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SEMISYNTHESIS OF CONJUGATES FROM MATRINE/CHOLESTEROL WITH PIPERIC ACID/PIPERIC ACIDS-LIKE AS INSECTICIDAL AND APHICIDAL AGENTS

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Abstract – To find natural-product-based pesticide candidates, a series of conjugates of cholesterol/matrine and piperic acid/piperic acids-like were semi-prepared as insecticidal and aphicidal agents against *Mythimna separata* Walker and *Aphis citricola* van der Goot. Notably, two steric structures were confirmed by single-crystal diffraction. Importantly, compounds **IIc**, **IVc** and **Vb** exhibited (3.2 and 3.5 folds promising insecticidal activity of cholesterol) more potent insecticidal activity than toosendanin. Compounds **Ia** (1.45-fold of matrine) and **IVb** (2.2-fold of cholesterol) showed potent aphicidal activity.

Matrine (**1**), a quinolizidine-type alkaloid, is extracted from the roots of *Sophora* species plants, and showed a high volume of medical and agricultural activities.[1] Cholesterol (**2**), a steroid alcohol extracted from animal lipids, displayed a variety of pharmaceutical properties such as anticancer,[2] antioxidant,[3] antileishmanial and antimicrobial activities,[4,5] and insecticidal activity.[6] Previously, oximinoesters of **1** [7] and esters/oximinoesters of **2** [8] showed more potent insecticidal activity than toosendanin. A simple alkaloid piperine (Figure 1) extracted from *Piper nigrum* Linn., possessed interesting medical and agricultural properties.[9] Additionally, some monosaccharide esters containing the fragment **A** (a piperic acid scaffold) of piperine showed potent insecticidal activities.[10] Therefore, to discover natural-product-based pesticide alternatives,[9,11] new conjugates **I**(**a**−**c**)−**V**(**a**−**c**) were semisynthesized by combination of cholesterol/matrine and piperic acid/piperic acids-like fragments via the esters/oximinoesters bonds. Their pesticidal activities were evaluated against two typical insect pests *Mythimna separata* (Lepidoptera: Noctuidae) and *Aphis citricola* (Hemiptera: Aphididae).[12-15]

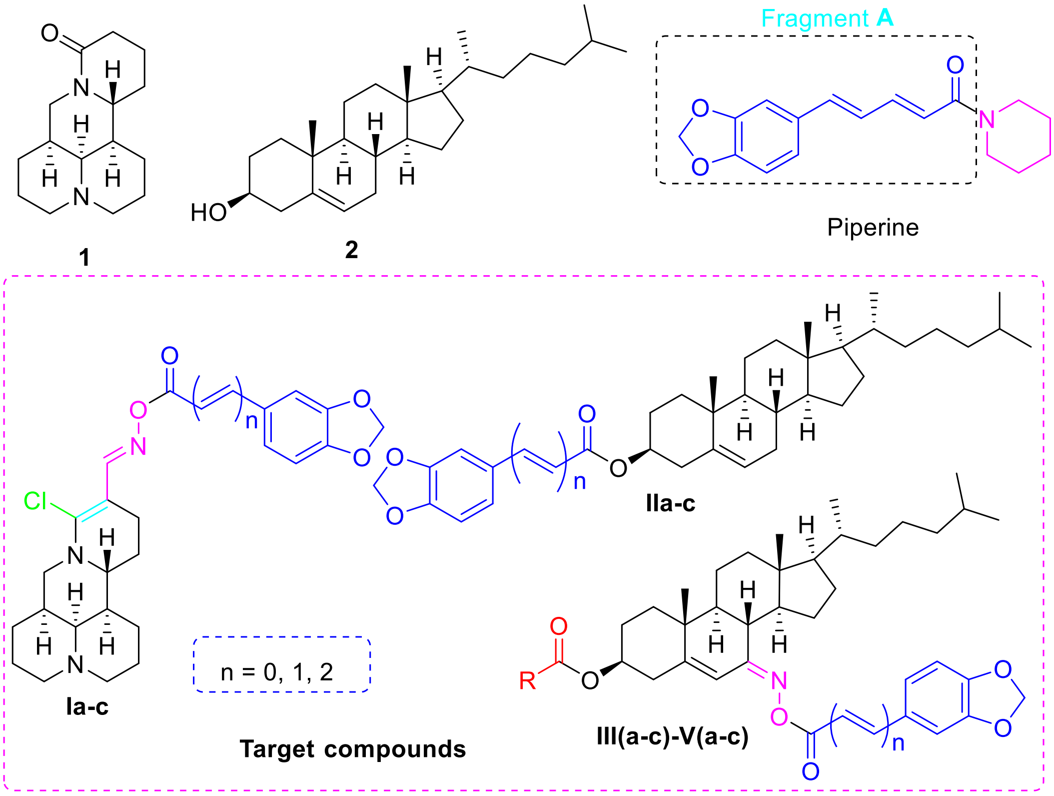
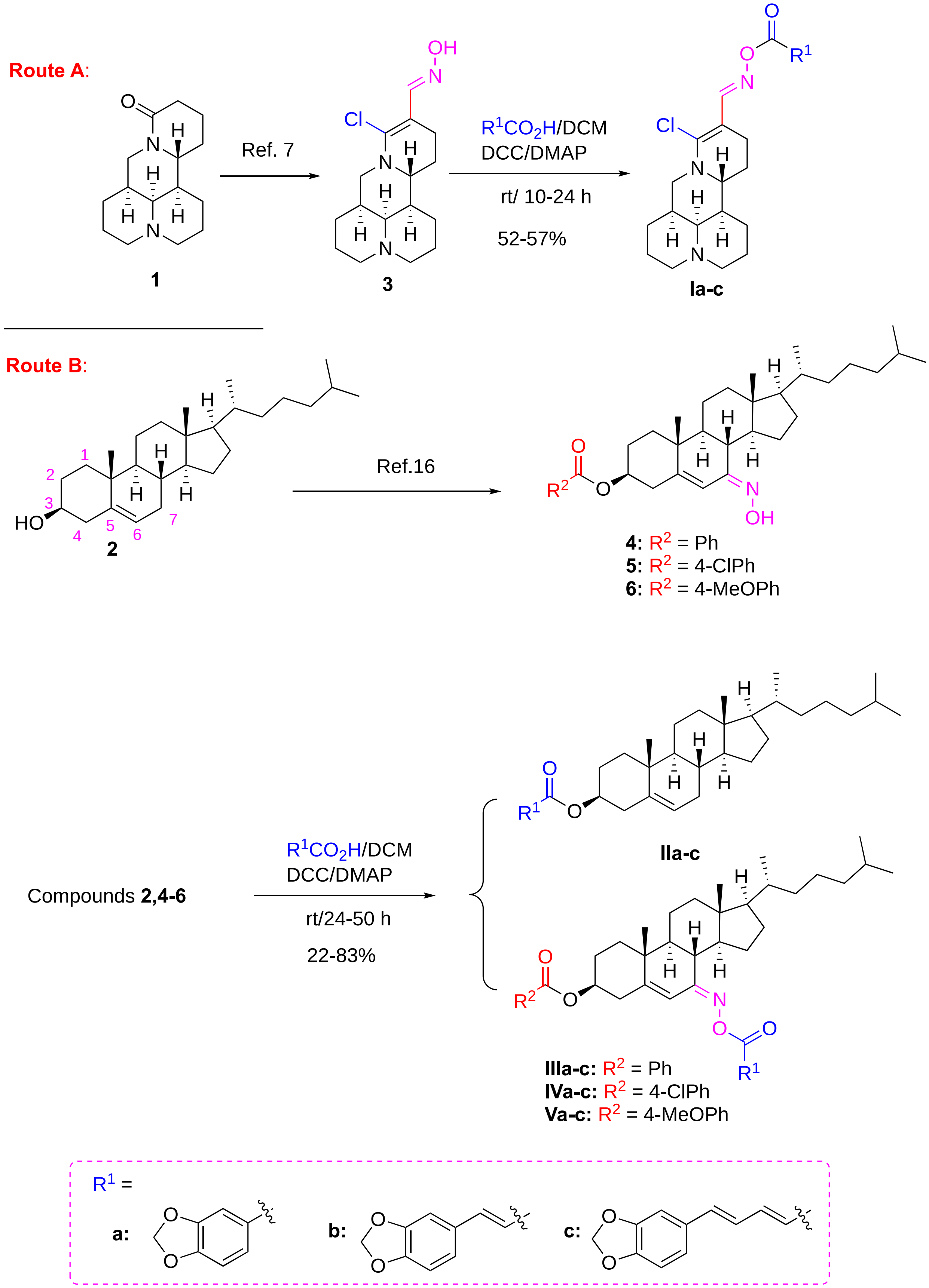


Figure 1. Design of new conjugates **I**(**a**–**c**)–**V**(**a**–**c**)

In Scheme 1, the intermediate **3** was semisynthesized from **1** as our previous method.[7] Then compound **3** reacted with piperonylic acid/3,4-methylenedioxycinnamic acid/piperic acid to afford **Ia-Ic** in 52–57% yields. Similarly, the intermediates **4**–**6** were firstly prepared from **2**,[16] then **II**(**a**–**c**)–**V**(**a**–**c**) were obtained in 22–83% yields by reaction of **2** and **4**–**6** with piperonylic acid/3,4-methylenedioxycinnamic acid/piperic acid. Their structures were characterized by HRMS, melting points, optical rotation and ^{1}H NMR. Particularly, steric structures of two derivatives **Ic** (CCDC: 2059786) and **IIa** (CCDC: 2059787) were measured by X-ray diffraction (Figures 2 and 3).

As shown in Table 1, the results of insecticidal activity of **I**(**a**–**c**)–**V**(**a**–**c**) against *M. separata* were presented. The toxic symptoms of *M. separata* treated with **I**(**a**–**c**)–**V**(**a**–**c**) during their three growth periods were found as exactly the same as previous reports.[7,8,16] When compared with their corresponding precursors such as matrine and cholesterol, except compound **Ib**, all derivatives showed the improved insecticidal activity. Moreover, the insecticidal activity of **IIc**, **IVb**, **IVc**, **Vb** and **Vc** was more potent than, or equivalent to that of toosendanin. Especially the corrected final mortality rate (CFMR) of **Vb** was 50%, whereas the CFMR of its precursor **2** was only 14.3%; obviously, compound **Vb** exhibited 3.5 times promising insecticidal activity of **2**. The CFMRs of **Ia**–**Ic** were 25.0%, 14.3% and 39.3%, respectively; it demonstrated that introduction of the piperic acid scaffold on the oxime ester fragment of **3** was important for the insecticidal activity (**Ic** vs. **1**), whereas 3,4-methylenedioxycinnamic acid scaffold introduced on



Scheme 1. Synthesis of **I(a-c)** (A), and **II(a-c)**−**V(a-c)** (B)

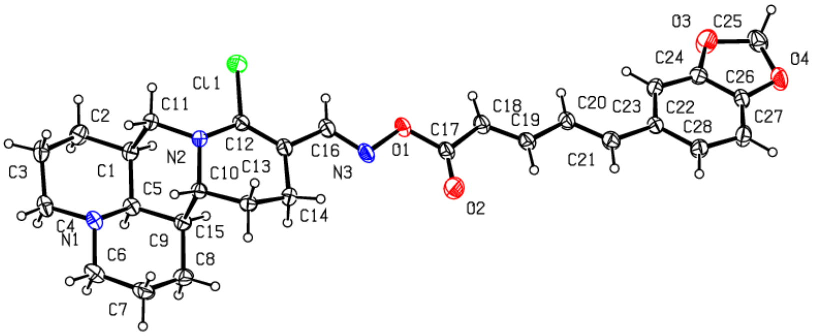


Figure 2. X-Ray crystal structure of **Ic**

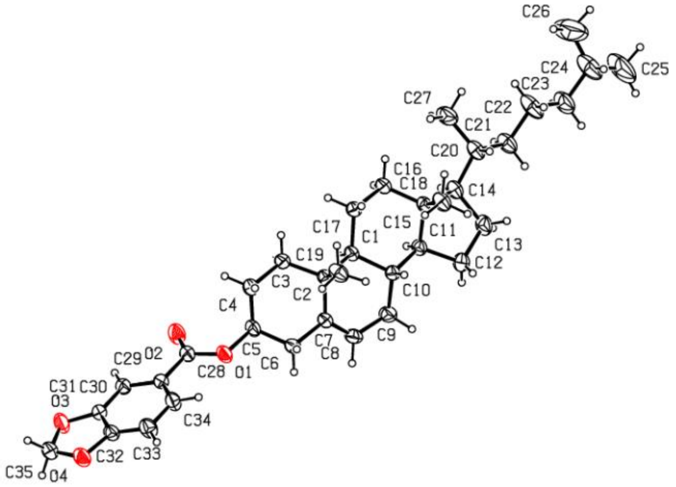


Figure 3. X-Ray crystal structure of **IIa**

Table 1. Insecticidal activity of compounds **I**(**a**−**c**)−**V**(**a**−**c**) against *M. separata* at 1 mg/mL

Compound	Final mortality rate (mean ± SE, %)[a]
1	21.4 ± 6.7
2	14.3 ± 5.8
Ia	25.0 ± 5.8
Ib	14.3 ± 5.8
Ic	39.3 ± 8.8
IIa	35.7 ± 5.8
IIb	28.5 ± 6.7
IIc	46.4 ± 5.8
IIIa	39.3 ± 6.7

IIIb	32.1 ± 3.3
IIIc	17.8 ± 3.3
IVa	39.3 ± 3.3
IVb	42.8 ± 6.7
IVc	46.4 ± 5.8
Va	28.5 ± 6.7
Vb	50.0 ± 3.3
Vc	42.8 ± 6.7
toosendanin	42.8 ± 3.3

[a] Values are mean ± SE of three replicates

the oxime ester fragment of **3** resulted in **Ib**, whose insecticidal activity was less potent than that of precursor **1**. To **IIa−IIc**, similarly, introduction of the piperic acid scaffold on compound **2** was also vital for the insecticidal activity. To **III**(**a**−**c**)−**V**(**a**−**c**), when R^2 was 4-ClPh or 4-MeOPh, generally, the corresponding derivatives displayed more promising insecticidal activity than those of R^2 as Ph. Among **IV**(**a**−**c**) and **V**(**a**−**c**), introduction of the 3,4-methylenedioxycinnamic acid/piperic acid scaffold on the oxime ester fragment was beneficial for the insecticidal activity.

Table 2. Aphicidal activity of compounds **I**(**a**−**c**)−**V**(**a**−**c**) against apterous adults of *A. citricola* treated at 0.04 μg/nymph

Compound	Corrected mortality rate (mean ± SE, %)[a]	
	24 h	48 h
1	17.8 ± 2.9	27.0 ± 2.9
2	10.0 ± 1.9	16.9 ± 1.1
Ia	7.8 ± 1.1	39.3 ± 3.3
Ib	5.6 ± 1.1	23.6 ± 1.1
Ic	6.7 ± 1.9	30.3 ± 1.1
IIa	5.6 ± 1.1	11.2 ± 1.1
IIb	10.0 ± 1.9	11.2 ± 2.9
IIc	2.2 ± 1.1	9.0 ± 3.3
IIIa	10.0 ± 1.9	15.7 ± 0
IIIb	7.8 ± 1.1	22.5 ± 1.9
IIIc	12.2 ± 1.1	14.6 ± 2.9
IVa	13.3 ± 1.9	24.7 ± 1.1
IVb	17.8 ± 1.1	37.1 ± 2.9
IVc	10.0 ± 1.9	13.5 ± 1.1
Va	8.9 ± 2.9	23.6 ± 1.1

Vb	8.9 ± 2.9	28.1 ± 1.1
Vc	7.8 ± 1.1	14.6 ± 2.9
methomyl[b]	53.4 ± 3.0	86.4 ± 1.9

[a]Values are mean ± SE of three replicates; [b] 0.004 μg/nymph.

As described in Table 2, among **I**(**a**−**c**)−**V**(**a**−**c**), against *A. citricola*, compounds **Ia** (R^1 as 3,4-methylenedioxyphenyl) and **IVb** (R^1 as 3,4-methylenedioxyphenylethylenyl; R^2 as 4-ClPh) showed the potent aphicidal activity with 48 h CMRs of 39.3% and 37.1%, respectively. To **Ia−Ic**, like their insecticidal activity, **Ib** (R^1 as 3,4-methylenedioxyphenylethylenyl) exhibited less potent aphicidal activity than the precursor **1**. However, unlike their insecticidal activity, the aphicidal activity of compounds **II**(**a**−**c**) was decreased compared with that of the precursor **2**; to **III**(**a**−**c**)−**V**(**a**−**c**), it indicated that introduction of R^1 as 3,4-methylenedioxyphenylethylenyl scaffold on the oxime ester fragment was vital for the aphicidal activity.

In summary, to discover natural-product-based pesticide candidates, new conjugates of cholesterol/matrine and piperic acid/piperic acids-like were semi-synthesized. Against *M. separata*, compounds **IIc**, **IVc** and **Vb** exhibited (3.2 and 3.5 times promising insecticidal activity of cholesterol) more potent insecticidal activity than toosendanin. Against *A. citricola*, compounds **Ia** (1.45 times of matrine) and **IVb** (2.2 times of cholesterol) showed potent aphicidal activity. Their structure-activity relationships suggested that introduction of R^1 as piperic acid scaffold on the oxime ester fragment was vital for the insecticidal activity; introduction of R^1 as 3,4-methylenedioxycinnamic acid scaffold on the oxime ester fragment was necessary for the aphicidal activity. These will pave the way for future preparation of cholesterol and matrine derivatives as botanical pesticidal agents in agriculture.

EXPERIMENTAL

The multi-generation sensitive strains of *M. separata* (3rd instar larvae) were raised in our lab. *A. citricola* were collected from the apple trees in morrow plots. Matrine and piperine were purchased from Baoji Haoxiang Biotechnology Co. Ltd. (Baoji, China). Cholesterol was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China).

General procedure for synthesis of target compounds I(a−c)−V(a−c)

Compounds **2**−**6** (1 mmol) reacted with acids R^1CO_2H (1.3 mmol) in dry CH_2Cl_2 (10 mL) in the presence of DCC (1.3 mmol) and DMAP (0.1 mmol) at room temperature for 10−50 h. Then the solution was diluted with CH_2Cl_2 (20 mL), washed with 0.1 N aq. HCl (10 mL), 5% aq. Na_2CO_3 (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, and purified by PTLC eluting with petroleum ether/EtOAc (2:1, v/v) to afford **I**(**a**−**c**)−**IV**(**a**−**c**) in 22–83% yields.

Data for Ia: Yield: 57%, yellow solid, mp 148-150 °C, $[\alpha]^{23}_{D}$ -54 (c 0.9 mg/mL, $CHCl_3$); ^{1}H NMR (500

MHz, $CDCl_3$) *δ*: 8.57 (s, 1H), 7.71 (d, *J* = 9.0 Hz, 1H), 7.52 (s, 1H), 6.85 (d, *J* = 8.5 Hz, 1H), 6.04 (s, 2H), 4.00 – 3.98 (m, 1H), 3.89 (d, *J* = 13.5 Hz, 1H), 3.52 (t, *J* = 13.0 Hz, 1H), 2.85 (dd, *J* = 24.5, 11.0 Hz, 2H), 2.53 – 2.51 (m, 2H), 2.16 (s, 1H), 2.01-1.94 (m, 3H), 1.89 – 1.81 (m, 2H), 1.73 – 1.65 (m, 3H), 1.62 – 1.53 (m, 3H), 1.46 (s, 2H), 1.36 (t, *J* = 13.5 Hz, 1H). HRMS [ESI]: calcd for $C_{24}H_{29}ClN_3O_4$ ($[M+H]^+$), 458.1847; found, 458.1833.

Data for Ib**:** Yield: 52%, yellow solid, mp 152-154 °C, $[\alpha]^{23}_D$ -40 (*c* 0.85 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ*: 8.50 (s, 1H), 7.70 (d, *J* = 15.5 Hz, 1H), 7.05 (s, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.35 (d, *J* = 16.0 Hz, 1H), 6.00 (s, 2H), 4.00 – 3.98 (m, 1H), 3.89 (d, *J* = 11.5 Hz, 1H), 3.52 (t, *J* = 13.0 Hz, 1H), 2.85 (dd, *J* = 24.5, 10.4 Hz, 2H), 2.51 (s, 2H), 2.15 (s, 1H), 2.01 – 1.94 (m, 3H), 1.89 – 1.80 (m, 2H), 1.73 – 1.68 (m, 3H), 1.59 (t, *J* = 17.0 Hz, 3H), 1.45 (s, 2H), 1.35 (t, *J* = 13.0 Hz, 1H). HRMS [ESI]: calcd for $C_{26}H_{31}ClN_3O_4$ ($[M+H]^+$), 484.2003; found, 484.1990.

Data for Ic**:** Yield: 56%, yellow solid, mp 156-158 °C, $[\alpha]^{23}_D$ -19 (*c* 0.75 mg/mL, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) *δ* 8.48 (s, 1H), 7.53 (dd, *J* = 15.6, 11.4 Hz, 1H), 7.00 (s, 1H), 6.92 (d, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 15.6 Hz, 1H), 6.79 (d, *J* = 7.8 Hz, 1H), 6.75 – 6.70 (m, 1H), 6.03 (d, *J* = 15.0 Hz, 1H), 5.98 (s, 2H), 4.00 – 3.97 (m, 1H), 3.87 (d, *J* = 10.2 Hz, 1H), 3.52 (t, *J* = 12.6 Hz, 1H), 2.85 (dd, *J* = 29.4, 11.4 Hz, 2H), 2.50 (t, *J* = 5.4 Hz, 2H), 2.15 (s, 1H), 2.01 – 1.94 (m, 3H), 1.88 (d, *J* = 13.8 Hz, 1H), 1.83-1.79 (m, 1H), 1.75 – 1.68 (m, 3H), 1.62 (d, *J* = 14.4 Hz, 3H), 1.45 (s, 2H), 1.35 (t, *J* = 13.8 Hz, 1H). HRMS [ESI]: calcd for $C_{28}H_{33}ClN_3O_4$ ($[M+H]^+$), 510.2160; found, 510.2151.

Data for IIa**:** Yield: 41%, white solid, mp 170-172 °C, $[\alpha]^{23}_D$ -5 (*c* 0.6 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.65 (d, *J* = 9.5 Hz, 1H), 7.47 (s, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.02 (s, 2H), 5.41 (d, *J* = 4.0 Hz, 1H), 4.83 – 4.78 (m, 1H), 2.44 (d, *J* = 8.0 Hz, 2H), 2.03 – 1.96 (m, 3H), 1.92 (d, *J* = 13.5 Hz, 1H), 1.72 (d, *J* = 12.0 Hz, 1H), 1.57 – 1.50 (m, 7H), 1.35 (d, *J* = 15.0 Hz, 3H), 1.25 (s, 2H), 1.18-1.09 (m, 6H), 1.06 (s, 3H), 1.02 (dd, *J* = 18.0, 10.5 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.87–0.85 (m, 6H), 0.68 (s, 3H). HRMS [ESI]: calcd for $C_{35}H_{50}O_4Na$ ($[M+Na]^+$), 557.3607; found, 557.3596.

Data for IIb**:** Yield: 55%, white solid, mp 170-172 °C, $[\alpha]^{23}_D$ 9 (*c* 0.7 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.59 (d, *J* = 16.0 Hz, 1H), 7.02 (s, 1H), 7.00 (d, *J* = 9.0 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.26 (d, *J* = 16.0 Hz, 1H), 6.00 (s, 2H), 5.40 (d, *J* = 4.0 Hz, 1H), 4.76 – 4.70 (m, 1H), 2.40 (d, *J* = 7.0 Hz, 2H), 2.03 (d, *J* = 13.5 Hz, 1H), 1.97 – 1.90 (m, 2H), 1.87 – 1.79 (m, 1H), 1.70 – 1.64 (m, 1H), 1.58 – 1.44 (m, 7H), 1.40 – 1.33 (m, 3H), 1.27 (dd, *J* = 20.5, 9.5 Hz, 2H), 1.17 – 1.09 (m, 6H), 1.04 (s, 3H), 1.01 – 0.95 (m, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.87 (dd, *J* = 6.5, 5.0 Hz, 6H), 0.68 (s, 3H). HRMS [ESI]: calcd for $C_{37}H_{52}O_4Na$ ($[M+Na]^+$), 583.3763; found, 583.3755.

Data for IIc**:** Yield: 71%, yellow solid, mp 157-159 °C, $[\alpha]^{23}_D$ 9 (*c* 0.9 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) *δ*: 7.42 (dd, *J* = 15.5, 11.0 Hz, 1H), 6.99 (s, 1H), 6.91 (d, *J* = 8.0 Hz, 1H), 6.82 (s, 1H), 6.79 (d, *J* = 7.5 Hz, 1H), 6.71 (dd, *J* = 15.0, 11.0 Hz, 1H), 5.98 (s, 2H), 5.93 (d, *J* = 15.5 Hz, 1H), 5.38 (s, 1H), 4.71 – 4.69 (m, 1H), 2.38 (d, *J* = 7.0 Hz, 2H), 2.00 (t, *J* = 12.5 Hz, 2H), 1.89 (t, *J* = 14.5 Hz, 3H), 1.60 – 1.42 (m, 7H), 1.35 – 1.29 (m, 3H), 1.25 (s, 2H), 1.16 – 1.09 (m, 6H), 1.04 – 0.96 (m, 6H), 0.92 (d, *J* = 6.0 Hz, 3H), 0.87 (d, *J* = 5.5 Hz, 6H), 0.68 (s, 3H). HRMS [ESI]: calcd for $C_{39}H_{54}O_4Na$ ($[M+Na]^+$), 609.3920; found, 609.3907.

***Data for IIIa*:** Yield: 68%, white solid, mp 143-145 °C, $[\alpha]^{23}_D$ -100 (*c* 0.85 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.05 (d, *J* = 7.0 Hz, 2H), 7.68 (d, *J* = 9.5 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.49 (s, 1H), 7.44 (t, *J* = 8.0 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.54 (s, 1H), 6.04 (s, 2H), 5.01 (m, 1H), 2.77– 2.73 (m, 1H), 2.70 – 2.61 (m, 2H), 2.50 (t, *J* = 11.5 Hz, 1H), 2.09 (t, *J* = 15.0 Hz, 2H), 2.03 (dd, *J* = 27.5, 13.5 Hz, 2H), 1.89 – 1.80 (m, 1H), 1.62 (s, 3H), 1.53 – 1.46 (m, 2H), 1.43 – 1.39 (m, 1H), 1.34 – 1.28 (m, 5H), 1.25 (s, 1H), 1.22 (s, 3H), 1.17 (dd, *J* = 15.0, 9.0 Hz, 5H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.88 (dd, *J* = 6.5, 2.0 Hz, 6H), 0.74 (s, 3H). HRMS [ESI]: calcd for $C_{42}H_{53}NO_6Na$ ($[M+Na]^+$), 690.3771; found, 690.3759.

***Data for IIIb*:** Yield: 38%, white solid, mp 85-87 °C, $[\alpha]^{23}_D$ -100 (*c* 0.95 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.05 (d, *J* = 7.5 Hz, 2H), 7.70 (d, *J* = 15.5 Hz, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.09 (d, *J* = 2.0 Hz, 1H), 7.06 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.56 (d, *J* = 2.0 Hz, 1H), 6.45 (d, *J* = 16.0 Hz, 1H), 6.00 (s, 2H), 5.00-4.94 (m, 1H), 2.78-2.74 (m, 1H), 2.66 – 2.58 (m, 2H), 2.47 (t, *J* = 11.5 Hz, 1H), 2.08 (t, *J* = 12.5 Hz, 2H), 2.03 – 1.93 (m, 2H), 1.89-1.81 (m, 1H), 1.61 – 1.58 (m, 3H), 1.53 – 1.39 (m, 4H), 1.34 – 1.28 (m, 5H), 1.21 (s, 3H), 1.17 – 1.03 (m, 5H), 0.95 (d, *J* = 6.0 Hz, 3H), 0.88 (dd, *J* = 6.5, 2.5 Hz, 6H), 0.73 (s, 3H). HRMS [ESI]: calcd for $C_{44}H_{55}NO_6Na$ ($[M+Na]^+$), 716.3927; found, 716.3912.

***Data for IIIc*:** (two isomers: 1/1)*:* Yield: 22%, white solid, mp 82-83 °C, $[\alpha]^{23}_D$ -57 (*c* 0.8 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.06 (d, *J* = 7.0 Hz, 2H), 7.91 (dd, *J* = 15.5, 12.0 Hz, 0.5H), 7.57 (t, *J* = 7.5 Hz, 1H), 7.54 (t, *J* = 11.0 Hz, 0.5H) 7.45 (t, *J* = 7.5 Hz, 2H), 7.00 (s, 0.5H), 6.94 (d, *J* = 7.0 Hz, 0.5H), 6.87 – 6.70 (m, 3.5H), 6.53 (s, 1H), 6.34(s, 0.5H) 6.17 (dd, *J* = 30.0, 15.0 Hz, 1H), 5.98 (s, 1H), 5.90 (s, 1H), 5.00 – 4.93 (m, 1H), 2.75 – 2.68 (m, 1H), 2.62 (m, 2H), 2.48-2.43 (m, 1H), 2.08 (t, *J* = 13.5 Hz, 2H), 2.02 (dd, *J* = 16.0, 4.0 Hz, 2H), 1.95 (t, *J* = 8.0 Hz, 1H), 1.62 – 1.51 (m, 6H), 1.43 – 1.41 (m, 2H), 1.34 – 1.29 (m, 4H), 1.21 (d, *J* = 3.0 Hz, 3H), 1.16 – 1.12 (m, 4H), 1.07 (d, *J* = 8.5 Hz, 1H), 0.95 (dd, *J* = 6.5, 2.0 Hz, 3H), 0.88 (dd, *J* = 7.0, 2.5 Hz, 6H), 0.73 (d, *J* = 3.0 Hz, 3H). HRMS [ESI]: calcd for $C_{46}H_{57}NO_6Na$ ($[M+Na]^+$), 742.4084; found, 742.4068.

***Data for IVa*:** Yield: 25%, white solid, mp 163-165 °C, $[\alpha]^{23}_D$ -64 (*c* 0.7 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 8.5 Hz, 2H), 7.68 (d, *J* = 5.0 Hz, 1H), 7.49 (s, 1H), 7.43 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.54 (d, *J* = 1.5 Hz, 1H), 6.04 (s, 2H), 5.01-4.95 (m, 1H), 2.75 – 2.72 (m, 1H), 2.69 – 2.60 (m, 2H), 2.50 (t, *J* = 11.5 Hz, 1H), 2.09 (t, *J* = 10.5 Hz, 2H), 2.03 – 1.92 (m, 2H), 1.87 – 1.80 (m, 1H), 1.61 (s, 3H), 1.54 – 1.38 (m, 4H), 1.36 – 1.28 (m, 5H), 1.25 (s, 1H), 1.22 (s, 3H), 1.19 – 1.03 (m, 5H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.87 (dd, *J* = 6.5, 2.5 Hz, 6H), 0.74 (s, 3H). HRMS [ESI]: calcd for $C_{42}H_{52}ClNO_6Na$ ($[M+Na]^+$), 724.3381; found, 724.3363.

Data for IVb**:** Yield: 24%, white solid, mp 76-78 °C, $[\alpha]^{23}_{D}$ -45 (*c* 0.9 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 8.5 Hz, 2H), 7.70 (d, *J* = 15.5 Hz, 1H), 7.43 (d, *J* = 8.5 Hz, 2H), 7.08 (s, 1H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.55 (s, 1H), 6.44 (d, *J* = 15.5 Hz, 1H), 6.00 (s, 2H), 4.98 – 4.93 (m, 1H), 2.76 – 2.73 (m, 1H), 2.65 – 2.57 (m, 2H), 2.46 (t, *J* = 11.5 Hz, 1H), 2.08 – 2.06 (m, 2H), 2.02-1.93 (m, 2H), 1.88 (dd, *J* = 25.5, 12.0 Hz, 1H), 1.61 – 1.58 (m, 3H), 1.56 – 1.49 (m, 3H),1.46 – 1.40 (m, 3H), 1.36 – 1.33 (m, 3H), 1.21 (s, 3H), 1.18 – 1.13 (m, 5H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.88 (dd, *J* = 6.5, 2.0 Hz, 6H), 0.73 (s, 3H). HRMS [ESI]: calcd for $C_{44}H_{54}ClNO_6Na$ ($[M+Na]^+$), 750.3537; found, 750.3522.

Data for Ivc**:** (two isomers: 1/1)*:* Yield: 26%, white solid, mp 86-88 °C, $[\alpha]^{23}_{D}$ -43 (*c* 0.95 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, *J* = 8.5 Hz, 2H, Ph-H), 7.91 (dd, *J* = 15.0, 11.5 Hz, 0.5H), 7.53 (dd, *J* = 15.0, 10.5 Hz, 0.5H), 7.43 (d, *J* = 8.5 Hz, 2H, Ph-H), 7.00 (s, 0.5H), 6.93 (d, 0.5H), 6.87 – 6.70 (m, 3.5H), 6.52 (d, *J* = 15.0 Hz, 1H), 6.33 (t, *J* = 11.5 Hz, 0.5H), 6.16 (dd, *J* = 30.5, 15.5 Hz, 1H), 5.98 (s, 1H, OCH_2O), 5.91 (s, 1H, OCH_2O), 4.99 – 4.91 (m, 1H, OCH), 2.74 – 2.67 (m, 1H), 2.62 (d, *J* = 10.0 Hz, 2H), 2.48-2.42 (m, 1H), 2.07 (t, *J* = 10.0 Hz, 2H), 2.01 – 1.93 (m, 2H), 1.83 (d, *J* = 12.5 Hz, 1H), 1.61 – 1.49 (m, 5H), 1.42 (d, *J* = 7.5 Hz, 2H), 1.34 – 1.29 (m, 5H), 1.20 (d, *J* = 3.0 Hz, 3H), 1.16 – 1.06 (m, 5H), 0.94 (dd, *J* = 6.5, 2.0 Hz, 3H), 0.88 – 0.86 (m, 6H), 0.73 (d, *J* = 3.0 Hz, 3H). HRMS [ESI]: calcd for $C_{46}H_{56}ClNO_6Na$ ($[M+Na]^+$), 776.3694; found, 776.3681.

Data for Va**:** Yield: 83%, white solid, mp 199-201 °C, $[\alpha]^{23}_{D}$ -63 (*c* 0.85 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 10.0 Hz, 2H, Ph-H), 7.69 (d, *J* = 10.0 Hz, 1H, Ph-H), 7.50 (s, 1H, Ph-H), 6.93 (d, *J* = 5.0 Hz, 2H, Ph-H), 6.88 (d, *J* = 10.0 Hz, 1H, Ph-H), 6.54 (s, 1H), 6.04 (s, 2H, OCH_2O), 5.00 – 4.93 (m, 1H, OCH), 3.87 (s, 3H, OCH_3), 2.76 – 2.72 (m, 1H), 2.70 – 2.59 (m, 2H), 2.50 (t, *J* = 10.0 Hz, 1H), 2.09 (d, *J* = 15.0 Hz, 2H), 2.02–1.93 (m, 2H), 1.87–1.80 (m, 1H), 1.62 (s, 3H), 1.55 – 1.39 (m, 3H), 1.36 – 1.26 (m, 6H), 1.22 (s, 3H), 1.19-1.03 (m, 5H), 0.95 (d, *J* = 5.0 Hz, 3H), 0.88 (dd, *J* = 6.5, 2.0 Hz, 6H), 0.74 (s, 3H). HRMS [ESI]: calcd for $C_{43}H_{55}NO_7Na$ ($[M+Na]^+$), 720.3876; found, 720.3861.

Data for Vb**:** Yield: 29%, white solid, mp 168-170 °C, $[\alpha]^{23}_{D}$ -43 (*c* 0.9 mg/mL, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 8.5 Hz, 2H, Ph-H), 7.70 (d, *J* = 16.0 Hz, 1H, Ph-H), 7.08– 7.04 (m, 2H, Ph-H), 6.93 (d, *J* = 6.5 Hz, 2H, Ph-H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.54 (s, 1H), 6.44 (d, *J* = 16.0 Hz, 1H), 6.00 (s, 2H, OCH_2O), 4.96 – 4.91 (m, 1H), 3.86 (s, 3H, OCH_3), 2.76 – 2.73 (m, 1H), 2.66 – 2.56 (m, 2H), 2.46 (t, *J* = 11.0 Hz, 1H), 2.08 (d, *J* = 12.0 Hz, 2H), 2.01 – 1.93 (m, 2H), 1.87 – 1.82 (m, 1H), 1.60 – 1.52 (m, 6H), 1.43 – 1.40 (m, 1H), 1.36-1.30 (m, 5H), 1.21 (s, 3H), 1.18 – 1.11 (m, 5H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.88 (dd, *J* = 6.5, 2.5 Hz, 6H), 0.73 (s, 3H). HRMS [ESI]: calcd for $C_{45}H_{57}NO_7Na$ ($[M+Na]^+$), 746.4033; found, 746.4018.

***Data for Vc*:** (two isomers: 0.67/1)*:* Yield: 24%, white solid, mp 78-80 °C, $[\alpha]^{23}_{D}$ -34 (*c* 0.85 mg/mL, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ: 8.01 (d, *J* = 8.5 Hz, 2H, Ph-H), 7.91(dd, *J* = 15.0, 12.0 Hz, 0.4H), 7.53 (dd, *J* = 15.5, 11.0 Hz, 0.6H), 7.00 (s, 0.5H), 6.93 (d, *J* = 8.5 Hz, 2.5H, Ph-H), 6.87 – 6.70 (m, 3.5H), 6.52 (d, *J* = 14.5 Hz, 1H), 6.33 (t, *J* = 11.5, 0.4H), 6.16 (dd, *J* = 30.5, 15.0 Hz, 1H), 5.98 (s, 1H), 5.91 (s, 0.6H), 4.97 – 4.88 (m, 1H), 3.86 (s, 3H, OCH_3), 2.74 – 2.67 (m, 1H), 2.64 – 2.56 (m, 2H), 2.48 – 2.42 (m, 1H), 2.07 – 2.04 (m, 2H), 2.01 – 1.91 (m, 2H), 1.85-1.77 (m, 1H), 1.62 – 1.50 (m, 5H), 1.47 – 1.40 (m, 2H), 1.36 – 1.27 (m, 5H), 1.20 (d, *J* = 3.0 Hz, 3H), 1.16 – 1.12 (m, 4H), 1.06-1.02 (m, 1H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.87 – 0.86 (m, 6H), 0.73 (d, *J* = 3.0 Hz, 3H). HRMS [ESI]: calcd for $C_{47}H_{59}NO_7Na$ ($[M+Na]^+$), 772.4189; found, 772.4175.

Biological assay

Insecticidal activity of compounds **I(a−c)−V(a−c)** *against early third-instar larvae of M. separata.*[16] Thirty early 3rd-instar larvae of *M. separata* were chosen as the tested insects for each compound. The solutions of compounds**1**, **2**, and **I**(**a**−**c**)−**V**(**a**−**c**) (toosendanin used as a positive control) were prepared in acetone at 1 mg/mL. After dipped into the corresponding solution for 3 sec, wheat leaf discs (1×1 cm) were taken out, and dried. Wheat leaf discs were treated by acetone alone as the blank control group (CK). Some above discs were added to each culture dish (ten insects per dish). Once the discs were consumed, additional ones were added. After 48 h, the rest of compound-soaked discs were cleaned out, and the untreated ones were added till the end of pupae (temperature: 25 ± 2 °C; RH: 65–80%; photoperiod: light/dark = 12 h/12 h). Their corrected final mortality rate values were calculated as follows: corrected mortality rate (%) = $(T - C) \times 100/(100\% - C)$; *C* is the mortality rate of CK, and *T* is the mortality rate of the treated *M. separata*.

Aphicidal activity of compounds **I**(**a**−**c**)−**V**(**a**−**c**) *against apterous adults of A. citricola.*[16] The solutions of compounds **1**, **2**, and **I**(**a**−**c**)−**V**(**a**−**c**) were prepared in acetone at 1 mg/mL. For each compound, 90 healthy and size-consistency of *A. citricola* (30 aphids per group) were selected out. The corresponding solution (0.04 μL) was added to the pronotum of aphids, which were then transferred into fresh apple leaves in 9 cm culture dish (30 aphids per dish). The aphids treated with acetone alone were used as CK, and methomyl was used as a positive control. The experiment was carried out at 25 ± 1 °C and 50 ± 7% relative humidity (RH), and on 14 h/10 h (light/dark) photoperiod. Their mortalities were recorded at 24 and 48 h after treatment. Their corrected mortality rate values were calculated as follows: corrected mortality rate (%) = $(T - C) \times 100/(100\% - C)$; *C* is the mortality rate of CK, and *T* is the mortality rate of the treated *A. citricola*.

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