Scientia Pharmaceutica www.scipharm.at

Research article Open Access

Defensive Agents of *Blaps femoralis*, a Traditional Mongolian Medicinal Insect

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Sci Pharm. 2009; 77: 597–604 doi:10.3797/scipharm.0905-18

Published: July 6th 2009 Received: May 26th 2009
Accepted: July 6th 2009

This article is available from: http://dx.doi.org/10.3797/scipharm.0905-18

Abstract

Knowledge about therapy with insects in Mongolian traditional medicine is less studied even they have been used broadly since ancient time. Several orthodox practitioners have surveyed the therapeutic potentials of defensive agents in *Blaps femoralis* known as "*stink beetle*" in the past.
We present results about content of defensive secretion and surface lipids, both a biologically active principle in insect, used in traditional Mongolian medicine. A combination of gas chromatography and mass spectroscopy was used for the identification of several *p*-benzoquinone derivatives accompanied by straight chain hydrocarbons and fatty acids. None of these compounds has been reported previously from this species.
An IR imaging of insect cuticle surface shows the possibility for the analytical characterization of fatty acids in tissue at high lateral resolution of few microns.

Keywords

Blaps femoralis • Traditional Mongolian medicine • Defensive secretion • Benzoquinone derivatives • Surface lipids • IR imaging

Introduction

Insects and their substances have been used as medicinal resources by different cultures since ancient time because of chemical compounds – e.g. pheromones, defensive sprays, venoms and toxins, which were sequestered from plants or prey and later concentrated or transformed for their own use [1, 2].

Numerous insect originated *materia medica* (Fig. 1) in Mongolian traditional medicine contribute this source of therapeutics and variety of ancient medical treatises by local authors as well as translations of renowned Ayurvedic medical books about animals as medicine exist [3].

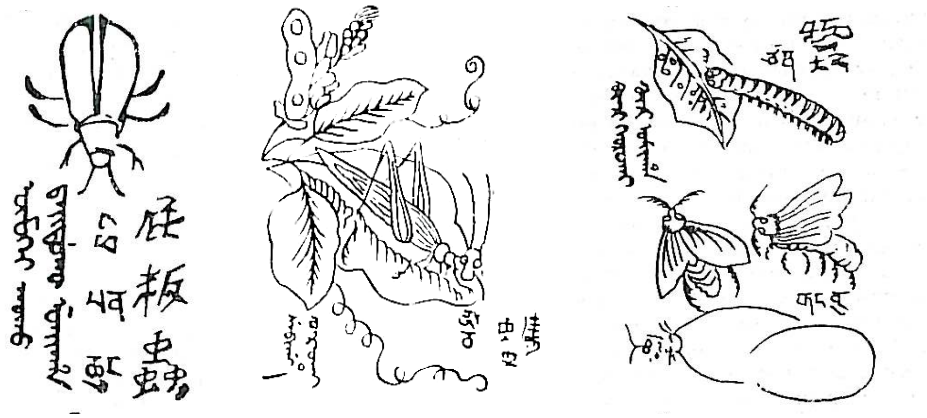

Fig. 1. Illustration of some insects used in traditional medicine in ancient medical book

Once banned by the post-revolutionary government, such medicines now valued by the practitioners of orthodox medicine, government as well as by the society.

Yet the scientific community has to give this major and crucial component of traditional Mongolian medicine the attention it deserves, scientific knowledge about biologically active principles within medicinal insect remain poorly unknown.

Omnivore*, Blaps femoralis (Tenebrionidae)*, [4] known as "*stink beetle*" is used to treat a great variety of locally diagnosed gastrointestinal ailments, e.g. peptic ulcer, inflammation and infection as well as acute abdominal pain and cholecystitis etc. [3] where conventional treatments have failed.

Defensive secretions of *Tenebrionid* beetles are known for their toxicity/irritancy based on *p*-benzoquinones (accompanied by straight chain hydrocarbon carriers) against predators [5]. On the other hand, an external cuticle of insect is covered with a thin layer of lipids in order to prevent the loss of body water, invasion of pathogenic micro organisms and irradiation by UV-light and to contain hydrocarbons with a role in chemical communication.

According to traditional prescription methods described in [3], either two or three insect must be immersed in alcohol and/or in combination with different other substances before they discharge their defensive secretion and not touching surface lipids, the most active principles in it. Thus, the importance of *B. femoralis* defensive agents goes far beyond their essential role in insect life.

Results and Discussion

The qualitative GC/MS analysis of *B. femoralis* defensive secretion showed the presence of *p*-benzoquinone (1), methyl-*p*-benzoquinone (2) and 2,3-dimethyl-*p*-benzoquinone (3), 2-ethyl-*p*-benzoquinone (4), hydroquinone (5), 2-methyl-hydroquinone (6), 2-ethyl-

hydroquinone (7) together with 1-tridecene (8) and 1-pentadecanol (9). Identifications were made by comparing the data (Fig. 2 A) with those from literatures [5–8] and authentic samples available.

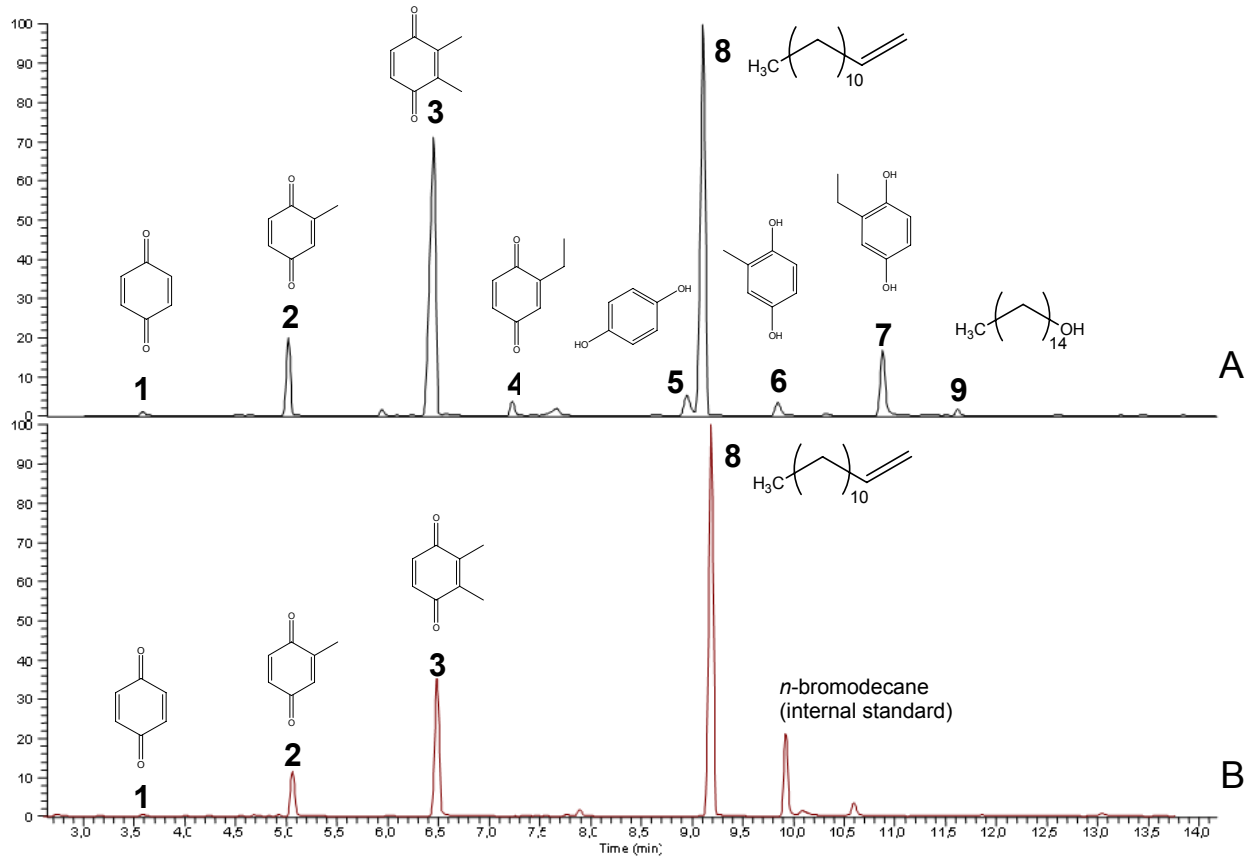

Fig. 2. Total ion chromatograms of defensive secretion (A) and volatile emission (B) of *B. femoralis*

Methyl- and ethylquinones are well established components of the defensive secretions in many arthropods and their presence vary between species and sexes [6]. Studies on the differences in the released defensive volatile by different species support their taxonomy [9, 10]. Therefore, relative content of released volatiles trapped onto charcoal (Fig. 2 B) was analyzed. However it showed slightly lower content of those compounds found very largely in taxa and supporting the conclusion from previous [7] workers that the chemical composition of defensive secretion is less valuable in *Tenebrionid* beetles. The compositions of the defensive secretion of male and female insects were identical (*data not included*).

Wax compounds embedded on insect cuticle surface were collected by using modification of cryo-adhesive method described by [11] and were analyzed with GC/MS.

The surface lipids of the insect was composed of tetradecanoic acid (1), pentadecanoic acid (2), hexadecanoic acid (3), heptadecanoic acid (4), *cis*-9,12-octadecadienoic acid (5), *cis*-octadecanoic acid (6), octadecanoic acid (7), eicosanoic acid (8) and hexacosane (9) (Fig. 3)

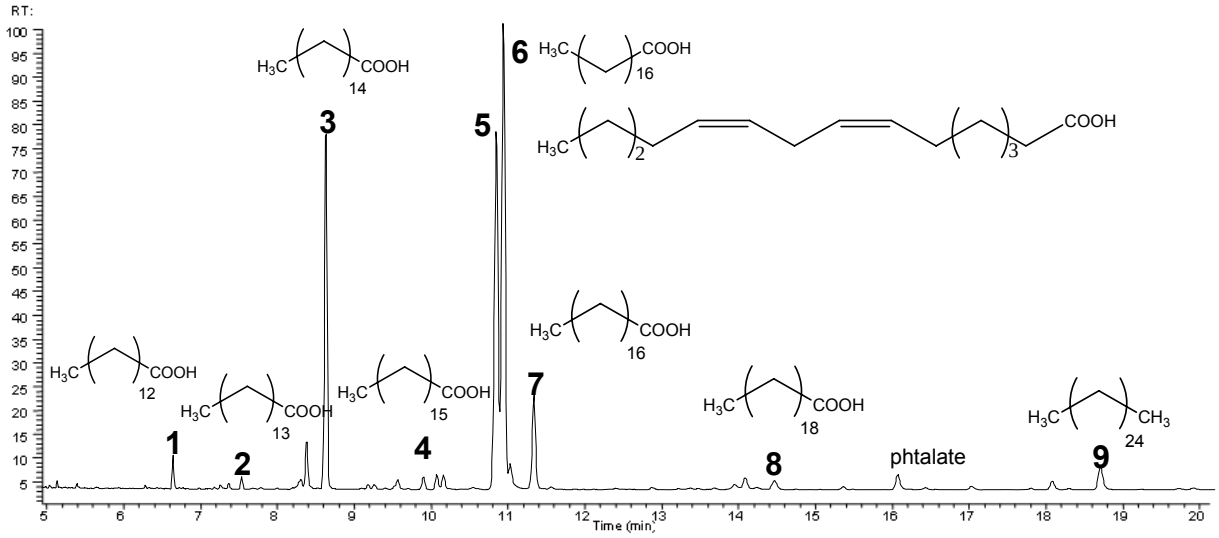

Fig. 3. Total ion chromatograms of *B. femoralis* surface lipid extract

Absorbance bands observed in the IR spectra from *B. femoralis* surface section (Fig. 4 A) were used for local analysis by spectral imaging, a very potent analytical technology which provides insight into the chemical and biological compositions of different tissue samples [12, 13].

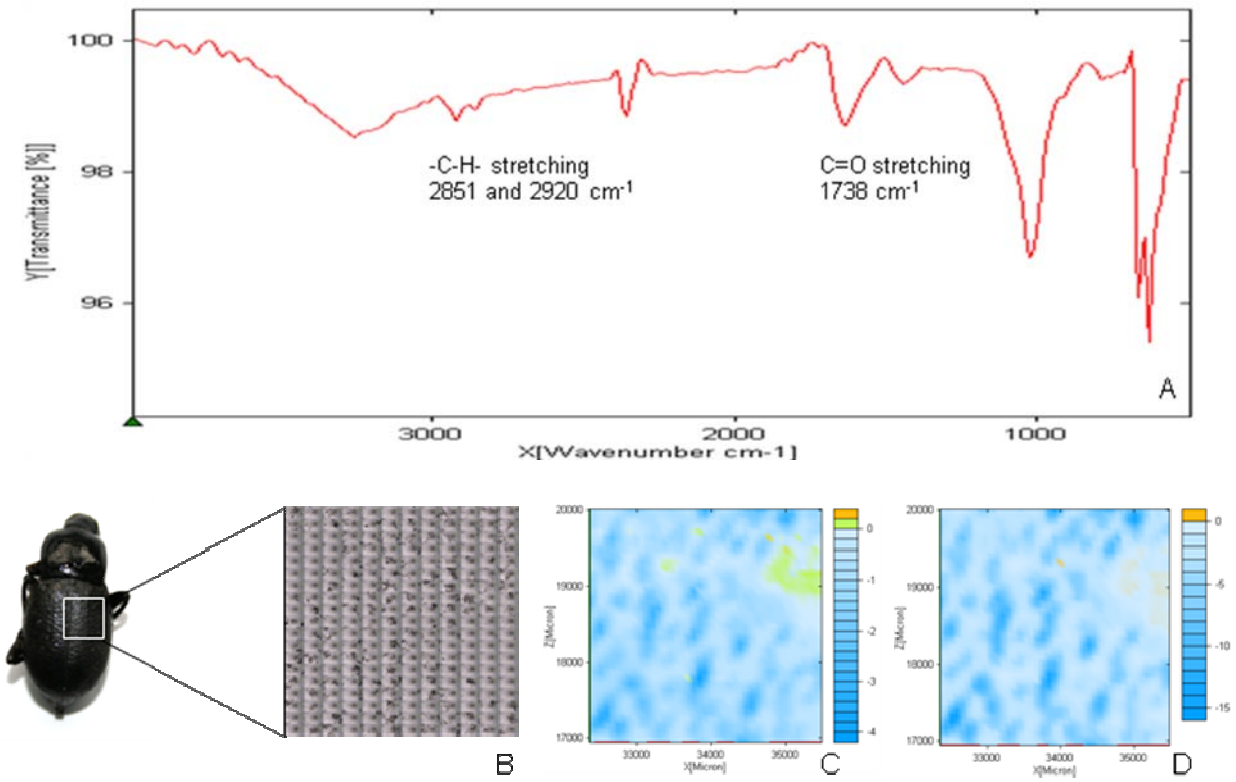

Fig. 4. IR imaging of *B. femoralis* surface lipids

Plotting the intensity distribution of carbonyl (1738 cm^{-1}), and the symmetric and asymmetric methylene stretching (2851 and 2920 cm^{-1}) vibrations in sample indicated that the structure is mainly composed of fatty acids identified with GC/MS (Fig. 3).

IR images (Fig. 4 C and D) were obtained by calculating the integral intensity of prominent bands and transforming it in a color code: dark blue means a high, blue color a low amount of the imaged component. Thus, the contrast in the infrared microscopic images represents the spatial distribution of infrared light absorbed by components of the sample at each wavelength. Because the absorption is proportional to the concentration of the sample, it was clear that the sample distribution is same throughout the area imaged. The vast majority of dark blue spots in image can be characterized by accumulation of lipids around gland cell ducts agreeing with a microphotograph (Fig. 4 B) of beetle armor cuticle.

In summary, this particular chemical investigation appears to be a very first scientific record about defensive agents of *B. femoralis*. However, it exhibit defensive secretion profile that are typical for many *Tenebrionid* beetles [9, 14]; containing methyl-*p*-benzoquinone, 2,3-dimethyl-*p*-benzoquinone, 2-ethyl-hydroquinone together with 1-tridecene, a carrier and surfactant that promotes spread of secretion.

Aliphatic constituents found in *B. femoralis* cuticle surface are very similar to those found in other species and even in plants [15], in profile, mostly consisting from fatty acids of antimicrobial properties [16]. They are mainly octadecanoids, which has recently been demonstrated their importance of the signaling function for plant-insect interactions [17]. A new approach to microscopic chemical imaging of the beetle cuticle using an IR spectroscopy showed relatively homogeneous distribution of protective layer of surface lipids.

Experimental

Insects

Samples of *Blaps femoralis* were collected in August, 2008 from a colony in sandy soil in Ovorkhangai, Mongolia. Living beetles were transferred immediately in a box with ice in order to avoid defensive secretion discharge.

The insects were kindly provided by Dr. Khishgee Dagva (Traditional Medical Science, Technology and Production Corporation of Mongolia) and specimens were deposited at the same Institution, Ulaanbaatar, Mongolia.

Extraction and isolation

For volatile analysis, a whole insect was enclosed in glass containers (2.5 l). The emitted volatiles were trapped onto charcoal traps (1.5 mg of charcoal, CLSA-Filter, Le Ruisseau deMontbrun, Daumazan sur Arize, France) while air circulated for 24 hours. The collected volatiles were eluted with dichloromethane (2 x 20 *µ*l) containing *n*-bromodecane as an internal standard (Fig. 2 B).

On the other hand, viscous defensive secretion of deep purple color was isolated with the help of fine capillaries from a defensive gland of *B. femoralis*, a pair of large sacs lie to the

side of hindgut in the posterior fourth of the abdomen and further diluted with chloroform and was analyzed (Fig. 2 A) with GC/MS.

Surface lipid compounds embedded on insect body surface were successively collected by using a slightly changed version of method described in [11]. Due to its high polarity, water was used as it is not a suitable solvent for waxes and thus dissolution can be excluded.

For cryo-adhesive based mechanical removal a droplet of approximately 50 *µ*l distilled water was mounted on a stainless steel plate. Then a whole cuticle was then gently pressed onto the droplet with another steel plate of 25 mm in diameter until the liquid covered entire surface. The whole arrangement was frozen by dipping the plate into liquid nitrogen for about 10 seconds. Finally, the still frozen cuticle was removed carefully and an imprint was dissolved in *n*-hexane and further analyzed.

Chemical analysis

The chemical components were identified by gas chromatography/mass spectrometry (GC/MS) in comparison with authentic references samples. Prior to gas chromatography analysis hydroxyl containing compounds were transformed to corresponding trimethylsilyl derivatives by reaction with *bis*-N, O-trimethylsilyltrifluoroacetamide (*Sigma Aldrich*) in pyridine for 1 hour at 60^{o}C. Samples were then analyzed on a ThermoQuest/Finnigan TRACE GC 2000 with a TRACE MS (Manchester, UK) equipped with an ECTM-5 capillary column (0.25 mm i.d. x 15 m with 0.25-mm Wlm, Alltech, DeerWeld, IL, USA). Injection volume: 1 *µ*l; split 1:100; 220°C. Ionization energy: 70 eV.

Compounds were eluted under programmed conditions starting from 40°C (2 min hold) and ramped up at 10°C min^{-}1 to 200°C followed by 30°C min^{-}1 to 280°C, which was held for 1 minute prior to cooling. Helium at a flow rate of 1.5 ml/min^{-}1 served as a carrier gas.

IR-mapping

Spectra were recorded with a Bruker IRscope II IR microscope (Bruker Optics Inc., Billerica, MA) equipped with a liquid nitrogen cooled detector, and a 36x IR objective. The IRscope II is coupled to a Bruker Equinox FTIR spectrometer, controlled by a personal computer. Spectral array data were processed and images constructed using Bruker's OPUS software. For FTIR mapping the rectangular aperture was set at 30 X 30 μm^2. The IR mapping data were collected in transmission mode by scanning the computer-controlled microscope stage in a raster pattern in increments of 10 *µ*m.

Acknowledgement

The authors are grateful for the financial support of the Max Planck Society and Dr. Khishgee Dagva for providing *Blaps femoralis* samples.

Authors' Statements

Competing Interests

The authors declare no conflict of interest.

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