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Synthesis and insecticidal activity of new deoxypodophyllotoxin derivatives modified in the D-ring

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ABSTRACT

In continuation of our program aimed at the discovery of new natural-product-based insecticidal agents, twenty-six deoxypodophyllotoxin derivatives modified in the D-ring were synthesized and evaluated as insecticidal agents against the pre-third-instar larvae of oriental armyworm, *Mythimna separata* (Walker) in vivo at 1 mg/mL. The configuration of three compounds **3**, **4**, and **IIIi** was unambiguously determined by single-crystal X-ray diffraction. It demonstrated that aminolysis of deoxypodophyllotoxin in the presence of pyrrolidine and piperidine could result in complete inversion of the configuration of the carbonyl group at its C-2 position. Five compounds **IIa**, **IIi–k**, and **IIIh** showed the equal or higher insecticidal activity than toosendanin. Especially **IIj** displayed the most potent insecticidal activity with the final mortality rate of 65.5%.

The naturally occurring aryltetralin lignan podophyllotoxin (compound **1**, Fig. 1) is isolated from the roots and rhizomes of *Podophyllum hexandrum* such as *P. hexandrum* and *Podophyllum peltatum*. Compound **1** has been used as the lead compound for the preparation of potent anticancer drugs such as etoposide, teniposide and etopophos,[1–3] and the insecticidal agents.[4–8] Recently, we have investigated the insecticidal activity of 2α/β-bromo- or 2β-chloropodophyllotoxin derivatives modified in the C ring and 4-deoxypodophyllotoxin (compound **2**, Fig. 1) derivatives modified in the E ring, and found some compounds showed the equal or higher insecticidal activity than toosendanin, a commercial botanical insecticide isolated from *Melia azedarach*.[9–13] On the other hand, as the pesticides originated from plant secondary metabolites may result in less or slower resistance development and lower pollution, so the discovery of new pesticidal agents from plant secondary metabolites, or by using them as the lead compounds for further structural modification, have recently been one of the important routes for research and development of new insecticides.[14–19] Recently, Xiao et al. found that introduction of pyrrolidine at the D-ring of **1** could lead to the potent compounds **I** (Fig. 1), which exhibited the good antitumor activity.[20] Encouraged by the above-mentioned results, and in continuation of our program aimed at the discovery and development of novel natural-product-based pesticides, in this Letters we designed and prepared a series of novel derivatives of deoxypodophyllotoxin **II** (Fig. 1) with an opened D ring as insecticidal agents by introduction of Part A of compounds **I** into compound **2**. Meanwhile, deoxypodophyllotoxin derivatives **III** (Fig. 1), where the piperidine moiety was substituted for the pyrrolidine one in the Part A of **II**, were prepared as the control.

As shown in Scheme 1, firstly, 4-deoxypodophyllotoxin (**2**) was prepared from podophyllotoxin (**1**) mediated by 10% palladium/carbon.[21] Then **2** reacted with pyrrolidine and piperidine to give two D-ring opened intermediates **3** and **4**, respectively. Finally, in the presence of *N,N′*-diisopropylcarbodiimide (DIC) and 4-dimethylaminopyridine (DMAP), the target products **IIa–n** and **IIIa–i,l–n** were obtained by the reaction of carboxylic acids with **3** and **4**, respectively. Moreover, the configuration of **3**, **4**, and **IIIi** was further unambiguously identified by single-crystal X-ray diffraction.[22] As shown in Figures 2–4, the substituents at the C-2 and C-3 positions of **3**, **4**, and **IIIi** were all in β configuration. It clearly demonstrated that aminolysis of **2** in the presence of pyrrolidine and piperidine could result in complete inversion of the configuration of the carbonyl group at its C-2 position.

As shown in Table 1, the insecticidal activity of compounds **IIa–n** and **IIIa–i,l–n** was evaluated against the pre-third-instar larvae of oriental armyworm, *Mythimna separata* (Walker) at the concentration of 1 mg/mL.[23] Toosendanin was used as the positive control at 1 mg/mL. Leaves treated with acetone alone were used as a blank control group. It was found that for the corresponding mortality rates of tested compounds after 35 days were usually higher than those after 10 and 20 days, these compounds exhibited the delayed insecticidal activity. Additionally, the symptoms of the

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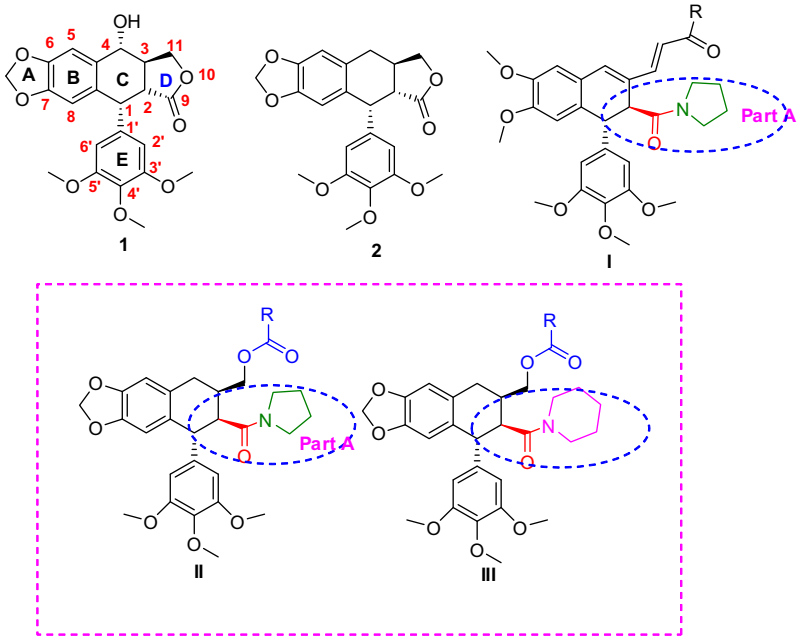
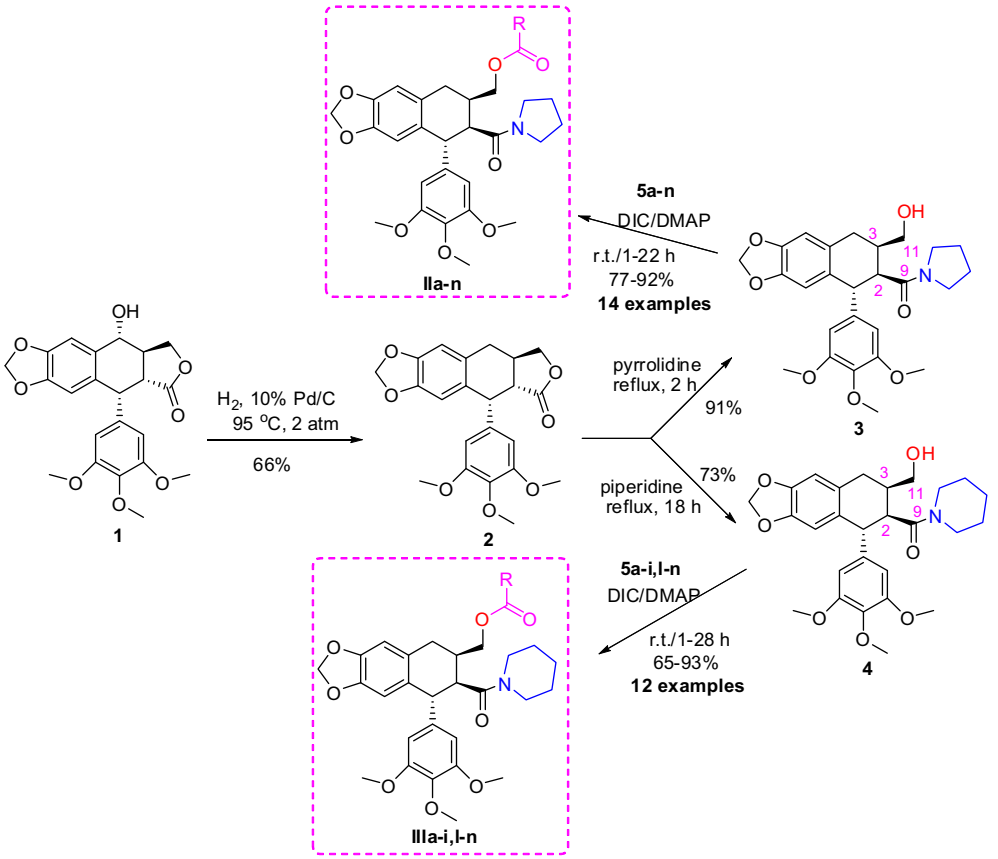


Figure 1. Chemical structures of podophyllotoxin (**1**), deoxypodophyllotoxin (**2**) and their derivatives (**I**–**III**).



Scheme 1. Synthetic route for preparation of deoxypodophyllotoxin derivatives **IIa**–**n** and **IIIa**–**i**,**l**–**n**.

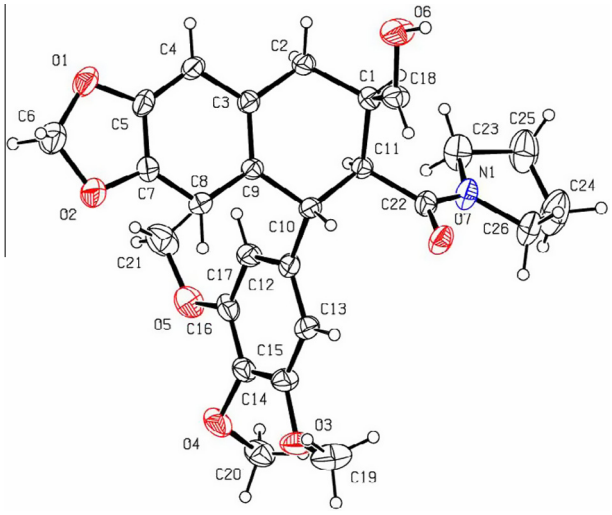


Figure 2. The X-ray crystal structure of **3**.

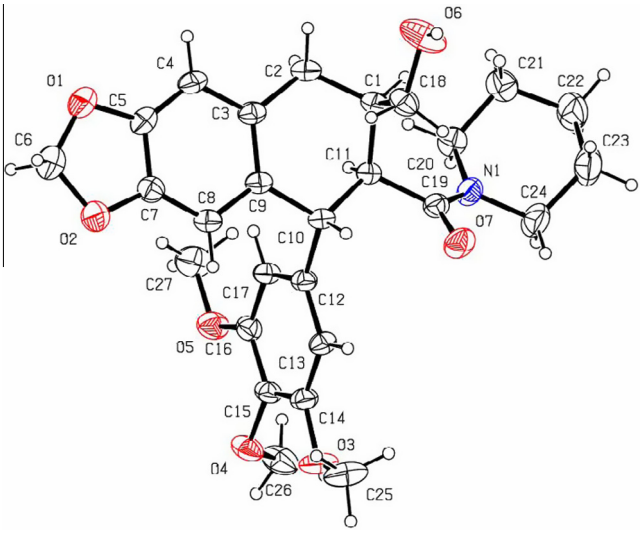


Figure 3. The X-ray crystal structure of **4**.

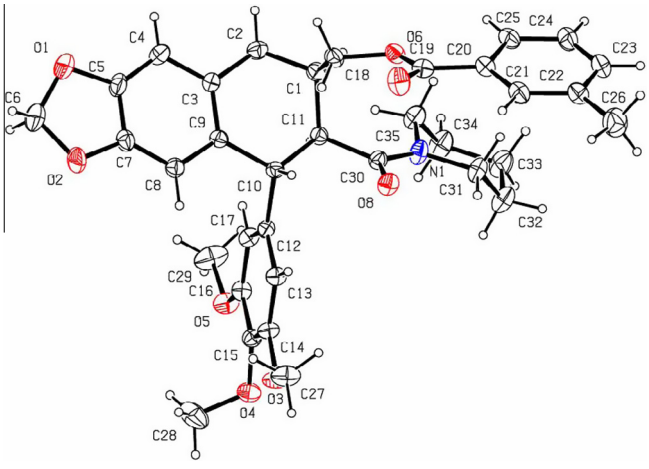


Figure 4. The X-ray crystal structure of **IIIi**.

tested *M. separata* were characterized by the same way as our previous reports.[9–13] Among all derivatives, five compounds **IIa**, **IIi–k**, and **IIIh** showed the equal or higher insecticidal activity than toosendanin. Especially **IIj** displayed the most potent insecticidal activity with the final mortality rate of 65.5%, whereas the final mortality rate of the precursor **1** was only 34.5%. In general, to alkylacyloxy series, the proper length of the side chain at the C-11 position of **IIa–d** and **IIIa–d** was important for their insecticidal activity. For example, the final mortality rates of **IIb** ($R = (CH_2)_6CH_3$), **IIc** ($R = (CH_2)_{10}CH_3$), **IId** ($R = (CH_2)_{13}CH_3$), **IIIb** ($R = (CH_2)_6CH_3$), **IIIc** ($R = (CH_2)_{10}CH_3$) and **IIId** ($R = (CH_2)_{13}CH_3$) were only 37.9%, 37.9%, 37.9%, 41.4%, 24.1% and 31%, respectively; whereas the final mortality rates of **IIa** ($R = CH_3CH_2$) and **IIIa** ($R = CH_3CH_2$) were 58.6% and 44.8%, respectively. Meanwhile, introduction of 3-pyridylacyloxy group at the C-11 position of **3** and **4** did not result in the more potent compounds. To arylacyloxy series, the electron effect on the phenyl ring of **IIi–n** to their insecticidal activity was obvious. For example, introduction of the electron-donating groups such as methyl and methoxy on the phenyl ring at the C-11 position of **IIe** led to the more potential compounds **IIi–k**, and the final mortality rates of **IIi–k** were 51.7%, 65.5% and 51.7%, respectively; whereas introduction of the electron-withdrawing groups such as fluorine, chlorine and bromine atoms on the phenyl ring at the C-11 position of **IIe** gave the less active compounds **III–n**, and the final mortality rates of **III–n** were 41.4%, 41.4% and 37.9%, respectively;

In summary, we have prepared twenty-six deoxypodophyllotoxin-based derivatives modified in the D-ring and evaluated their insecticidal activity against the pre-third-instar larvae of *M. separata* in vivo at 1 mg/mL. It suggested that aminolysis of deoxypodophyllotoxin in the presence of pyrrolidine and piperidine could lead to complete inversion of the configuration of the carbonyl group at its C-2 position. Among all derivatives, five compounds **IIa**, **IIi–k**, and **IIIh** showed the equal or higher insecticidal activity

Table 1
Insecticidal activity of **IIa–n** and **IIIa–i,l–n** against *M. separata* on leaves at a concentration of 1 mg/mL

Compound	Corrected mortality rate (%)		
	10 days	20 days	35 days
1	16.7 ± 4.7	24.1 ± 9.4	34.5 ± 4.7
2	30.0 ± 8.2	34.5 ± 4.7	48.3 ± 8.2
3	20.0 ± 8.2	17.2 ± 8.2	31.0 ± 4.7
4	16.7 ± 9.4	24.1 ± 9.4	27.6 ± 8.2
IIa	6.7 ± 9.4	41.4 ± 4.7	58.6 ± 8.2
IIb	10.0 ± 8.2	13.8 ± 4.7	37.9 ± 8.2
IIc	3.3 ± 4.7	27.6 ± 0	37.9 ± 8.2
IId	13.3 ± 9.4	24.1 ± 4.7	37.9 ± 8.2
IIe	23.3 ± 4.7	37.9 ± 0	44.8 ± 4.7
IIf	10.0 ± 8.2	24.1 ± 4.7	44.8 ± 4.7
IIg	13.3 ± 9.4	34.5 ± 9.4	48.3 ± 8.2
IIh	10.0 ± 8.2	27.6 ± 8.2	44.8 ± 4.7
IIi	13.3 ± 9.4	34.5 ± 4.7	51.7 ± 9.4
IIj	13.3 ± 4.7	48.3 ± 8.2	65.5 ± 4.7
IIk	16.7 ± 4.7	44.8 ± 9.4	51.7 ± 4.7
III	10.0 ± 8.2	17.2 ± 8.2	41.4 ± 9.4
IIm	16.7 ± 4.7	31.0 ± 4.7	41.4 ± 4.7
IIn	13.3 ± 4.7	27.6 ± 8.2	37.9 ± 8.2
IIIa	13.3 ± 4.7	34.5 ± 9.4	44.8 ± 4.7
IIIb	10.0 ± 0	34.5 ± 9.4	41.4 ± 4.7
IIIc	3.3 ± 4.7	17.2 ± 8.2	24.1 ± 4.7
IIId	6.7 ± 9.4	24.1 ± 4.7	31.0 ± 4.7
IIIe	6.7 ± 4.7	27.6 ± 8.2	37.9 ± 8.2
IIIf	13.3 ± 9.4	37.9 ± 8.2	48.3 ± 0
IIIg	13.3 ± 12.5	27.6 ± 8.2	41.4 ± 4.7
IIIh	6.7 ± 4.7	41.4 ± 9.4	55.2 ± 9.4
IIIi	13.3 ± 4.7	34.5 ± 9.4	48.3 ± 8.2
IIIl	10.0 ± 8.2	37.9 ± 0	44.8 ± 4.7
IIIm	13.3 ± 9.4	31.0 ± 4.7	37.9 ± 8.2
IIIn	3.3 ± 4.7	24.1 ± 4.7	27.6 ± 8.2
Toosendanin[a]	33.3 ± 4.7	44.8 ± 4.7	51.7 ± 4.7
Blank control	0 ± 0	3.3 ± 4.7	3.3 ± 4.7

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

than toosendanin. Especially **IIj** displayed the most potent insecticidal activity with the final mortality rate of 65.5%. To arylacyloxy series, the electron effect on the phenyl ring of **IIi–n** to their insecticidal activity was observed. It demonstrated that, to alkylacyloxy series, the proper length of the side chain at the C-11 position of **IIa–d** and **IIIa–d** was important for their insecticidal activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.07.076.

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22. Crystallographic data (excluding structure factors) for the structure of **3**, **4** and **IIIi** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 995275, 995273 and 995274, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
23. *Biological assay:* The insecticidal activity of compounds **IIa–n** and **IIIa–i,l–n** against the pre-third-instar larvae of *Mythimna separata* Walker was assessed by leaf-dipping method. Toosendanin, a commercial insecticide isolated from *Melia azedarach*, was used as a positive control and supplied by Research & Development Center of Biorational Pesticide, Northwest A&F University, Shaanxi province, China. For each compound, 30 larvae (10 larvae per group) were used. Acetone solutions of all the above tested compounds, and toosendanin were prepared at the concentration of 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, where every 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 and on 12 h/12 h (light/dark) photoperiod. The insecticidal activity of the tested compounds was calculated by the following formula: 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.