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Analysis of chemical profiles of insect adhesion secretions by gas chromatography–mass spectrometry

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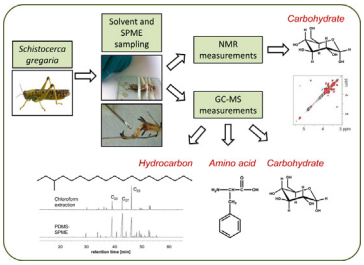
HIGHLIGHTS

- Adhesion secretions of desert locust analyzed by GC–MS.
- Insect secretions are composed of apolar and polar constituents.
- Sampling simplified with contact SPME as compared to solvent sampling.
- Thin-film SPME-GC–MS revealed complex alkane patterns for insect secretions.
- Differences in tarsal (feet) secretions and samples from tibiae (upper legs) identified.

GRAPHICAL ABSTRACT



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ABSTRACT

This article reports on the chemical analysis of molecular profiles of tarsal secretions of the desert locust *Schistocerca gregaria* (Forsskål, 1775) by gas chromatography hyphenated with quadrupol mass spectrometry (GC–MS) as well as ^{1}H-nuclear magnetic resonance (^{1}H NMR) spectroscopy. Special focus of this study was to elaborate on sampling methods which enable selective microscale extraction of insect secretions in a spatially controlled manner, in particular tarsal adhesive secretions and secretions located on cuticle surfaces at the tibia. Various solvent sampling procedures and contact solid-phase microextraction (SPME) methods were compared in terms of comprehensiveness and extraction efficiencies as measured by signal intensities in GC–MS. Solvent sampling with water as extraction solvent gave access to the elucidation of chemical profiles of polar compound classes such as amino acids and carbohydrates, but is extremely tedious. Contact SPME on the other hand can be regarded as a simplified and more elegant alternative, in particular for the lipophilic compound fraction. Many proteinogenic amino acids and ornithine as well as carbohydrate monomers arabinose, xylose, glucose, and galactose were detected in tarsal secretions after acid hydrolysis of aqueous extracts. Qualitatively similar but quantitatively significantly different molecular profiles were found for the lipid fraction which contained mainly *n*-alkanes and internally branched monomethyl-, dimethyl-, and trimethyl-alkanes in the C23–C49 range as well as long chain fatty acids and aldehydes. Especially, hydrocarbons with >C40 carbon numbers have previously been rarely reported for insect secretions. The results suggest that the investigated insect secretions are complex emulsions which allow the attachment of tarsi on

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various otherwise incompatible materials of smooth and rough surfaces. The solid consistence of the established alkanes at ambient temperatures might contribute to a semi-solid consistence of the adhesive, amalgamating partly opposing functions such as slip resistance, tarsal release, desiccation resistance, and mechanical compliance. The methods developed can be extended to other similar applications of studying compositions of insect secretions of other species.

1. Introduction

In the past years, research on adhesive systems in animals, especially mussels, insects, and geckos, has experienced substantial popularity. Of particular interest are insects. Insects are able to adhere to smooth surfaces and walk on all kinds of substrates. This remarkable capability has fascinated scientists for years. Yet the mechanisms of the outstanding ability of insects for fast, strong, and reversible attachment to vertical surfaces or even ceilings is not yet fully understood.

Physical and geometric properties of the insect's adhesive system play a major role in the attachment and locomotion procedure [1]. Legs (tibiae) and feet (tarsi) exhibit special properties that allow this sophisticated attachment. Over time two different forms of adhesive organs have evolved independently from each other several times in nature, namely hairy (e.g., in flies, beetles) and smooth, flexible (e.g., in ants, bees, cockroaches, grasshoppers) tarsal pads [2–4]. Both systems are elastically soft and ensure close contact between the tarsus and the substrate [5,6]. Additionally, insects emit a fluid from their tarsal glands [7]. This secreted liquid was found to support the attachment of the insect to the substrate filling contact cavities and establishing maximum area of real contact [8], which is especially important for smooth pads [6]. Capillary and viscous adhesion is enhanced due to this secretion. Several authors showed that the fluid of some insects is of biphasic nature, containing both hydrophobic and hydrophilic components [7,9,10]. Vötsch and coworkers suggest that this emulsion might act as a bonding agent bringing together surfaces of different polarity [5,9]. Other authors propose that the secreted emulsion of the stick insect *Carausius morosus* exhibits non-Newtonian characteristics, such benefiting from both wetting properties upon shearing and increased viscosity in static condition [11,14].

Previous studies showed that the lipid fraction of the outer body layer of insects (epicuticle) resembles the lipid composition of their footprints or general cuticular hydrocarbon fraction [15–17]. It was proposed that this congruence is a general principle in beetles [17]. There are a number of studies which have examined the chemical composition of the nonpolar or lipid fraction of insect cuticular secretions. Hydrocarbons, fatty acids, wax esters, alcohols, triglycerides, and some other unassigned substances have been identified [12,15,16,18–21]. A significant number of studies dealt with insect hydrocarbons, in particular on cuticles [21–29]. Studies which report polar constituents are less common. However, not surprisingly, as constituents of the polar fraction e.g., of the footprints of *Locusta migratoria* carbohydrates and amino acids have been identified [9].

The most critical step in the analysis of insect secretions is the sampling procedure which should be representative for the composition of the respective specimen and for the type of information to be derived. Sample discrimination should be avoided, spatially controlled sampling would be desirable to eliminate blurring of chemical information from mixing of components of different locations, and last but not least enrichment would be desirable owing to the small sample volumes. Solvent extraction was the most common sample preparation method in the past. For example, insects were killed by freezing with nitrogen. External lipids have then been extracted by hexane, pentane, petroleum ether, chloroform, dichloromethane, or mixtures of chloroform/methanol (Folch method: 2:1, v/v; Bligh–Dyer method: 1:2; v/v) [23]. Hexane, pentane, petroleum ether extraction have the advantage of less cross-contamination from internal lipids such as fatty acids.

Another sampling strategy was the so-called solid injection technology [21,30,31]. Thus, pieces of the insect such as cuticle or single exocrine glands were inserted in sealed glass capillaries directly into the GC injector.

Recently, solid-phase microextraction (SPME) [32–36], developed in the 1990s by Arthur and Pawliszyn [37], has become an accepted alternative sampling technique for studying lipid compositions of insects [38,39]. Volatile components can be sampled by head-space SPME [40–43]. For instance, hydrocarbons up to C29 have been determined in termite cuticles [42]. Since this method is discriminative in favor of low molecular weight compounds, other researchers tested to use SPME sampling by direct contact of the SPME fiber with the specimen's surface [24,44]. Thus, surface hydrocarbon profiles have been analyzed by rubbing SPME fiber on the cuticle surface of the Colorado potato beetle *Leptinotarsa decemlineata* while the specimen was glued onto a handheld [24]. The advantage is that higher hydrocarbons can also be extracted e.g., up to C37 in [24].

Herein, we report on compositions of surface secretions of the locust *Schistocerca gregaria* Forskål (Orthoptera, Acrididae). The goal was to elucidate both the lipid fraction as well as the polar constituents. Of particular interest was the comparison of compositions between tarsal adhesive secretions and normal cuticular secretions. Spatially controlled sampling at the tarsal adhesive organs (euplantulae), the praetarsal adhesive organ (arolium), and the tibiae is necessary for this purpose. However, in the following text, we do not distinguish between tarsal and pretarsal adhesion secretions. Various sampling methods have, therefore, been examined and were compared including solvent sampling with aqueous and apolar extraction media as well as contact SPME.

2. Materials and methods

2.1. Study animals and preparation of specimens

Mature locusts (*Schistocerca gregaria* Forskål, Fig. 1a) were taken from a laboratory breeding fed with graminiferous plants and lettuce. They were immobilized on a glass plate by bonding their feet (Fig. 1b) with a wire to this glass plate while their body was fixed to the plate by an adhesive tape (Fig. 1c). Before sampling, the immobilized locust's feet and legs were cleaned thoroughly by washing them with the respective solvent used for the sampling and depending on which analytes were in the focus, respectively, i.e., water, toluene or chloroform. In other words, the first fractions of solvent sampling were discarded.

2.2. Solvent sampling

Solvent sampling was carried out with a 10 μL Hamilton syringe, and various solvents depending on which analytes were of interest