SYNTHESIS AND INSECTICIDAL ACTIVITY OF NOVEL DIMERS OF CELANGULIN-V AND PODOPHYLLOTOXIN

Ji-wen Zhang,[1,2] Sheng-kun Li,[1,2] Zhi-qin Ji,[1] Zhao-nong Hu,[1] and Wen-jun Wu[1,2*]

UDC 547.66

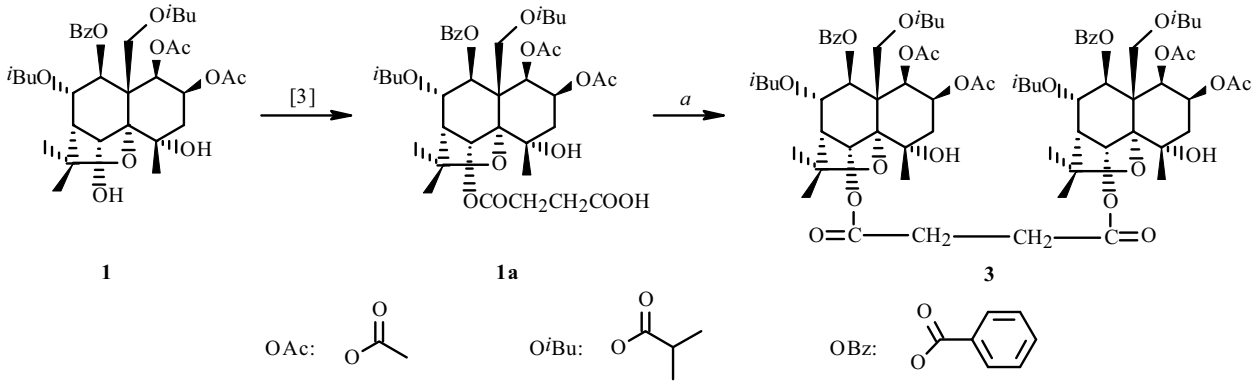
*Celangulin-V (**1**) and podophyllotoxin (**2**) are both natural compounds with significant insecticidal activity. The identical and nonidentical dimers of celangulin-V and podophyllotoxin (**3**, **4**, **5**) were synthesized using succinic acid as the linker, and their insecticidal activities against 3rd instar larvae of* Mythimna separata *were tested. Only the identical dimer of celangulin-V (**3**) show some insecticidal activity.*

Key words: celangulin, podophyllotoxin, dimers, insecticidal activity.

Natural products have been a continuous source of new lead compounds for drugs and pesticides. In recent years, much interest has centered on the role of natural products influencing the association of plants and insects and development of botanical pesticides possessing high insecticidal activity, low toxicity in nontarget organisms, and safety in the environment [1, 2].

Celangulin-V (**1**) and podophyllotoxin (**2**) are two important bioactive natural compounds from *Celastrus angulatus* M. and *Juniperus sabina* L. that showed antifeedant activity and stomach poisonous effects against *Mythimna separata* [3–5]. As lead compounds for pesticides, a large number of derivatives of podophyllotoxin and celangulin-V have been prepared, some of which displayed significant insecticidal activity [5, 6]. To our knowledge, there are no reports on the insecticidal activity and synthesis of dimers of celangulin-V and podophyllotoxin, although some dimeric chemical structures have especially high biological activities in drug research. Examples of identical twin drugs and nonidentical twin drugs include artemisinin [7, 8], protein kinase C (PKC) [9], and paclitaxel–camptothecin [10].

The aim of this work was to investigate whether the two insecticidal compounds link together to make dimers displaying multiple activity or improved activity. We report in this paper the synthesis and insecticidal activity of the identical **3** and **4** and nonidentical **5**.



a. DCC, DMAP, DCM, stired at 20°C for 3 h.

Scheme 1. Synthesis pathway of compound **3**.

1) Institute of Pesticide, Northwest A&F University, Yangling, Shaanxi 712100, China, e-mail: nwzjw@yahoo.com.cn; 2) College of Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 431–433, July–August, 2009. Original article submitted February 7, 2008.

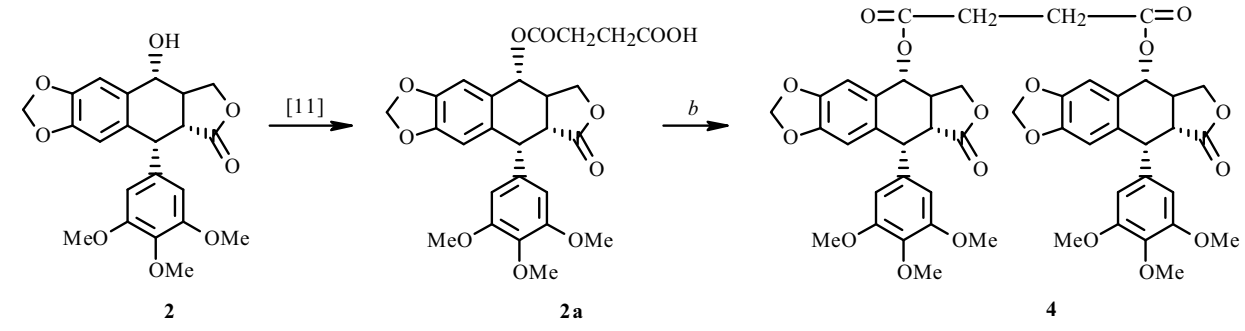
0009-3130/09/4504-0507

TABLE 1. Experimental Data of the Three Dimmers

Compound	Formula	HRMS/([M+Na]$^+$) Calcd. (Found)	$[\alpha]_D^{20}$ ° (c, mg/mL)	Mp, °C	Yield, %
3	$C_{72}H_{94}O_{28}$	1429.5829(1429.5833)	+2/(0.6)	233–235	63.6
4	$C_{48}H_{46}O_{18}$	933.2582(933.2571)	–50/(0.4)	180–182	60.4
5	$C_{60}H_{70}O_{23}$	1181.4206(1181.4218)	–62/(0.5)	176–178	54.6

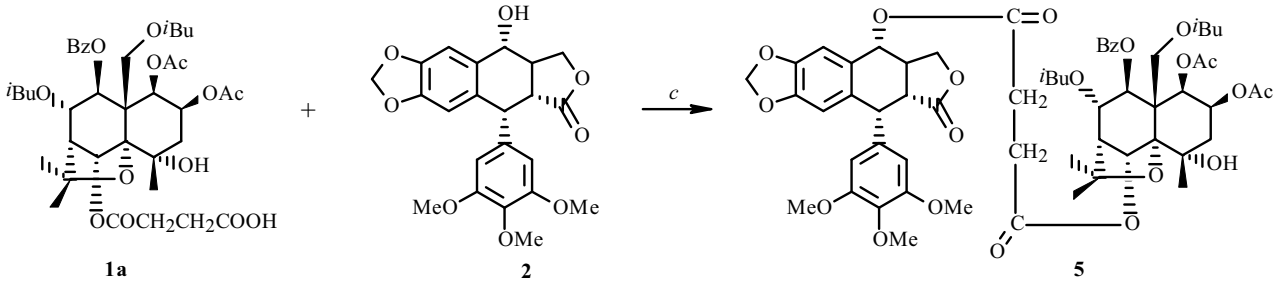
TABLE 2. Insecticidal Activities Data of Compounds **1–5**

Test, %	CK	**1**	**2**	**3**	**4**	**5**
Mortality rate	0	46.7	13.3	16.7	0	0
Antifeedant index	0	26.2	67.3	0	0	0



b. DCC, DMAP, DCM, stired at 20°C for 3 h.

Scheme 2. Synthesis pathway of compound **4**.



c. DCC, DMAP, DCM, stired at 20°C for 3 h.

Scheme 3. Synthesis pathway of compound **5**.

The synthesis pathways of compounds **3**, **4**, and **5** are shown in Scheme 1, 2, and 3. The experimental data of the dimers are shown in Table 1.

All the structures of the dimers were certified by ^{1}H NMR, ^{13}C NMR, DEPT, and HR-ESI-MS. Besides the monomers, the methylene and carbonyl signals of the succinic acid linker can been found in the NMR spectra of the dimers. Signals for the linker of compound **3** appear at δ 3.47 (4H, m), 29.3 (CH_2), and 170.5 (C=O); signals for compound **4** appear at 2.77 (4H, t, J = 3.5), 29.1 (CH_2), and 173.6 (C=O); and signals for compound **5** appear at 3.48 (2H, m), 2.73 (2H, m), 29.5 (CH_2), 33.9 (CH_2), 170.5 (C=O), and 173.7 (C=O). Insecticidal activities against 3rd instar larvae of *Mythimna separata* of the three dimers were tested. Unfortunately, they do not show any antifeedant activities at concentration of 5%, and only compound **3** shows some stomach poisonous activity. The insecticidal activity data of the dimers are shown in Table 2.

EXPERIMENTAL

Celangulin-V (**1**) was provided by the Institute of Pesticides, Northwest A&F University. Podophyllotoxin (**2**) was provided by Prof. Xuan Tian, Lanzhou University.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-500 spectrometer in $CDCl_3$ with TMS as internal standard. Melting points were recorded on an electrothermal digital apparatus made in Beijing and were uncorrected. HR-MS were recorded under ESI condition using a APEX II 49e (ESI) instrument. Optical rotation was measured by a Perkin–Elmer Model 241 instrument. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel), and spots were visualized with H_2SO_4–EtOH. Separation and purification were carried out by column chromatography on silica gel (200–300 mesh) (Qingdao Haiyang Chemical Group Co., P. R. China). Yields were not optimized. Succinic acid celangulin-V ester (**1a**) and succinic acid podophyllotoxin ester (**2a**) were prepared by the literature method [3, 11]. Solvents were dried by standard methods and distilled. The title compounds were synthesized under a nitrogen atmosphere.

Insecticidal activity was investigated using a leaf disc choice method. The concentration of the tested compounds was 5% in acetone [12].

Synthesis of 3. A mixture of celangulin-V (**1**) (0.079 g, 0.12 mmol) and succinic acid celangulin-V ester (**1a**) (0.076 g, 0.1 mmol), dimethylaminopyridine (DMAP, 0.012 g, 0.1 mmol), and *N*,*N*-dicyclohexylcarbodiimide (DCC, 0.042 g, 0.02 mmol) in dry dichloromethane (DCM, 20 mL) was stirred for 3 h at 20°C. The resulting suspension was filtered, and saturated NH_4Cl (30 mL) was added into the DCM layer. After extracting with dichloromethane (25 mL × 3), the organic layer was dried and concentrated to give the crude product, which was then purified by silica gel column. A yield of 63.6% (0.089 g) of a white powder was obtained, mp 233–235°C, $[\alpha]_D^{20}$ +2° (*c* 0.6, MeOH), $C_{72}H_{94}O_{28}$ HRMS/([M+Na]$^+$) Calcd. (Found) 1429.5829 (1429.5833).

^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm, J/Hz): 5.47 (2H, d, J = 3.5), 5.36 (2H, d, J = 3.5), 1.96, 2.09 (4H, m), 6.51 (2H, s), 2.53 (2H, d, J = 3.5), 5.77 (2H, dd, J = 3.5, J = 9.5), 6.04 (2H, d, J = 9.5), 4.88, 4.66 (4H, dd, J = 13.5), 1.72 (6H, s), 1.60 (6H, s), 1.47 (6H, s), 2.92 (2H, m), 2.37 (2H, m), 1.38 (12H, m), 0.97 (12H, m), 7.86 (4H, d, J = 8.0), 7.41 (4H, dd, J = 7.5, J = 8.0), 7.55 (2H, dd, J = 7.5, J = 8.0), 2.09 (6H, s), 1.54 (6H, s), 3.47 (4H, m).

^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 75.5 (CH), 67.7 (CH), 42.1 (CH_2), 69.6 (C), 92.2 (C), 75.9 (CH), 52.3 (CH), 73.4 (CH), 75.2 (CH), 51.5 (C), 84.4 (C), 61.8 (CH_2), 24.7 (CH_3), 29.8 (CH_3), 25.9 (CH_3), 176.8 (C=O), 175.6 (C=O), 169.6 (C=O), 169.5 (C=O), 165.6 (C=O), 21.1 (CH_3), 20.5 (CH_3), 133.5 (CH), 128.7 (2 × CH), 129.3 (C), 129.4 (2 × CH), 34.2 (CH), 34.1 (CH), 19.1 (CH_3), 19.0 (CH_3), 18.6 (CH_3), 18.5 (CH_3), 170.5 (C=O), 29.3 (CH_2).

Synthesis of 4 and 5. Compound **4** was prepared from podophyllotoxin (**2**) and compound **5** from podophyllotoxin (**2**) and succinic acid celangulin-V ester (**1a**) in the same way.

Compound **4** was obtained as a white powder, yield 60.4%, mp 180–182°C, $[\alpha]_D^{20}$ –50° (*c* 0.4, MeOH), $C_{48}H_{46}O_{18}$ HRMS/([M+Na]$^+$) Calcd. (Found) 933.2582 (933.2571).

^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm, J/Hz): 4.12 (2H, m), 4.62 (2H, d, J = 4.0), 6.55 (2H, s), 5.97 (2H, d, J = 1.5), 5.99 (2H, d, J = 1.5), 6.81 (2H, s), 5.95 (2H, d, J = 9.0), 2.92 (2H, m), 4.39 (2H, m), 4.19 (2H, m), 6.40 (4H, s), 3.76 (12H, s), 3.81 (6H, s), 2.77 (4H, m).

^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 173.6 (C=O), 45.5 (CH), 43.7 (CH), 128.0 (C), 106.9 (CH), 148.2 (C), 101.7 (CH_2), 147.6 (C), 109.8 (2 × CH), 132.3 (C), 74.2 (CH), 38.6 (CH), 71.2 (CH_2), 134.8 (C), 108.2 (CH), 152.7 (2 × C), 137.3 (C), 56.2 (2 × CH_3), 60.8 (CH_3), 172.9 (C=O), 29.1 (CH_2).

Compound **5** was obtained as a white powder, yield 54.6%, mp 176–178°C, $[\alpha]_D^{20}$ –62° (*c* 0.5, MeOH), $C_{60}H_{70}O_{23}$ HRMS/([M+Na]$^+$) Calcd. (Found) 1181.4206 (1181.4218).

^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm, J/Hz): 5.48 (1H, d, J = 3.0), 5.36 (1H, d, J = 3.0), 1.95, 2.09 (2H, m), 6.53 (1H, s), 2.53 (1H, d, J = 3.0), 5.77 (1H, dd, J = 3.0, J = 9.5), 6.05 (1H, d, J = 9.5), 4.88, 4.67 (2H, dd, J = 13.0), 1.73 (3H, s), 1.59 (3H, s), 1.51 (3H, s), 2.38 (1H, m), 2.81 (1H, m), 0.95 (6H, m), 1.38 (6H, m), 2.08 (3H, s), 1.55 (3H, s), 7.86 (2H, d, J = 8.0), 7.41 (2H, dd, J = 8.0, J = 7.5), 7.56 (1H, dd, J = 8.0, J = 7.5), 3.48 (2H, m), 2.73 (2H, m), 4.17 (1H, m), 4.61 (1H, d, J = 4.0), 6.53 (1H, s), 5.99 (2H, m), 6.79 (1H, s), 5.93 (1H, d, J = 9.0), 2.70 (1H, m), 4.39 (1H, m), 4.19 (1H, m), 6.40 (2H, s), 3.77 (6H, s), 3.82 (3H, s).

^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 75.5 (CH), 67.6 (CH), 42.1 (CH_2), 69.6 (C), 92.1 (C), 75.9 (CH), 52.3 (CH), 73.4 (CH), 75.2 (CH), 51.4 (C), 84.4 (C), 61.8 (CH_2), 24.7 (CH_3), 29.8 (CH_3), 25.6 (CH_3), 176.8 (C=O), 175.6 (C=O), 169.6 (C=O), 169.5 (C=O), 165.6 (C=O), 21.2 (CH_3), 20.5 (CH_3), 133.5 (CH), 128.7 (2 × CH), 129.4 (2 × CH), 129.5 (C), 34.3

(CH), 34.2 (CH), 19.0 (CH_3), 18.9 (CH_3), 18.6 (CH_3), 18.4 (CH_3), 170.5 (C=O), 29.5 (CH_2), 33.9 (CH_2), 173.7 (C=O), 172.8 (C=O), 45.6 (CH), 43.8 (CH), 128.2 (C), 106.9 (CH), 148.2 (C), 101.6 (CH_2), 147.6 (C), 109.8 (2 × CH), 132.3 (CH), 73.9 (CH), 38.6 (CH), 71.2 (CH_2), 134.8 (C), 108.2 (CH), 152.7 (2 × C), 137.2 (C), 56.2 (2 × CH_3), 60.8 (CH_3).

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (No. 30800729) and the 973 project of China (2003CB114404).

REFERENCES

1. Murray B. Isman, *Pest Manag. Sci.,* **64**, 8 (2008).
2. Leonard G. Copping and Stephen O. Duke, *Pest Manag. Sci*., **63**, 524 (2007).
3. Zhijun Qi, Xiaoping Xue, Wenjun Wu, Jiwen Zhang, and Runya Yang, *J. Agric. Food Chem.,* **54**, 20, 7600 (2006).
4. Jin-Ming Gao, Wen-Jun Wu, Ji-Wen Zhang, and Yasuo Konishi, *Nat. Prod. Rep.*, **24**, 1153 (2007).
5. Ying-Qian Liu, Liu Yang, Yanqing Liu, Xudong Di, Hang Xiao, Xuan Tian, and Rong Gao, *Nat. Prod. Res.*, **21**, 11, 967 (2007).
6. Zhang Jiwen, Ji Zhiqin, and Wu Wenjun, *J. Northwest A&F. Univ*., **32**, 10, 99 (2004).
7. J. Prince Jeyadevan, Patrick G. Bray, James Chadwick, Amy E. Mercer, Aoife Byrne, Stephen A. Ward, B. Kevin Park, Dominic P. Williams, Rick Cosstick, Jill Davies, Adrian P. Higson, Ed Irving, Gary H. Posner, and Paul M. O′Neill, *J. Med. Chem.*, **47**, 1290 (2004).
8. Yonghong Yuan, Dongzhi Wei, Yanhua Lu, and Yuling Dou, *Chem. Nat. Comp.,* **43**, 539 (2007).
9. Jayalakshmi Sridhar, Zhi-Liang Wei, Ireneusz Nowak, Nancy E. Lewin, Jolene A. Ayres, Larry V. Pearce, Peter M. Blumberg, and Alan P. Kozikowski, *J. Med. Chem.*, **46**, 4196 (2003).
10. Hironori Ohtsu, Yuka Nakanishi, Kenneth F. Bastow, Fang-Yu Lee, and Kuo-Hsiung Lee, *Bioorg. Med. Chem.,* **11**, 1851 (2003).
11. Xu Dunming, Yu Xiangyang, Liu Yingqian, Feng Juntao, Ma Junqing, Liu Xianjin, and Zhang Xing, *Acta Pharm. Sin.*, **40** (6), 518 (2005).
12. M. Agarwal, S. Walia, S. Dhingra, and B. P. S. Khambay, *Pest Manag. Sci.*, **57**, 289 (2001).