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Short Report

Potential Insecticidal Activity of Steroidal C-17 Pyrazolinyl Derivatives

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Agrochemical research, over the last two decades, has resulted in the discovery of chemically novel insecticides, of which steroids-based compounds that mimic the action of hormones have been considered as safe insecticides. In this study, eight steroidal C-17 pyrazolinyl derivatives were resynthesized through molecular hybridization and their insecticidal activities against 4th instar larvae of *Mythimna separate* were evaluated. These results showed that some compounds exhibited significant insecticidal activities and the susceptibility assays were expressed as the median lethal dose (LD_{50}), of which one of the compounds exerted the most potent insecticidal activity (LD_{50} = 296 μg g^{-1}), comparable to that of the natural product insecticide, celangulatin V (LD_{50} = 260 μg g^{-1}). This strategy led to a promising candidate for the development of new steroidal insecticidal agents.

Keywords: steroid, progesterone, pyrazoline, insecticidal activity

Introduction

The routine use of a wide variety of synthetic chemical insecticides in agriculture has now become an accepted practice; however, the increasing application of those insecticides over the years has resulted in the resistance of insect pest populations and environmental problems. Therefore, development of new effective, selective and safe pesticides is highly desirable. Due to steroids that mimic the action of juvenile hormones could be used as safe insecticides; consequently, numerous steroids-based derivatives were designed and synthesized.[1-4] The pyrazoline nucleus is an important class of heterocyclic structure found in many synthetic products with a wide range of pharmacological properties, such as antimicrobial,[5,6] antitumor,[7,8] antimalarial activity,[9] and pyrazoline-type insecticides were strongly studied in the last years.[1,10]

Molecular hybridization is a prevailed concept in drug design. It usually occurs in two or more pharmacophores or chemical entities either linked or fused together to create a new molecule which exhibits synergistic or additive pharmacological activities.[11,12] For example, steroidal derivatives in which D-ring is modified with pyrazolines showed potential anticancer activity,[13,14] however, there is no insecticidal activity report yet about this class of compounds.

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In order to obtain biologically potent insecticidal compounds with diverse structures, in the present study, a series of steroidal C-17 pyrazolinyl derivatives were re-prepared from the commercially available progesterone (**1**),[15] and their insecticidal property against 4th instar larvae of *Mythimna separate* were tested. Moreover, a preliminary structure-activity relationship was discussed.

Experimental

Chemistry

Melting points were measured on an X-4 micromelting point apparatus. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance III 500 instrument in $CDCl_3$ with tetramethylsilane (TMS) as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm) and coupling constants are given in Hz. Mass spectra were recorded on an ESI-esquire 3000 Bruker Daltonics instrument. Column chromatography (CC) was performed over silica gel (200-300 mesh, Qingdao Marine Chemical Ltd.). The progress of all reactions was monitored by thin layer chromatography (TLC) on 2 cm × 5 cm precoated silica gel 60 F_{254} plates of thickness of 0.25 mm (Qingdao Marine Chemical Group, Co.). Spots were visualized at 254 and 366 nm under

UV and iodine. All commercially available solvents and reagents were used without further purification.

Synthesis

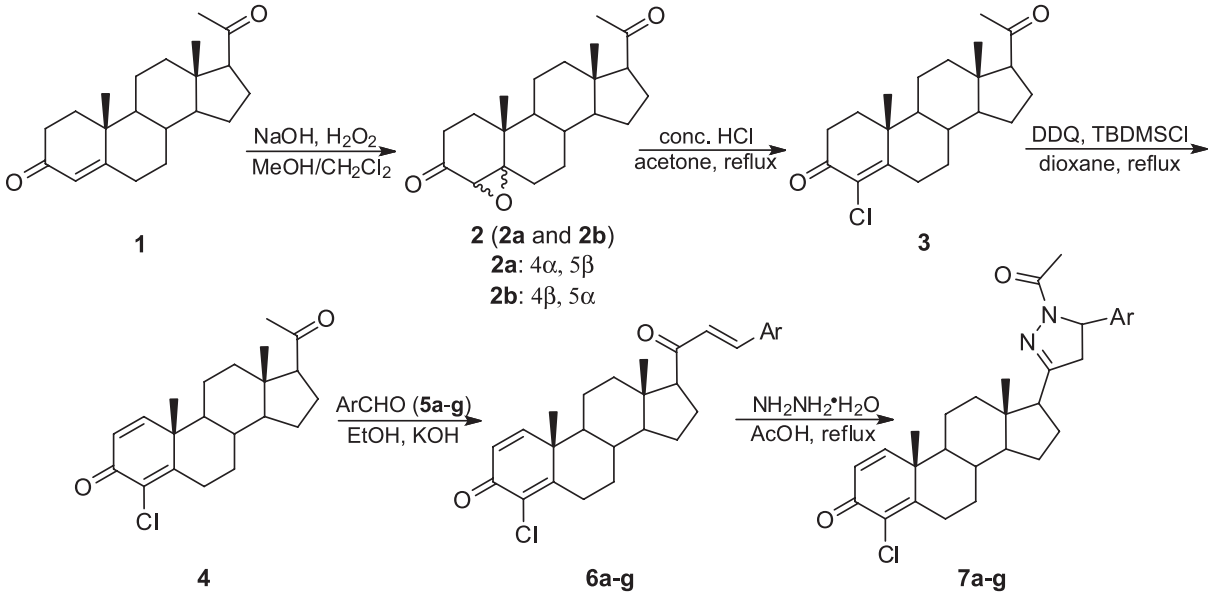
The synthetic route is presented in Schemes 1 and 2. The full details of the chemical and structural elucidation of the steroidal C-17 pyrazolinyl derivatives have been described previously.[15]

Insecticidal activity screening assay

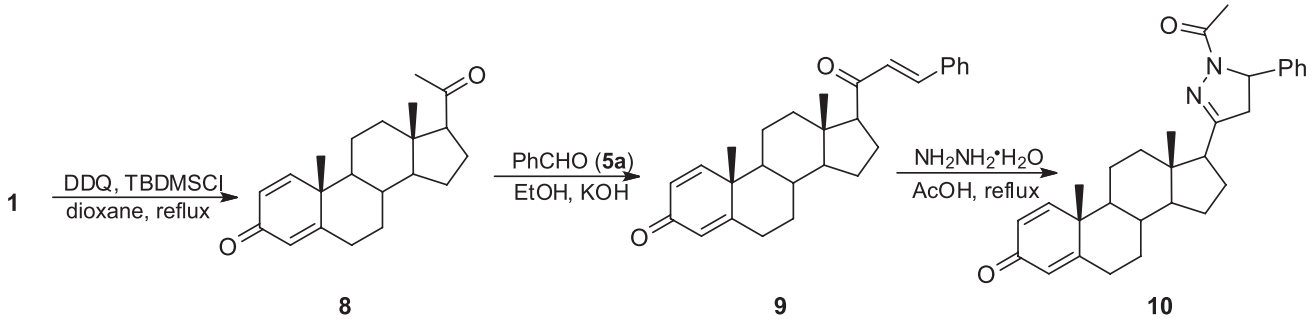
The insecticidal activities of compounds **1**, **4**, **7a-g**, **8** and **10** against 4th instar larvae of *M. separata* were evaluated by the leaf disc method.[16] Leaf discs (0.5 × 0.5 cm) were treated with 10 μg *per* disc of the test samples dissolved in acetone (acetone and celangulin V[17] was used as negative and positive control, respectively). The 4th instar larvae of *M. separate* were fed with the discs for 24 h (repeated 10 times for each sample) and kept in a growth chamber in the dark at 27 ± 1 °C and 60% relative humidity under a 16L:8D photoperiod. After 24 h, the numbers of dead larvae (symptoms: the larvae were knocked down, serious loss of body fluid and death) were recorded, and mortality percentage was calculated.

Median lethal dose (LD_{50})

The toxicity for the active compounds was further ascertained by estimating the median lethal dose (LD_{50}, killing 50%), and was tested following the same procedures described above. Briefly, leaf discs of known area were treated with known amounts of the test samples (5, 10, 15, 20 and 25 μg *per* disc). The 4th instar larvae of *M. separate* (weight 14-20 mg) were fed with the treated leaves and kept in a growth chamber. All experiments and the respective controls were carried out in three replicates. After 24 h, the eaten area of a leaf was measured under a binocular microscope by counting l-mm squares exposed when the partially eaten disc was placed on a circle the exact size of the disc drawn on mm-ruled paper, simultaneously. Then the dose of the chemical was calculated based on the weight and consumed areas of

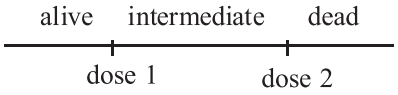


Scheme 1. Synthesis of C-17 pyrazolinyl derivatives **7a-g** from progesterone.



Scheme 2. Synthetic routine of derivative **10** from progesterone.

larvae, and divided into three parts (alive, intermediate and dead groups) based on dose.



Finally, the LD_{50} values were account as follows:

$$LD_{50} = \frac{A + B}{2}$$

where A is the average dose of alive larvae, and B is the average dose of dead larvae in intermediate section.

Statistical analysis

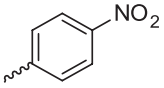
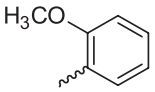
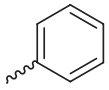
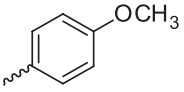
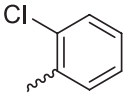
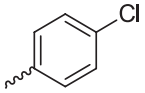
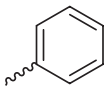
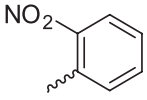
The data of insecticidal assay were expressed as mean ± standard deviation (SD) of triplicates and ten *M. separata* larvae in each group. The difference in values at $p \leq 0.01$ when compared with control was considered as statistically significant. The analysis of variance (ANOVA) was performed using Graph Pad Prism 5 software.

Results and Discussion

In our previous studies, we prepared a series of new progesterone derivatives with a pyrazoline nucleus at D-ring that showed good cytotoxic activities.[14] In order to detect whether they have the insecticidal activity, in this study, these compounds were also selected for a research program with the aim of identifying new steroidal derivatives with insecticidal activity. The rational design was based on the consideration that progesterone has a steroidal skeleton and pyrazoline is an important class of pharmacophore. Thus, eight pyrazolinyl derivatives were synthesised with progesterone **1** as starting material. As shown in Scheme 1, C-17 pyrazolinyl derivatives **7a**-**g** with chlorine atom (Cl) at C-4 position were high yield synthesized from intermediate **4**.[15] In order to study the effect of Cl of the A ring on the activity, correspondingly, compound **10** can be obtained from the other intermediate **8** (Scheme 2).

The 4th instar larvae of *Mythimna separata* were used as test insects and the concentrations of the tested compounds were 10 mg mL^{-1}. The well-known plant-derived natural product insecticide celangulatin V,[17] which was isolated from *Celastrus angulatus*, was used as the positive control. As summarized in Table 1, **7a**-**g** and **10** displayed weak to strong insecticidal activity with mortality rates of 8% to 100% at 10 mg mL^{-1}, which were stronger than progesterone (**1**) and their parent compounds **4** or **8** (mortality rates are 0%). Among the compounds tested, mortality rates of **7a**, **7c** and **7d** were higher than 60%, and **7a**, **7c** and **7d** were selected for median lethal dose (LD_{50}) assay. A preliminary structure-activity relationship (SAR) could be proposed: (*i*) the pyrazoline nucleus might be a pharmacophore involved in the insecticidal activities of these series of compounds, for example, **7a**-**g** *vs.* **4** and **10** *vs.* **8**; (*ii*) compound **7a** (mortality rate is 100%),

Table 1. The mortality (at 10 mg mL^{-1}) against the 4th instar larvae of *M. separata* of compounds **7a**-**g** and **10**

Compound	Ar	Death rate / %	Compound	Ar	Death rate / %
1	–	0 ± 0	**7e**		33 ± 3
4	–	0 ± 3	**7f**		25 ± 7
7a		100 ± 7	**7g**		33 ± 3
7b		17 ± 3	**8**	–	0 ± 0
7c		83 ± 3	**10**		8 ± 3
7d		67 ± 7	celangulatin V	–	100 ± 3

with no substituent aromatic ring, was found to be more active than **7b**-**g**, thereby indicating that the introduction of *ortho*- or *para*-substituents into the aromatic ring may not be a good strategy to improve their insecticidal activity. It also demonstrated the importance of the pyrazoline for insecticidal activity; (*iii*) compounds **7a** exhibited insecticidal activity with mortality rates of 100%, which are stronger than **10**. It implies that introducing a chlorine atom into C-4 position may be significantly important to improve insecticidal activity.

Moreover, we determined the median lethal dose (LD_{50}) of the most active compounds **7a**, **7c**, and **7d** for further evaluation of their insecticidal activity. As shown in Table 2, LD_{50} for **7a** was 296 μg g^{-1}, comparable to that of celangulatin V (LD_{50} = 260 μg g^{-1}), whereas **7c** (587 μg g^{-1}) and **7d** (881 μg g^{-1}) showed weaker activity than the reference. This suggested that **7a**, bearing non-substituted phenyl, was the most active. It is evident from the data that changes in the *para*- or *ortho*-position of the same substituent in the aromatic ring have significant effects on the activity. This may be attributed to their differences in either polarity changing their lipophilicity or conformation altering the target protein binding properties present within insects. Therefore, it can be concluded that the toxicity of **7a**-**g** is sensitive to very small structural changes, especially to different substituents.

Table 2. Median lethal dose (LD_{50}) of **7a**, **7c** and **7d** against 4th instar larvae of *M. separata*

Compound	LD_{50}[a] / (μg g^{-1})
7a	296 ± 26
7c	587 ± 38
7d	881 ± 50
celangulatin V	260 ± 16

[a]LD_{50}: the compound dose enough to cause 50% lethality of instar larvae of *M. separate*.

Conclusion

Through rational design based on the hybridization of steroidal molecules and pyrazoline, we demonstrated that this strategy led to a substantial increase in insecticidal activity. Compound **7a** exhibited the most potent insecticidal activity (LD_{50} = 296 μg g^{-1}), comparable to that of the natural product insecticide, celangulatin V (LD_{50} = 260 μg g^{-1}). This is the first report of the insecticidal activity of steroidal C-17 pyrazolinyl derivatives. This study may contribute to the development of new pharmaceutical prototypes derived from steroidal sources with insecticidal activity.

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