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Identification of an insecticidal polyacetylene derivative from *Chrysanthemum macrotum* leaves

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ABSTRACT

Compounds responsible for insecticidal properties of *Chrysanthemum macrotum* (D.R.) Ball. leaves against *Spodoptera littoralis* Boiduval caterpillars have been investigated. The screening of the insecticidal activity was performed by incorporating methanol, buthanol or ethyl acetate extracts, or some chromatographic fractions to the caterpillars' artificial diet. It was noted that extracts and fractions ameliorated or disturbed nutritional indexes, being not always toxic for caterpillars. Among the tested fractions, one pure compound with a high insecticidal activity (percentage of mortality 66.7%) was purified. The nuclear magnetic resonance study allowed its identification as a polyacetylene derivative, in particular a spiroketal enol ether one.

1. Introduction

Insect pests have mainly been controlled with synthetic insecticides in the last fifty years. Most insecticidal compounds fall within four main classes, the organochlorines, organophosphates, the carbamates and pyrethroids. Out of these the major classes in use today are organophosphates and carbamates. There are problems of pesticide resistance and negative effects on non-target organisms including man and the environment (Kabaru and Gichia, 2001). Since few decades, many investigations on plant insecticide potential were carried in order to substitute chemical molecules. More than 2000 plant species are a rich source of novel insecticides (Klocke, 1989), where *Meliaceae*, *Rutaceae*, *Asteraceae*, *Annonaceae*, *Labiatae* and *Canellaceae* are the promising families (Wheeler and Isman, 2001), the two famous compounds are azadirachtin extract from the Indian neem tree (*Azadirachta indica*) (Jilani and Saxena, 1990) and pyrethrum from *Chrysanthemum cinerareafolium* (Prakash and Rao, 1997). Recently, over than 2000 polyacetylenes are known, with more than 1100 in the plant family *Asteraceae* (Minto and Blacklock, 2008). These compounds are fatty acid derivatives characterized with long hydrocarbon chains (Baek et al., 1995) they are generally stored in secretory plant cells, called laticifers (Ellis, 1997), secreting latex. Most of them have an insecticidal, ovicidal, larvicidal, fungicidal, nematocidal and phytotoxicity proprieties (Stevens et al., 1990). Some polyacetylenes, such as the relatively stable α-terthienyl and phenylheptatriyne are phototoxic to insects and fungi at very low concentrations when introduced into insect diets or tested against cultured fungi by using the disk diffusion method (Towers and Champagne, 1987; Downum, 1992). Early study realized by Wrang and Lam (1975) identified fifteen polyacetylenes from *Chrysanthemum leucanthemum*, from which five are new in this herb. Bohlmann and Zdero (1975) have also described four new polyacetylenes from *Chrysanthemum macrotum*: 8-(2-thienyl)-3t,5t-ocradien-7-in-1-ol-acetat, 8-(2-thienyl)-3t,5t-uctadien-7-in-1-ol, 2t,4t-undecadien-8,10-diinsaure-4,6-heptadiinylester and 1-(2,3-dihydro-2-furyl)-4-(2-thienyl)-1t-buten-3-in. Nevertheless, no biological activity investigation of these compounds has been made. Some years later, Bowers and Aregullin (1987), Cũnat et al. (1990), Sanz et al. (1990) and Song et al. (2005) identified nine polyacetylenes in *Chrysanthemum coronarium*, from which four compounds have an insect antijuvenile hormone activity against *Oncopeltus fasciatus*. The biological properties of these compounds make them of a great interest to plant pathologists and pharmacologists. Many of the polyacetylenes and related compounds require UV-light (300–400 nm) for being toxic and having other biological activities (Christensen and Lam, 1990).

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As part of our search within the *Chrysanthemum* genus and after a preliminary study on eight *Chrysanthemum* species (Haouas et al., 2003, 2005, 2008, 2009) we focus to identify a polyacetylene(s) derivative from *C. macrotum* leaves endowed with insecticidal activity against *Spodoptera littoralis* caterpillars.

2. Materials and methods

2.1. Plant material

C. macrotum (D.R.) Ball. leaves were collected in 2007 during its flowering stage in April from the region of Zaghouan (Tunisia). The sampling was made on the rocky mountain slopes (36°23′13.15″N, 10°07′51.49″E) at about 305 m above the sea level which belong to the sub-humid bioclimatic stage. Voucher specimens were deposited in the National Gene Bank of Tunisia. Leaves were dried in open air in the shade.

2.2. Chemical study

One hundred gramme of the dried powdered leaves of *C. macrotum* were extracted at room temperature with MeOH (1 l × three times during 3 days). After filtering, extracts were combined and dried at reduced pressure. 45 g of residues were re-dissolved in methanol–water (3:1) and partitioned in a separatory funnel with EtOAc and BuOH, in the order. After solvents removal at reduced pressure, respective EtOAc (8.7 g), BuOH (3.6 g) and MeOH–H_2O (32.4 g) extracts were obtained. After bio-insecticidal activity evaluation of these three crude extracts, it was established that the most effective was the EtOAc one. Thus, a portion of this latter extract (7 g) was re-dissolved in MeOH and submitted to size-exclusion chromatography on a Sephadex LH-20 column, eluting with 100% MeOH. According to TLC (Merck Kieselgel 60 F254) analysis, chromatograms were visualized under UV light at 254 and 366 nm and/or sprayed with cerium sulphate or Naturstoffereagenz A-PEG reagents. Collected fractions were combined in nine homogeneous fractions (AC_I–AC_{IX}). All of them were tested for their insecticidal activity. Only AC_I and AC_{II} (0.98 g) fractions resulted effective and were further fractionated on a silica gel 60 column, eluting with $CHCl_3$: MeOH mixtures (9:1, 8.5:1.5, 7:3, 5:5, to 100% MeOH). After TLC analysis, 21 homogeneous fractions (AC_{S1}–AC_{S24}) were obtained. Finally, a pure secondary metabolite (**1**, 3.5 mg), endowed with a high insecticidal activity was isolated (Flamini et al., 1997, 2004; Song et al., 2005).

Structural determination of **(1)** was performed by spectroscopic analysis. Melting points (uncorrected) were determined with Kofler apparatus; 1H and 13C NMR spectra were obtained with a Bruker Avance II 250 spectrometer in $CDCl_3$, using TMS as internal standard. All experiments were performed using the standard Bruker library of microprograms. Known compounds were identified by comparison of their spectral data to those of the literature (Flamini et al., 1997, 2004).

2.3. Insects

Insects were obtained from a culture of *S. littoralis* and maintained under standard conditions of temperature (27 ± 1 °C), photoperiod (L16:D8) and relative humidity (60–70%). caterpillars were reared in Petri dishes with small artificial diet cubes based on wheat germ (Poitout and Bues, 1974), while adults with a 15% honey water solution. The culture was continuously supplemented with wild moths captured with a light trap in High Institute of Agronomy, Tunisia.

2.4. Insect assay

2.4.1. Extracts and fractions effects on insect survival

Thirty 3rd instar caterpillars were individually placed in glass Petri dishes (1 cm high and 9 cm in diameter) and provided with appropriate artificial diet added with 0 (control), 0.1, 1 and 10 mg/g of *C. macrotum* leaves methanol crude extract, or 1 mg/g of semi-purified BuOH, EtOAc or MeOH–H_2O extracts. Fractions obtained from the Sephadex column were tested at 1 mg/g, while those from silica gel at 0.1 mg/g. All fractions are dried and well mixed with the artificial diet after preparation and cooling according to the procedure of Martinez and Van Emden (2001). Insect mortality was recorded in the end of experiment and adjusted for control using Abbott's correction (Abbott, 1925).

$$\mathrm{Mc} = \frac{\mathrm{Mo} - \mathrm{Me}}{100 - \mathrm{Me}} \times 100$$

Mo = mortality rate of treated insects (%); Me = mortality rate of control (%); Mc = corrected mortality rate (%).

2.4.2. Extracts and fractions effects on food consumption and utilization

The effect of extracts and fractions on food consumption and utilization by third instar caterpillars was investigated using reared caterpillars on control diet after the second molt (<24 h). They were weighed and individually placed in Petri dishes. They were fed with known weights of diets containing 0, 0.1, 1 and 10 mg/g of extract (n = 30 and five replications for each concentration) and left to feed for 2 days, a period slightly shorter than instar duration. At the end of the experiment, caterpillars and faeces were weighed and food consumption was determined. Nutritional indices, namely relative consumption rate (RCR), relative growth rate (RGR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and approximate digestibility (AD) were calculated as follows:

$$\mathrm{RCR} = I/B_a T$$
$$\mathrm{RGR} = \Delta B/B_a T$$
$$\mathrm{ECI} = (\Delta B/I) \times 100$$
$$\mathrm{AD} = [(I - F)/I] \times 100$$
$$\mathrm{ECD} = [\Delta B/(I - F)] \times 100$$

where: I = weight of consumed food; B_a = arithmetic mean of insect weight during the experiment = [(PF − PI)/log (PF/PI)]; PF = caterpillars final weight (mg); PI = caterpillars starting weight (mg); T = feeding period in hours; ΔB = change in body weight; F = weight of faeces produced during the feeding period (Waldbauer, 1968; Farrar et al., 1989).

2.5. Statistical analyses

Nutritional indexes obtained after treatments with methanol and ethyl acetate extracts and fractions from sephadex column, as well as mortality, for all different treatments were compared using analysis of variance (ANOVA) followed by Duncan test for multiple-comparison when significant differences were observed at P = 0.01. Nutritional indexes resulting on caterpillars treated on fractions obtained from silica gel column were analyzed using the principal compound analysis (Tanagra version 1.4.38).

3. Results

3.1. Effect of methanol extract

After preliminary study related to the insecticidal activity of flowers and leaves *Chrysanthemum* powders and their methanolic extracts against *S. littoralis* caterpillars (Haouas et al., 2008; Haouas,