Synthesis of Novel Environmentally Friendly Dehydronor-Cantharidin Insecticides

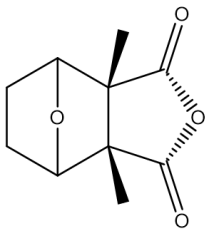
Jianxin Sun[1][a], Mengjing Feng[1][b] and Liangzhong Xu[1,2][c]
[1]*College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China*
[2]*State Key Laboratory Base of Eco-chemcal Engineering, Qingdao 266042, China*

Keywords: Dehydronorcantharidin, Synhtesis, Biological Activity.

Abstract: In order to find new environmentally friendly compounds with insecticidal activity, a series of nornorcantharidin dihydrazide derivatives were synthesized by active substructure splicing method based on the structure of norcantharidin and dihydrazide. the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS. The results of biological activity test showed that compound I_2 had the highest activity (52% at the concentration of 1 mg/L against *plutella xylostella* and 70% at the concentration of 10 mg/L against *Tetranychus cinnabarinus*).

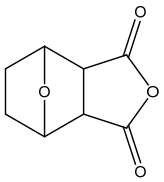
1 INTRODUCTION

Insecticides from natural products are environmentally compatible, so they are ideal sources for developing environmentally friendly insecticides. Cantharides (Zhang 2018) is a traditional Chinese medicine, which comes from the dried bodies of meloidae insects. It is mainly used in the treatment of malignant tumors and tinea (Song 2020). It is difficult to synthesize cantharidin artificially (Wang 2014), so the main source of cantharidin is dry *Mylabris phalerata Pallas*, so the main way to obtain cantharidin is still natural extraction.



Cantharides

These factors seriously affect the development and application of cantharidin. Norcantharidin (Zhou 2020) is one of the important derivatives of cantharidin. It can be obtained from maleic anhydride and furan by two steps of Diels-alder reaction and catalytic hydrogenation reaction (Chi 2019, Ogura 2021). The synthesis route is simple, the atom economy is good and the synthesis cost is low. It can be used in large-scale industrial production. In addition, there are some similarities in biological activities between the two compounds (Yang 2016). This paper refers to dihydrazide pesticides as the main reference structure, through the use of dehydronorcantharidin (Jin 2015, Li 2014) part and dihydrazide key active intermediate tert butyl hydrazine condensation, dehydronorcantharidin (He 2020) derivatives were obtained, and then applied the active substructure splicing theory to synthesize a series of compounds.



Norcantharidin

[a] https://orcid.org/0000-0002-2070-2443
[b] https://orcid.org/0000-0002-6785-9245
[c] https://orcid.org/0000-0002-8391-2739

Sun, J., Feng, M. and Xu, L.
Synthesis of Novel Environmentally Friendly Dehydronor-cantharidin Insecticides.
DOI: 10.5220/0011297200003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 833-837
ISBN: 978-989-758-595-1

2 SYNTHESIS OF THE TARGET COMPOUNDS

2.1 Synthesis of the Target Compounds

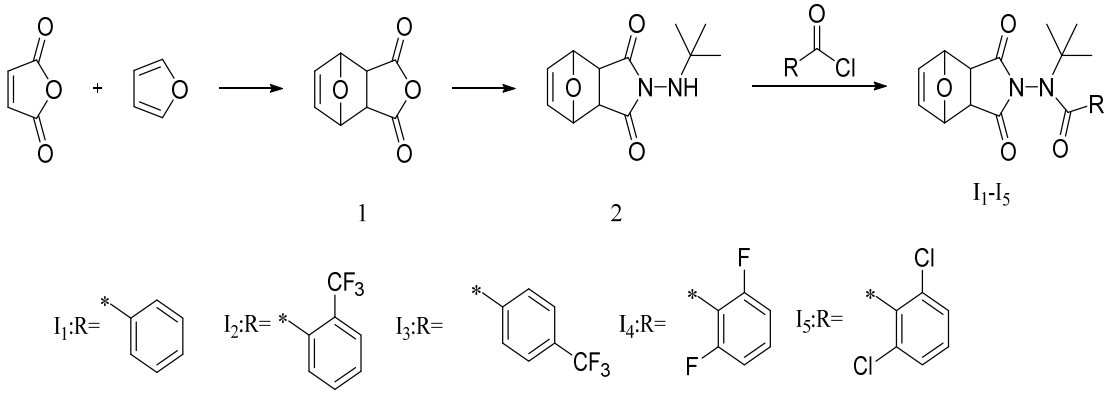


Figure 1: Synthetic route of target compounds I_1-I_5.

2.1.1 General Information for Synthesis

All reagents and solvents were used as received from commercial sources. All reactions were carried out under air atmosphere and monitored by thin-layer chromatography (TLC) performed on 0.25 mm silica gel plates (GF254) purchased from Haiyang Chemical Industry Co., Ltd (Qingdao, China). The TLC plates were visualized with a X-4 ultraviolet analyser. Column chromatographic purifications were carried out on silica gel (200-300 mesh) using petroleum ether (PE) and ethyl acetate (EA) as eluents. All ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AV-500 instrument. Chemical shifts (δ) were expressed in parts per million (ppm) with TMS used as an internal standard. High resolution electro-spray ionization mass spectra (HRMS-ESI) were obtained using a Waters G2-XS instrument.

2.1.2 Synthesis of Nornorcantharidin (1)

A 250 mL three-necked round bottom flask equipped with mechanical stirrer, addition funnel and thermometer, then added 9.8 g (0.1 mol) maleic anhydride and 80 mL ethyl acetate respectively, keep the room temperature, then 34.3 g (0.5 mol) furan was added, while slowly rise to 40℃, continue stirring for 8 hours, during which A lot of white solid appeared. The reaction mixture was filtered and dried to obtain 15.3 g (compound 1). as a white solid.

2.1.3 Synthesis of Compound 2

To a solution of 18.7 g tert butylhydrazine hydrochloride in 80 mL ethanol, 15.2 g of trimethylamine was added dropwise. while keeping internal temperature between 35℃-40℃), 16.6 g compound 1 was added in portions, continue stirring for 4h, 80 mL of water was added in one portion, then a lot of white solid appeared. The reaction mixture was filtered and dried to obtain 21.3 g (compound 2).as a white solid.

2.1.4 Synthesis of Compound I_1

To a solution of 2.36 g compound 2 and 1.32 g Potassium pyrophosphate in 40 mL acetonitrile added 1.5 g benzoyl chloride dropwise, then maintaining gentle reflux, after compound 2 disappears, 40 g water was added. The reaction mixture was filtered and the filter cake was dried to give a crude product. After purified by the flash chromatography, 2.8 g I_1 was obtained as a white solid. (compound I_2-I_5 were obtain by the same method).

2.2 Data of Compounds

Data for the I_1:white solid, yield 88%, m.p.169-170℃. ^{1}H NMR (500 MHz, DMSO-d_6), δ(ppm), 7.85-7.89, (m, 2H, Ar-H), 7.48-7.51(m, 3H, Ar-H), 6.15(s, 2H, CH), 4.76(s, 2H, CH), 2.97(s, 2H, CH), 1.40(s, 9H, CH3). ^{13}C NMR (125 MHz, DMSO-d_6), δ(ppm), 177.28, 177.28, 168.82, 139.46, 139.46, 139.44, 130.81, 128.12, 128.12, 128.00, 128.00, 80.21, 80.21, 58.14, 43.73, 43.73, 26.84, 26.84,

26.84. HRMS: Calcd for $C_{19}H_{21}N_2O_4^+[M+H]^+$: 341.1511; Found: 340.1420.

Data for the I_2: white solid, yield 67%, m.p.155-157℃. ^{1}H NMR (500 MHz, DMSO-d_6), δ(ppm), 7 .65-7.99(m, 4H, Ar-H), 6.15 (s, 2H, CH), 4.76 (s, 2H, CH), 2.97 (s, 2H, CH), 1.41 (s, 9H, CH3). ^{13}C NMR (125 MHz, DMSO-d_6), δ(ppm), 176.50, 176.50, 171.10, 139.46, 139.46, 134.50, 132.06, 129.46, 129.00, 128.18, 127.08, 126.15, 80.21, 80.21, 59.65, 43.73, 43.73, 26.28, 26.28, 26.28. HRMS Calcd for $C_{20}H_{20}F_3N_2O_4^+[M+H]^+$: 409.1375; Found: 409.1382.

Data for the I_3: white solid, yield 89%, m.p.166-168℃. ^{1}H NMR (500 MHz, DMSO-d_6)δ (ppm) 8.27-7.76 (m, 4H, Ar-H), 6.15(s, 2H, CH), 4.76 (s, 2H, CH), 2.97 (s, 2H, CH), 1.40 (s, 9H, CH3). ^{13}C NMR (125 MHz, DMSO-d_6)δ 177.28, 177.28, 167.11, 142.85, 139.46, 139.46, 132.91, 130.83, 125.88, 125.88, 124.90, 80.20, 80.20, 58.14, 43.73, 43.73, 26.84, 26.84, 26.84. HRMS: Calcd for $C_{20}H_{20}F_3N_2O_4^+[M+H]^+$: 409.1375; Found: 408.1370.

Data for the I_4: white solid, yield 70%, m.p.138-139℃. ^{1}H NMR (500 MHz, DMSO-d_6), δ(ppm), 7.63-7.05(m, 3H, Ar-H), 6.15(s, 2H, CH), 4.76(s, 2H, CH), 2.97(s, 2H, CH), 1.42(s, 9H, CH3). ^{13}C NMR (125 MHz, DMSO-d_6), δ(ppm), 176.86, 176.86, 163.94, 159.76, 159.76, 139.46, 139.46, 132.31, 114.90, 111.44, 111.44, 80.21, 80.21, 58.22, 43.73, 43.73, 26.48, 26.48, 26.48. HRMS Calcd for $C_{19}H_{19}F_2N_2O_4^+[M+H]^+$: 371.1313; Found: 376.1307.

Data for the I_5:white solid, yield 85% m.p.140-142℃. 1H NMR (500 MHz, DMSO-d_6), δ(ppm), 7.91-7.52 (m, 3H, Ar-H), 6.15(s, 2H, CH), 4.76(s, 2H, CH), 2.97(s, 2H, CH), 1.46(s, 9H, CH3). 13C NMR (125 MHz, DMSO-d_6), δ(ppm), 176.62, 176.62, 166.00, 139.46, 139.46, 137.61, 133.57, 133.57, 131.75, 127.57, 127.57, 80.21, 80.21, 57.34, 43.73, 43.73, 26.80, 26.80, 26.80. HRMS Calcd for $C_{19}H_{19}Cl_2N_2O_4^+[M+H]^+$: 409.0722; Found: 408.0727.

3 INSECTICIDAL ACTIVITY

According to the methods from literatures, tested the insecticidal activities against *plutella xylostella* and *Tetranychus cinnabarinus*. The main contents of the method were as follows: a certain concentration of compound mother liquor was prepared with DMF, and then diluted with 0.1% Tween-80 solution according to different concentration gradients, so as to prepare different concentrations of drug solutions. Take out the fresh and clean cabbage leaves with a punch with a diameter of 5 cm and uniform size and shape. Place the cabbage leaves in different concentrations of liquid medicine and soak them for 10-20 s. after that, put the dry cabbage leaves into a Petri dish padded with two layers of filter paper. The filter paper was wetted with clean water to ensure the humidity in the Petri dish. 30 diamondback moth larvae in the same growth state were cultured in a Petri dish for 48 hours (temperature controlled at 25℃, photoperiod: L: D = 16:8, relative humidity maintained at 60%). Checked and recorded the death number of *Plutella xylostella* larvae and calculate the mortality. During the experiment, clear water was set as the blank control group, and each group was set with three groups of repeated tests. The final experimental results were the average of the three groups of parallel tests.

Using spray method as test method, taking *Tetranychus cinnabarinus* as test object. At first, DMF was used to dilute the target compound into different concentrations of liquid medicine, then spray the leaves of the broad bean leaves with the same number and growth form. Then the leaves were placed in the observation room, and the condition of *Tetranychus cinnabaris* was recorded after 48 h. The death judgment method is to touch the mite body with a brush, and if there is no response, it is regarded as dead. Three groups of controls are set for each concentration, and the average mortality of each three parallel groups was taken as the mortality of this concentration.

4 RESULTS AND DISCUSSION

The data in Table 1 showed that all compounds had certain insecticidal activities. When the concentration of compound was 10 mg/L, the insecticidal activities of compound I_1, I_2 and I_3 were higher than 90%. When the concentration was reduced to 1 mg/L, the insecticidal activities against *plutella xylostella* of compound I_2 were higher than 52%.

The data in Table 2 showed that at the concentration of 100 mg / L, the acaricidal activities of compounds I_1, I_2, I4 and I_5 reached 100%, and the acaricidal activities of I_2 and I_5 were more than 80%. When the concentration of compound continued to decrease to 50 mg / L, the acaricidal activities of compounds I_2 and I_5 were 87% and 81% respectively. According to the relationship between structure and activity, the activity of o-trifluoromethylphenyl compound is higher than that of other compounds. Therefore, it shows that fluorine-containing groups can significantly improve the activity of compounds, and the position of fluorine-containing group

substituents is very important for insecticidal and acaricidal activity. When the concentration was reduced to 10 mg / L, the activity of I_2 was 70%, and it still had high activity.

Table 1: Insecticidal activities against *plutella xylostella* of target compounds.

No.	compound	lethality rate against *plutella xylostella*(%)				
		50 mg/L	20 mg/L	10 mg/L	5 mg/L	1 mg/L
1	I_1	100	100	95	72	33
2	I_2	100	100	96	83	52
3	I_3	100	100	93	43	9
4	I_4	100	100	86	76	33
5	I_5	82	56	37	0	0

Table 2: The insecticidal activity against *Tetranychus cinnabarinus* results of target compounds.

No.	Compound	lethality rate against *Tetranychus cinnabarinus* (%)		
		100 mg/L	50 mg/L	10 mg/L
1	I_1	100	78	33
2	I_2	100	87	70
3	I_3	79	56	37
4	I_4	100	76	55
5	I_5	100	81	65

5 CONCLUSIONS

In summary, 5 novel types of norcantharidin derivatives were designed and synthesized, the preliminary insecticidal activity against *plutella xylostella* and *Tetranychus cinnabarinus* test showed that all 5 target compounds had certain insecticidal activities. Among them, the compound containing o-trifluoromethylphenyl (I_2) had the highest insecticidal activity against plutella xylostella, and the insecticidal activity was 52% at the concentration of 1 mg/L and the acaricidal activity against Tetranychus cinnabarinus was 70%, at the concentration was reduced to 10 mg / L. The synthesis of these compounds had high atom utilization rate, less waste and high insecticidal activity. Besides, norcantharidin had good biocompatibility and degraded easily in nature, so norcantharidin derivatives were ideal environmental protection insecticides. This work also revealed that compound I_2 could be used as novel lead structures for further research.

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