduced at the C2′ or C2′ and C6′ positions on the E ring of podophyllotoxin was important for obtaining the potent derivatives. For example, the final mortality rates of **5b**,**d**,**k**,**l**, **6b**,**d**,**k**,**l**, and **7b**,**d**,**k**,**l** were 57.7%/57.7%/57.7%/38.5%, 57.7%/57.7%/53.8%/53.8%, and 55.6%/51.9%/59.3%/51.9%, respectively; however, the final mortality rates of **8b**,**d**,**k**,**l** were 38.5%, 34.6%, 38.5%, and 34.6%, respectively. For 4α-(alkylacyl)oxy derivatives, the effect of the length of their side chain at the C-4 position on the insecticidal activity was not very obvious. However, introduction of the (chloroacetyl)oxy group at the C-4 position of **2** or **4** resulted in less potent compounds (e.g., 42.3% for **5c** and 33.3% for **7c**). Similarly, for 4α-(arylacyl)oxy derivatives, the electronic effect of the substituents on their phenyl ring at the C-4 position on the insecticidal activity was generally not very clear. Interestingly, introduction of a (1-naphthylacetyl)oxy group at the C-4 position of **2** or **4** could also lead to more potent compounds (e.g., 69.2% for **5n** and 70.4% for **7n**) in the same way as described in our previous papers.[24,26,29] As compared with 2β-chloropodophyllotoxin derivatives (**I**), 2′(2′,6′)-(di)chloropodophyllotoxin derivatives **5** and **6** generally exhibited more potent insecticidal activity.[24,26] For example, the final mortality rate of 2β-chloropodophyllotoxin was 48.1%; whereas the final mortality rates of 2′-chloropodophyllotoxin (**2**) and 2′,6′-dichloropodophyllotoxin (**3**) were 65.4% and 61.5%, respectively. Similarly, the final mortality rates of 4α-(acetyloxy)-2β-chloropodophyllotoxin and 4α-(propanoyloxy)-2β-chloropodophyllotoxin were 40.7%, but the final mortality rates of 4α-(acetyloxy)-2′-chloropodophyllotoxin (**5b**) and 4α-(propanoyloxy)-2′,6′-dichloropodophyllotoxin (**5d**) were 57.7%. In general, 2′-bromopodophyllotoxin derivatives (**7**) displayed more promising insecticidal activity than the 2α/β-bromopodophyllotoxin derivatives (**II** and **III**).[27,28] For example, the final mortality rates of 2α-bromopodophyllotoxin and 2β-bromopodophyllotoxin were 41.7%, and 45.8%, respectively; whereas the final mortality rate of 2′-bromopodophyllotoxin (**4**) was 55.6%. Likewise the final mortality rates of 4α-(acetyloxy)-2α-bromopodophyllotoxin, 4α-[(*m*-nitrobenzoyl)oxy]-2α-bromopodophyllotoxin, and 4α-[(1-naphthylacetyl)oxy]-2α-bromopodophyllotoxin were 37.5%, 58.3%, and 50%, respectively; however, the final mortality rates of 4α-(acetyloxy)-2′-bromopodophyllotoxin (**7a**), 4α-[(*m*-nitrobenzoyl)oxy]-2′-bromopodophyllotoxin (**7n**), and 4α-[(1-naphthylacetyl)oxy]-2′-bromopodophyllotoxin (**7w**) were 59.3%, 70.4%, and 70.4%, respectively.

In summary, three series of novel 4α-(acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin derivatives modified in the C and E rings of podophyllotoxin were synthesized and evaluated for their insecticidal activity against the pre-third-instar larvae of

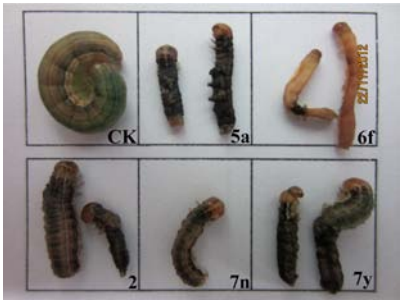

Figure 7. Representative abnormal larvae pictures of **2**, **5a**, **6f**, **7n**, and **7y** during the larval period (CK = blank control group).

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Table 1. Insecticidal Activity of Three Series of 4α-(Acyloxy)-2′(2′,6′)-(di)halogenopodophyllotoxin Derivatives (5a–o,s,w,a′,b′, 6b,d–g,i,k,l, and 7a–d,f,j–w,a′) at 1 mg/mL against *M. separata*

compd	corrected mortality rate (%)		
	10 days	20 days	30 days
1	13.3 ± 3.3	27.6 ± 0	37.0 ± 3.3[a]
2	26.7 ± 3.3	39.3 ± 6.7	65.4 ± 0
3	23.3 ± 3.3	42.9 ± 3.3	61.5 ± 6.7
4	13.3 ± 3.3	41.4 ± 3.3	55.6 ± 0
5a	56.7 ± 3.3	60.7 ± 3.3	69.2 ± 3.3
5b	46.7 ± 3.3	53.6 ± 6.7	57.7 ± 3.3
5c	43.3 ± 3.3	39.3 ± 3.3	42.3 ± 5.8
5d	46.7 ± 3.3	53.6 ± 3.3	57.7 ± 3.3
5e	23.3 ± 3.3	34.5 ± 3.3	50.0 ± 3.3
5f	53.3 ± 3.3	57.1 ± 5.8	61.5 ± 3.3
5g	30.0 ± 0	41.4 ± 3.3	53.6 ± 3.3
5h	26.7 ± 3.3	44.8 ± 3.3	50.0 ± 3.3
5i	33.3 ± 3.3	46.4 ± 5.8	53.8 ± 0
5j	20.0 ± 5.8	35.7 ± 5.8	61.5 ± 6.7
5k	50.0 ± 0	53.6 ± 6.7	57.7 ± 3.3
5l	36.7 ± 3.3	32.1 ± 3.3	38.5 ± 3.3
5m	26.7 ± 3.3	32.1 ± 3.3	61.5 ± 3.3
5n	43.3 ± 3.3	46.4 ± 0	69.2 ± 3.3
5o	50.0 ± 0	53.6 ± 3.3	61.5 ± 3.3
5s	26.7 ± 3.3	28.6 ± 3.3	50.0 ± 3.3
5w	46.7 ± 3.3	50.0 ± 6.7	53.8 ± 0
5a′	30.0 ± 0	50.0 ± 3.3	57.7 ± 3.3
5b′	26.7 ± 3.3	28.6 ± 3.3	38.5 ± 3.3
6b	23.3 ± 3.3	39.3 ± 6.7	57.7 ± 3.3
6d	36.7 ± 3.3	46.4 ± 5.8	57.7 ± 3.3
6e	30.0 ± 8.2	48.3 ± 8.2	51.9 ± 4.7
6f	41.6 ± 3.3	50.8 ± 3.3	59.3 ± 3.3
6g	23.3 ± 3.3	48.3 ± 0	64.3 ± 3.3
6i	46.7 ± 3.3	46.4 ± 5.8	53.8 ± 5.8
6k	20.0 ± 0	46.4 ± 5.8	53.8 ± 0
6l	50.0 ± 0	46.4 ± 0	53.8 ± 5.8
7a	3.3 ± 3.3	34.5 ± 3.3	59.3 ± 3.3
7b	10.0 ± 5.8	31.0 ± 3.3	55.6 ± 0
7c	6.7 ± 3.3	27.6 ± 0	33.3 ± 0
7d	13.3 ± 3.3	44.8 ± 3.3	51.9 ± 3.3
7f	6.7 ± 3.3	48.3 ± 0	74.1 ± 3.3
7j	10.0 ± 0	24.1 ± 3.3	37.0 ± 3.3
7k	20.0 ± 0	37.9 ± 0	59.3 ± 3.3
7l	10.0 ± 0	34.5 ± 3.3	51.9 ± 3.3
7m	20.0 ± 0	44.8 ± 3.3	59.3 ± 3.3
7n	10.0 ± 0	37.9 ± 0	70.4 ± 3.3
7o	16.7 ± 3.3	27.6 ± 0	51.9 ± 3.3
7p	10.0 ± 0	27.6 ± 0	48.1 ± 3.3
7q	20.0 ± 5.8	27.6 ± 5.8	55.6 ± 0
7r	16.7 ± 3.3	27.6 ± 0	59.3 ± 3.3
7s	13.3 ± 3.3	24.1 ± 3.3	33.3 ± 0
7t	3.3 ± 3.3	20.7 ± 3.3	48.1 ± 3.3
7u	10.0 ± 5.8	20.7 ± 3.3	40.7 ± 3.3
7v	13.3 ± 3.3	24.1 ± 3.3	48.1 ± 3.3
7w	10.0 ± 0	48.3 ± 0	70.4 ± 3.3
7x	23.3 ± 6.7	41.4 ± 3.3	48.1 ± 3.3
7y	20.0 ± 5.8	37.9 ± 0	51.9 ± 3.3
7z	23.3 ± 3.3	44.8 ± 3.3	63.0 ± 3.3
7a′	13.3 ± 6.7	44.8 ± 3.3	55.6 ± 0
8b	30.0 ± 0	35.7 ± 0	38.5 ± 3.3[a]
8d	33.3 ± 3.3	35.7 ± 0	34.6 ± 3.3[a]
8k	33.3 ± 3.3	35.7 ± 5.8	38.5 ± 3.3[a]
8l	30.0 ± 0	28.6 ± 3.3	34.6 ± 3.3[a]
toosendanin[b]	20.0 ± 0	31.0 ± 3.3	51.9 ± 3.3[a]

[a]After 33 days. [b]Toosendanin was used as a positive control at 1 mg/mL.

Figure 8. Representative malformed pupae pictures of **2**, **5i**, **6g**, **7f**, and **7w** during the pupation period (CK = blank control group).

Figure 9. Representative malformed moth pictures of **3**, **5j**, **5n**, **7f**, and **7n** during the emergence period (CK = blank control group).

M. separata in vivo at a concentration of 1 mg/mL. These derivatives likely displayed the antimolting hormone effect. Among all the derivatives, especially compounds **5a**, **5n**, **7f**, **7n**, and **7w** exhibited the most potent insecticidal activity with final mortality rates of 70% or so. This suggested that a chlorine or bromine atom introduced at the C2′ or C2′ and C6′ positions on the E ring of podophyllotoxin was necessary for obtaining the potent compounds. This will pave the way for further design, structural modification, and development of podophyllotoxin derivatives as insecticidal agents.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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

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Notes

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