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DETECTION OF CANTHARIDIN-RELATED COMPOUNDS IN *Mylabris impressa* (COLEOPTERA: MELOIDAE)

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ABSTRACT: Cantharidin is mainly found in the beetle families Meloidae and Oedemeridae (Insecta: Coleoptera) which are the natural producers of this terpene anhydride. Most studies to date have focused on cantharidin distribution in blister beetles, with few reports on recently found cantharidin-related compounds (CRCs). Using gas chromatography-mass spectrometry (GC-MS), the present work reported cantharidin and two CRCs, palasonin and cantharidinimide from *Mylabris impressa stillata* (Baudi, 1878) which was collected from Toyserkan county, Hamedan Province, Iran. Ionization provided mass spectra with characteristic fragments of cantharidin at *m/z* 96 and 128, demethylcantharidin at *m/z* 82 and 114, and cantharidinimide at *m/z* 70, 96 and 127. This is the first time that cantharidin and the two CRCs are found in the genus *Mylabris* which in turn is new to the field of venomous insects.

KEY WORDS: Cantharidin, CRC, palasonin, cantharidinimide, blistering beetle, Meloidae, *Mylabris*.

CONFLICTS OF INTEREST: There is no conflict.

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INTRODUCTION

Cantharidin ($C_{10}H_{12}O_4$), which is mainly found in blister beetles (Coleoptera: Meloidae), is among the most widely known insect natural products (5, 16). It is highly toxic to most animals (LD_{50} for humans: 10–60mg/kg; intraperitoneal LD_{50} for mice: 1mg/kg) (5). Its reputation principally derives from descriptions of its physiological activities as an aphrodisiac and a blistering agent for humans and livestock. For more than 2000 years, blister beetles in powdered or tincture form have been used medicinally in Europe, China and elsewhere. The ancient Greeks and Romans consumed cantharides as a diuretic and abortifacient as well as an aphrodisiac. Its mode of action as an aphrodisiac is by inhibition of phosphodiesterase and protein phosphatases (PPs) activity and stimulation of ß-receptors which irritates the genital mucosa, therefore enhancing sensation (21). In mammalian tissue, at least four types of PPs have been identified: PP_1, PP_{2A}, PP_{2B} and PP_{2C} (4, 12). Cantharidin and CRCs inhibit the activity of both PP_1 and PP_{2A} (11-15). In China and South Korea, cantharidin has been commercially formulated along with laboratory evaluation and clinical trials to be prescribed as anti-tumor and anti-cancer agent in humans (11, 19, 22).

Most chemical studies to date have focused on cantharidin distribution in blister beetles, with few reports on recently found CRCs. Working on meloid beetles, the present study reported two further CRCs from *Mylabris impressa stillata* (Baudi, 1878), which is new to the field of venomous insects.

MATERIALS AND METHODS

Beetle Collection

Specimens of *Mylabris impressa stillata* (Baudi, 1878) were manually collected from Toyserkan county, Hamedan province, Iran, by inspection while they were sitting on flowers or stems of different wild shrubs of the family Asteraceae. The specimens were placed in small net ported plastic boxes (18X13X6cm), bottom covered with a layer of wet kitchen paper, and transferred to the laboratory where they were immediately frozen at -30°C.

Extract Preparation

Tissue samples were put into test tubes and their dry weight determined after 36h of freeze-drying (-50°C, $9X10^{-2}$mbar) using a LYOVAC GT2-E freeze-dryer (AMSCO/

FINN-AQUA Co. Ltd.). Body fragments were hydrolyzed in small fused test tubes using 100–300µl 6N hydrochloric acid (Technical HCl, 31–33%, AUG. Headinger, Stuttgart, Germany) at 120°C for 4h in order to remove biomatrix and to set the bound cantharidin free. Following a short period of cooling down, an equivalent amount of chloroform (100–300µl) was added and each sample was vigorously shaken on a Vortex mixer for 60s. Afterwards, samples were centrifuged (Medifuge centrifuge, Heraeus Sepatech GmbH, Osterode, Germany) at 3000rpm for 5min. Using Pasteur pipette, the organic phase (chloroform-based compounds which stand at the bottom) of each tube was filtered and transferred into a conical 3-dram lip glass vial (10). All glassware used had been already silanized for 24h by dimethyldichlorosilane solution I in heptane 5% ($C_2H_6Cl_2Si$, Fluka).

Quantitative Gas Chromatography-Mass Spectrometry

To detect CRCs, GC-MS was used and 0.5µl of each sample splitlessly injected by a 1µl microsyringe (SGE, Australia) into the injector. Authentic cantharidin (purity 98%, SIGMA-ALDRICH Chemical Co., UK) served as standard for identification. Relatively high volatility and good thermal stability are those characters of cantharidin which makes GC analysis the method of choice. Capillary GC sensitivities are very good and the typical high resolution achieved with capillary GC permits analyses of substances from biomatrices with minimal sample preparation. Instrumental analyses were performed using a Hewlett-Packard 6890 series gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a J&W Scientific (Agilent Technologies, Wilmington, DE) DB-5 capillary column (film thickness: 0.25µm, inner diameter: 0.32mm, length: 30m) connected to a flame ionization detector. The temperature program used for analysis went from 60 to 160°C at a rate of 10°C/m, holding for 3min, then to 300°C at the rate of 10°C/m and a final hold at 300°C for 5min. Mass spectra were taken at 70eV with scanning speed of 1 scan/s from *m/z* 50 to 250 while the detector delayed for 5min. Helium (carrier gas) flow was 3.8ml/min and the injector and detector temperatures respectively set at 250 and 300°C. Ionization provided mass spectra with characteristic fragments of cantharidin at *m/z* 96 and 128 (Figure 1), demethylcantharidin at *m/z* 82 and 114 (Figure 2), and cantharidinimide at *m/z* 70, 96 and 127 (Figure 3). Integration of chromatographic peak areas was performed using Chemstation (revision A.07.01; Hewlett Packard).

RESULT AND DISCUSSION

In the animal kingdom, cantharidin is only produced by blister beetles (Coleoptera: Meloidae) and smaller oedemerid beetles (Coleoptera: Oedemeridae), in which it is found in hemolymph and various tissues (2, 3, 5, 7, 9). Cantharidin also acts as a potent attractant to minute fractions within various insect taxa. Living and especially dead meloids and oedemerids and even their cantharidin-containing feces are highly attractive to these so-called canthariphilous insects. They sequester the compound but cannot produce it *de novo*.

Cantharidin has not been discovered in plants; however, insecticidal seeds of the Himalayan tree, *Butea frondosa* (Leguminosae), contain demethylcantharidin (palasonin), in which the 3-methyl group of cantharidin is missing (1, 5, 20). Palasonin is the first CRC that has been so far recorded from the meloids. Dettner *et al*. (6) were the first who reported palasonin from the South African blister beetle, *Hycleus lunatus*. Nikbakhtzadeh (18) detected palasonin in *Hycleus polymorphus* and *Mylabris quadripunctata* from southern France and *Cyaneolytta* sp. from the Nairobi's suburbs in East Africa. Unlike the plant source, the beetle-derived palasonin is of low enantiomeric excess with (*R*)-(+)-enantiomer prevailing (8). Dettner *et al*. (6) also reported the second CRC, palasoninimide, from *H. lunatus*. Another CRC is cantharimide whose anhydride oxygen atoms are replaced by the basic amino acids $_L$-lysine, $_L$-ornithine and $_L$-arginine moieties and was reported from *Mylabris phalerata* Pall. (17). Apart from cantharidin and palasonin, low amount of cantharidinimide could be traced in the extract of *Mylabris impressa stillata*. Although toxicity is decreased in CRCs, all of them remain toxic to most birds, reptiles and, in particular, mammals and are still counted as capable feeding inhibitors.

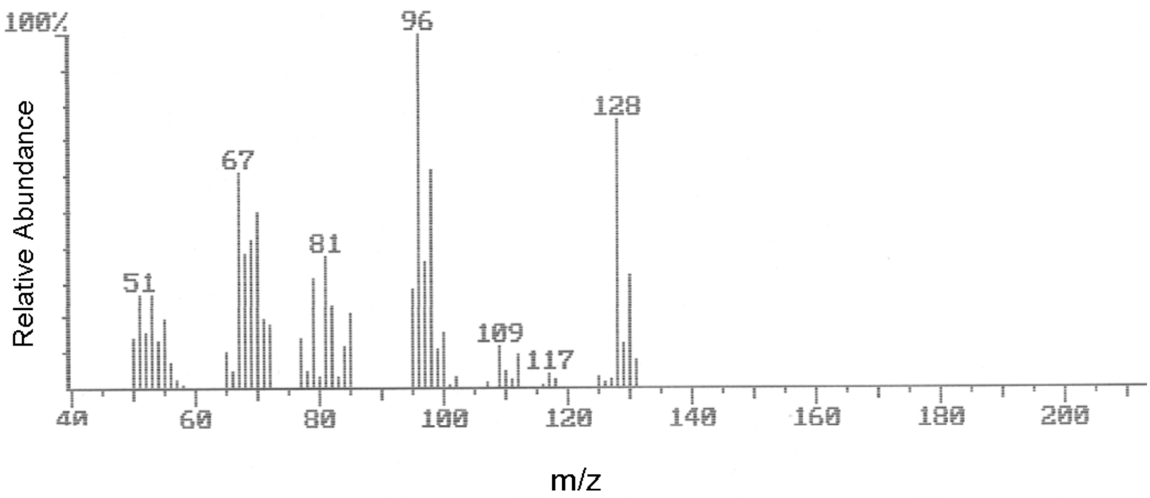

Figure 1. Mass spectra of cantharidin with base peaks at *m/z* 128 and 96 according to a Hewlett-Packard gas chromatograph.

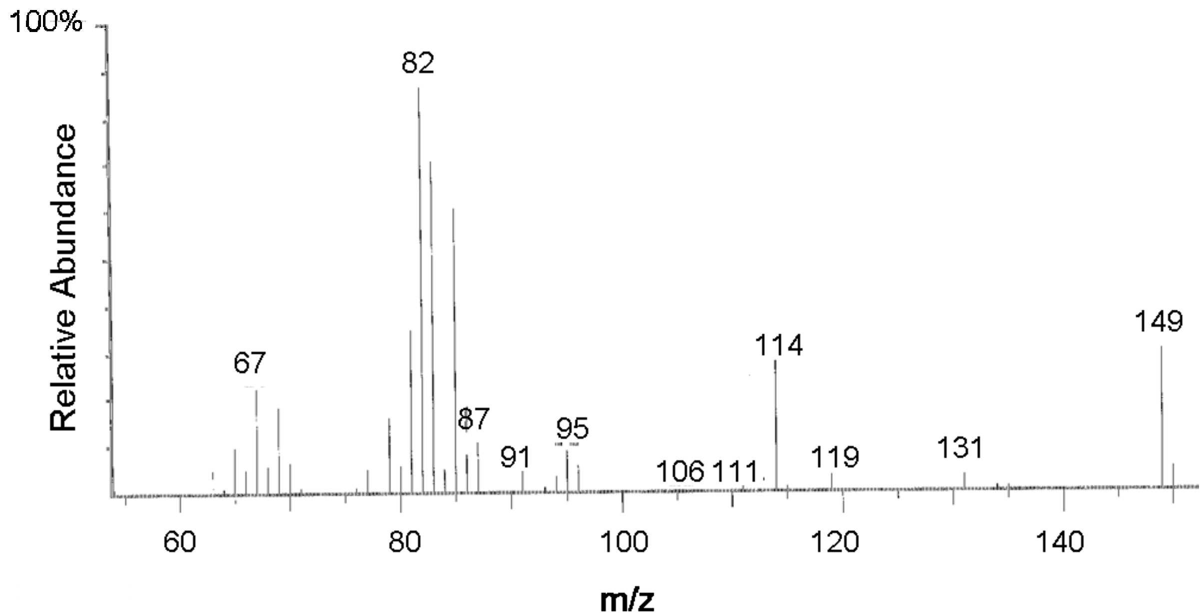

Figure 2. Mass spectra of demethylcantharidin with base peaks at *m/z* 114 and 82 according to a Hewlett-Packard gas chromatograph.

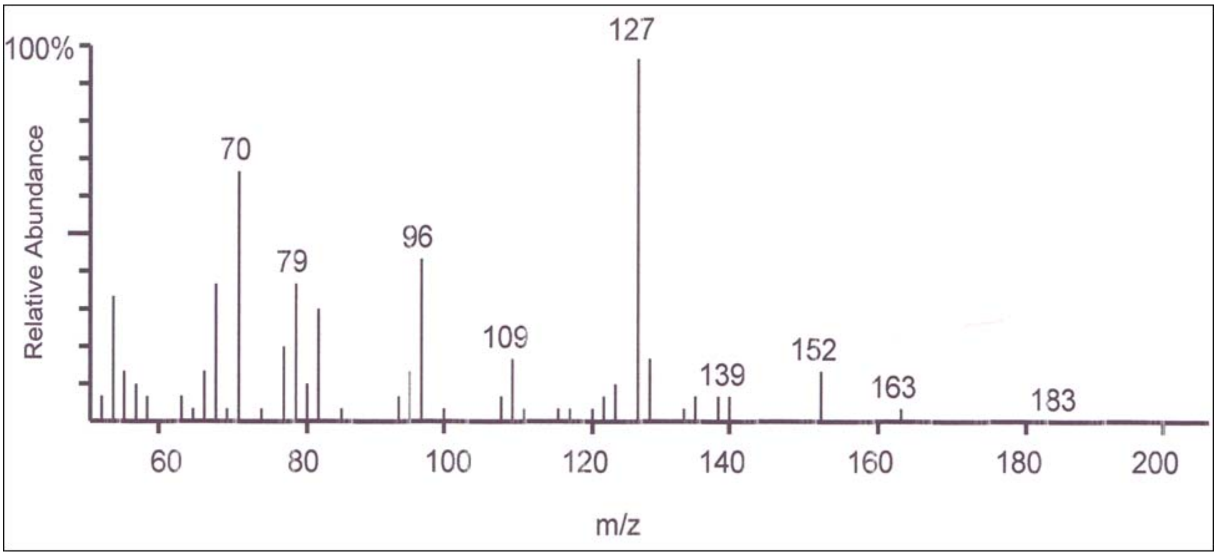

Figure 3. Mass spectra of cantharidinimide with base peaks at *m/z* 70, 96 and 127 according to a Hewlett-Packard gas chromatograph.

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