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Essential oil and polyacetylenes from *Artemisia ordosica* and their bioactivities against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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ABSTRACT

Five polyacetylenes (**1**-**5**) were isolated from *Artemisia ordosica*, one of the main arido-active shrubs growing in the arid areas in China. Their molecular structures were identified with NMR spectroscopy. The compounds were confirmed as capillene, capillin, capillinol, *cis*-dehydromatricaria ester and *trans*-dehydromatricaria ester. It was found that three compounds (**1**-**3**) had similiar structural skeleton of 2,4-hexadiyn-1-ylbenzene. The repellent and fumigant activities against *Tribolium castaneum* adults were investigated for compounds isolated and the essential oil. Bioassays showed that the essential oil extracted from the aerial parts of *A. ordosica* possessed fumigant toxicity of LC_{50} = 18.65 mg/L air, and compounds (**1**-**4**) had stronger activities from LC_{50} = 4.06 mg/L air to 6.16 mg/L air against *T. castaneum* adults. At the tested concentrations (62.91 and 12.58 nL/cm^2), the crude essential oil and compounds (**1**, **2** and **4**) showed the same repellent activity ($p > 0.05$) with DEET against *T. castaneum* adults at both 2 h and 4 h after exposure. Compound **2** showed strong repellence (PR = 100%) at tested concentrations of 62.91, 12.58 and 2.52 nL/cm^2 at 2 h after exposure. Moreover, at tested concentration (62.91, 12.58, 2.52 and 0.5 nL/cm^2), the PR value of compound **4** was all higher than 90% at both 2 h and 4 h after exposure. The results showed that the crude essential oil and the isolated polyacetylenes possessed fair repellent and fumigant activities. Furthermore, potential relationships between structure and bioactivity were also discussed. It might be found that the existence of acetylenic bonds was the principal factor of fumigant activity, and with the increase of acetylenic bonds, the fumigant activity would enhance. In addition, it was believed that oxygenous groups connected to the acetylenic bonds might contribute to fumigant toxicity against *T. castaneum* adults.

1. Introduction

Among the variety of nature's ecosystem services, the natural pest control is a principal aspect. The economic importance of the major stored-product insect species is highlighted with a loss of more than one billion US Dollars per year worldwide (Boyer et al., 2012). In fact, natural pest control can protect crops and minimize the economic loss. Currently, pesticide is one of the most important measures to control pests, and the widely used insecticidal compounds are organochlorines, pyrethroids, organophosphates and carbamates. However, these kinds of insecticides have led to the increasingly serious problems, such as disturbances of the environment and pest resurgence. Due to widespread public concern for long-term health and environmental effects of insecticides, efforts are continuing to seek new structure types as safer alternatives.

Polyacetylenes are widely distributed in living organisms and their acetylenic bonds are considered to be one of the most important functional groups. Acetylenic functions have readily been found in a series of natural products, especially in plant species (Christensen, 1998; Bohlmann et al., 1973; Lamberth, 2009). More than 2000 polyacetylenes have been found, and more than 1100

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have been isolated from the plants of Asteraceae family (Minto and Blacklock, 2008). The most of these compounds show various biological activities such as insecticide, ovicide, larvicide and fungicide (Stevens et al., 1990). A review has described that many alkyne derivatives play important roles in the control of insects, e.g. pyrethroids derived from the flower *Tanacetum cinerariaefolium* are used as voltage-dependent sodium channel blockers against pests; phenylacetylene derivatives possess highly efficient broad-spectrum insecticidal activity as GABA-gated chloride channel blockers (Lamberth, 2009). In addition to alkyne derivatives, alkyne functions of interesting insecticidal activities are also exhibited in several natural products. Such as capillene, isolated from the essential oil of *Artemisia capillaris* has been found to possess insecticidal properties (Xu and Zhao, 1993) and the 1,2-dithiin thiarubrine C, isolated from the flower *Rudbeckia hirta* has fair toxic against larvae of *Aedes atropalpus* (mosquito) and *Manduca sexta* (tobacco hornworm) (Guillet et al., 1997). Structure types of polyacetylenes should play important roles in the control of insects, and should be used as safer alternatives.

Artemisia ordosica is one of the main arido-active shrubs growing in the arid and semi-arid areas of the north China including Inner Mongolia, Ningxia, Gansu and Shanxi. The aerial parts of this plant have been used as folk medicine for expelling rheumatism, clearing heat, and dispelling swelling (Zhao et al., 2005). It has been found that the essential oil of *A. ordosica* contains rich and various chemical compounds, such as terpenoids, alcohols, esters, ketones, and carboxylic acids (Abad et al., 2012; Zhang et al., 2013). However, it has been rarely reported that polyacetylenes exist in its essential oil.

In this work, five polyacetylenes of the essential oil extracted from *A. ordosica* were isolated and identified. There are many different research methods to control stored-product insects, however, repellent and fumigant activities were more suitable to explore the mechanism of insect-resistant activity of essential oil due to its high volatility (Papachristos and Stamopoulos, 2003). So we assessed the repellent activity of essential oil and three compounds (**1**, **2** and **4**), meanwhile the fumigant activity of essential oil and four compounds (**1**-**4**) were also tested against *T. castaneum* adults.

2. Materials and methods

2.1. Essential oil extraction

The fresh aerial parts of *A. ordosica* were collected from Kubuqi Desert, Inner Mongolia Province, China (40°17′ E longitude, 109°44′ N latitude, altitude 1210 m) in August 2015. The species was identified by Dr. Liu, Q.R. and the voucher specimen (BNU-dushushan-20151208) was deposited at the Herbarium (BNU) of College of Resources Science and Technology, Beijing Normal University. The aerial parts were air-dried. The sample was weighed and transferred into a modified Clevenger type apparatus, and the hydrodistillation was carried out for 6 h to get the essential oil. The extra water was removed by adding anhydrous sodium sulphate. The essential oil was stored in airtight containers in refrigerator at 4 °C.

2.2. Polyacetylenes isolation

The crude essential oil (18 mL) of *A. ordosica* was chromatographed on a silica gel column (160–200 mesh, Qingdao Marine Chemical Plant, Shandong province, China) (column length 43.3 cm, i.d. 5 cm), eluting with a gradient of petroleum ether-ethyl acetate (from 80:1 to 0:100). Each 150 mL of eluate was collected as a fraction. With the monitoring of thin layer chromatography (TLC) profiles, the similar fractions were combined and at last 24 fractions were obtained. Among them, fractions (4, 8, 9, 16, 18) were pooled and further purified by silica gel column chromatography and five compounds were isolated from them. The isolated compounds were elucidated based on nuclear magnetic resonance. ^{1}H and ^{13}C NMR were performed on a 500 NMR spectrometer (Avance III, Bruker) at the temperature of 25 °C in the denatured chlorform ($CDCl_3$).

2.3. Gas chromatography and mass spectrometry

Components of the essential oil of *Artemisia ordosica* aerial parts were identified by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC–MS) using an Agilent 6890N gas chromatograph hooked to an Agilent 5973N mass selective detector. The same column and analysis conditions were used for both GC-FID and GC–MS. They were equipped with a HP-5MS (30 m × 0.25 mm × 0.25 μm) capillary column. The column temperature was programmed at 50 °C for 2 min, then increased at 2 °C/min to the temperature of 150 °C and held for 2 min, and then increased at 10 °C/min until the final temperature of 250 °C was reached, where it was held for 5 min. The injector temperature was maintained at 250 °C and the volume injected was 1 μL of 1% solution (diluted in *n*-hexane). The carrier gas was helium at flow rate of 1.0 mL/min. Spectra were scanned from 50 to 550 *m*/*z*. Most constituents were identified by comparison of their retention indices with those reported in the literatures. The retention indices were determined in relation to a homologous series of *n*-alkanes (C9–C30) under the same operating conditions. Further identification was made by comparison of their mass spectra with those stored in NIST 05 and Wiley 275 libraries or with mass spectra from literature (Adams, 2001). Relative percentages of the individual components of the essential oil were obtained by averaging the GC-FID peak area% reports.

2.4. Insects culture

T. castaneum adults were maintained in the dark incubator at 28–29 °C, 70–80% relative humidity for 2 preceding years before use. The insects were reared in glass containers (0.5 L) containing wheat flour at 12–13% moisture content mixed with yeast (10:1, w/w). The mixed-sex insects used in the bioassays were 1- to 2-weeks old.

2.4.1. Repellence tests

The repellent activity of *T. castaneum* adults was tested based on the area preference method (You et al., 2015; Guo et al., 2015). The testing materials were dissolved separately in *n*-hexane to prepare serials of testing solutions (3000ul) with five concentrations (62.91, 12.58, 2.52, 0.50, and 0.10 nL/cm^2), and *n*-hexane was used as the negative control. Petri dishes (9 cm in diameter) were used as experimental containers during the experiment. Filter paper (9 cm in diameter) was cut in half and 500 μL of each concentration was applied separately to half of the filter paper as uniformly as possible with a micropipette. The other half was treated with 500 μL of *n*-hexane. Both the treated half and the control half were then air-dried to evaporate the solvent completely for 30 s. The treated side was then joined to the control side by tape and placed in the glass Petri dishes with the seam oriented in randomly selected different directions to avoid any insecticidal stimuli affecting the distribution of insects. Twenty insects were released in the center of each filter paper disk, and a cover was placed over the Petri dish. A commercial repellent, DEET (*N*, *N*-diethyl-3-methylbenzamide), was purchased from Dr. Ehrenstorfer, Germany and used as a positive control. Five replicates were used and the experiment was repeated three times. Counts of the insects present on each side of the filter paper were