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Molecules of Interest

Aphid sex pheromones: from discovery to commercial production

Michael A. Birkett, John A. Pickett*

Biological Chemistry Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

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Abstract

This review charts the progress made with aphid sex pheromone chemistry, from initial identification of cyclopentanoid nepetalactones, for example (4a*S*,7*S*,7a*R*)-nepetalactone (**1**) and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**2**), to commercial production from a renewable non-food crop, the catmint, *Nepeta cataria* (Lamiaceae). The availability of aphid sex pheromone components is now facilitating the development of new aphid pest control strategies, incorporating the use of other semiochemicals, particularly in the manipulation of populations of aphid parasitoids and aphid predators such as lacewings, which can utilise the nepetalactones and closely related molecules to locate their hosts and prey. This is the first example of a plant resource being developed as a feedstock for the production of a commercially valuable insect pheromone. The development of a plant-based production route highlights the tremendous potential that higher plants offer as cheap and renewable resources for the production of insect semiochemicals, through the wide array of secondary metabolites that they can generate.

Keywords: Aphid; Sex pheromone; (4a*S*,7*S*,7a*R*)-Nepetalactone; (1*R*,4a*S*,7*S*,7a*R*)-Nepetalactol; *Nepeta cataria*; Renewable resource; Parasitoid; Lacewing

1. Introduction and life-cycle

Aphids (Homoptera, Aphididae) are the main insect pests of northern European agriculture. Aphid species within the sub-family Aphidinae, which contains the majority of pest aphid species, often alternate between a winter (or primary) host, which is usually a tree or shrub, and a summer (or secondary) herbaceous host, including crops, which grows rapidly during the summer (Fig. 1). Aphids generally reproduce sexually on the winter host, with mated females laying cold-resistant overwintering eggs (a). The stem mother, or fundatrix, which hatches from this egg (b), gives rise to a succession of asexual (parthenogenetic) stages on the winter host, giving birth to live wingless and winged females. Winged forms (c) then migrate to the summer host, and continue to reproduce asexually. As autumn approaches, the asexually reproducing aphids on the summer host respond to the reduced daylight hours by producing winged males and females (d). Females (gynoparae) migrate to the primary host, and produce wingless sexual females (oviparae), which release sex pheromones from glandular epidermal cells lying beneath scent plaques on the tibiae of the hind legs. Winged male aphids that fly separately detect the released sex pheromones via olfactory receptors (secondary rhinaria) situated mainly on the third segment of the six-segmented antennae.

2. Discovery of aphid sex pheromones

During the earlier part of the twentieth century, there were a number of suggestions that sexual female aphids (oviparae) might produce a sex attractant, i.e. a sex pheromone. The role of the aphid sex pheromone was considered to be no more than a close range aphrodisiac by various entomologists, until Pettersson (1970) published the first definitive evidence for an aphid sex pheromone and showed that male aphids in a behavioural bioassay responded to volatiles produced by sexual females. Since these early studies, sex pheromones have been identified, in pioneering studies at Rothamsted, from a number of aphid species, all of which are in the

* Corresponding author. Tel.: +44-1582-763133; fax: +44-1582-762595.

E-mail address: john.pickett@bbsrc.ac.uk (J.A. Pickett).

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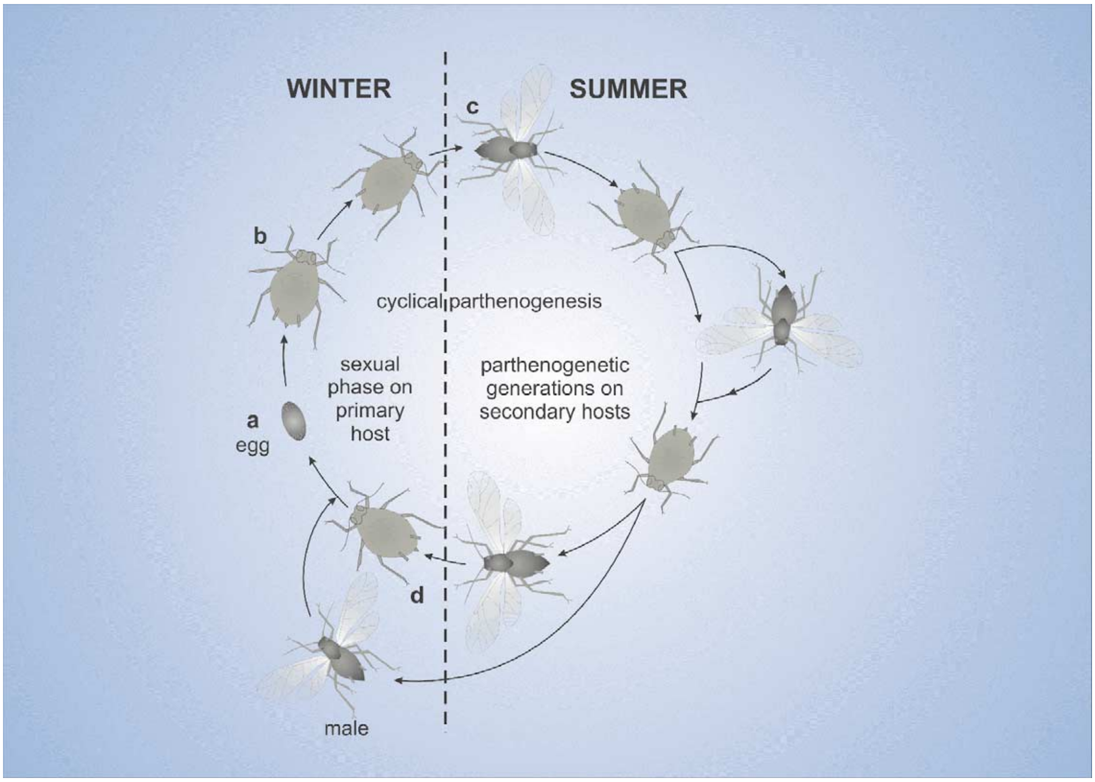


Fig. 1. The life-cycle of winter and summer host-alternating aphids within the sub-family Aphidinae.

subfamily Aphidinae (Hardie et al., 1999). The first chemical identification of an aphid sex pheromone was for the vetch aphid, *Megoura viciae* Buckton (Dawson et al., 1987). Innovative use of electrophysiological recording techniques with this very small insect using finely sharpened tungsten microelectrodes (Boeckh, 1962) from single olfactory cells in the secondary rhinaria of male aphids directly coupled to gas chromatography (GC) pinpointed two compounds from solvent elutions of female hindlegs. The biologically active components, present at sub-nanogram levels, were located on capillary column GC by coupling the effluent to the electrophysiological preparation and, simultaneously, to a flame-ionization detector (FID) (Fig. 2; Wadhams, 1990). Tentative identification by GC–mass spectrometry (GC–MS) and confirmation by comparison with synthetic materials authenticated by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy gave (4a*S*,7*S*,7a*R*)-nepetalactone (**1**) and, with further X-ray crystallography studies, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**2**), which are monoterpenoids in the cyclopentanoid series (Fig. 3). The sex pheromones for other aphid pest species in the Aphidinae have been identified and principally comprise **1** and **2** (see Table 1; Hardie et al., 1999). However, an investigation of the damson-hop aphid, *Phorodon humuli* (Schrank), showed that the pheromone of this species comprised neither compound **1** or **2**, but a mixture of the two diastereoisomers (1*S*)- and (1*R*,4a*R*,7*S*,7a*S*)-nepetalactols **3** and **4** (Fig. 3; Campbell et al., 1990). The availability of authentic samples of aphid sex pheromone components has allowed further studies to be conducted. In-depth behavioural studies conducted in the laboratory and in the field have since demonstrated relatively long-range attraction of male aphids (e.g. Hardie et al., 1992, Gabrys et al., 1997). Furthermore, the aphid sex pheromone component **1** attracts not only males of certain aphid species, but is also employed as a foraging stimulant by parasitic wasps (parasitoids) that develop from eggs laid in the aphid, subsequently destroying their host (Glinwood et al., 1998).

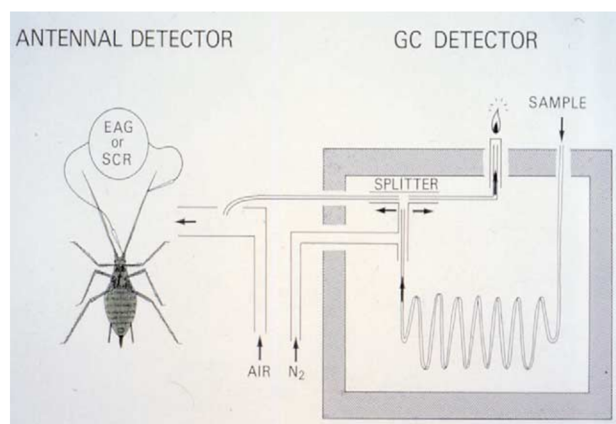


Fig. 2. The coupled GC-electrophysiology system used for the identification of aphid sex pheromone components.

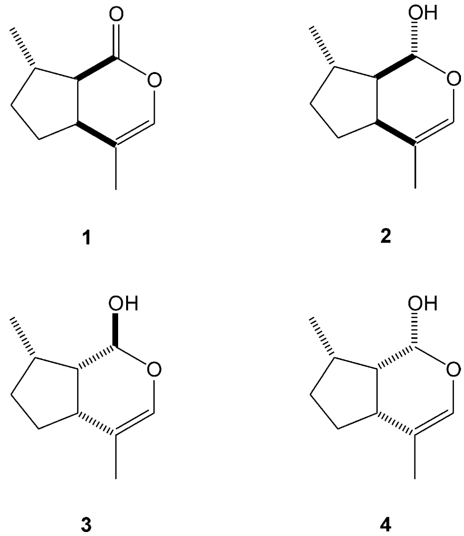


Fig. 3. Aphid sex pheromone components: (4a*S*,7*S*,7a*R*)-nepetalactone (**1**); (1*R*, 4a*S*,7*S*,7a*R*)-nepetalactol (**2**); (1*S*,4a*R*,7*S*,7a*S*)-nepetalactol (**3**); (1*R*,4a*R*,7*S*,7a*S*)-nepetalactol (**4**).

Table 1
Composition of aphid sex pheromones identified (taken from Hardie et al., 1999)

Sex pheromone blend	Aphid species	Reference
High **1**: Low **2**	*Aphis fabae*	Dawson et al. (1990), Isaacs (1994)
	Megoura viciae	Dawson et al. (1987), Hardie et al. (1990)
Low **1**: High **2**	*Schizaphis graminum*	Dawson et al. (1988)
	Cryptomyzus spp. (x 2)	Guldemond et al. (1993)
	Dysaphis plantaginea	Wadhams, unpublished data
1 and **2** equal	*Acyrthosiphon pisum*	Dawson et al. (1990)
	Myzus persicae	Dawson et al. (1990)
1 only	*Sitobion avenae*	Lilley et al. (1994/95)
	Sitobion fragariae	Hardie et al. (1992)
	Brevicoryne brassicae	Gabrys et al. (1997)
2 only	*Rhopalosiphum padi*	Wadhams, unpublished data
3 and **4**	*Phorodon humili*	Campbell et al. (1990)

3. Commercial production

For further biological studies involving aphids and their parasitoids that required authentic samples of sex pheromone components, a synthetic route to the nepetalactone **1** and nepetalactol **2** was initially developed from citronellol (Dawson et al., 1996). However, these compounds possess a number of chiral centres, and subsequent laboratory and field trials demonstrated that high enantiomeric purity was required for biological efficacy (Hardie et al., 1997). The strict stereochemical requirement for field efficacy necessitated the use of starting material of high enantiomeric purity, which would result in a cost exceeding £1000/g of pheromone for raw materials alone. Thus, commercial pheromone production from this route was not economically viable. Furthermore, the route utilised the highly toxic selenium dioxide and large amounts of organic solvents, aggravating other difficulties with scale up.

The nepetalactone **1** is produced by the catmint, *Nepeta cataria* (Lamiaceae = Labiatae), and can be obtained in high yield from fresh plant material following extraction. A collaborative project ("SEMIOCHEM"), [1] funded under the BBSRC/DEFRA LINK CIMNFC (Competitive Industrial Materials from Non-Food Crops) programme and involving Rothamsted, was set up with the aim of developing *Nepeta* spp. as renewable plant resources for the production of the commercially valuable aphid sex pheromone components. Initially, seeds of commercially available cultivars of *N. cataria* were obtained from a number of sources and the yields and enantiomeric purities were obtained. Two cultivars were selected from these studies for further development and small plots (0.1 acre) were established on two sites in the UK. Seeds were propagated at a nursery, hardened off as modules, and then planted using a module planter. Based on these data, one cultivar was selected for further development and was planted on a commercial acreage. Following co-distillation with cyclohexane, less than 35 t of biomass yielded over 30 kg of oil, the composition of which was investigated in relation to harvest date by GC–MS, with batches ranging from 85 to 97.5% of **1**. Based on the purities and yields, two harvests of *N. cataria* can be achieved each year. For commercial extraction, the tub trailer containing harvested material is connected directly to the steam distillation plant, which is fitted with an injection pump at the first point of entry to enable co-distillation (Fig. 4). Steam is applied whilst injecting cyclohexane, to extract and collect the volatile oil from the plant material. The cyclohexane and steam then travel through the horizontal pipework to a condenser, where they condense into a cyclohexane fraction and water in the separator. The cyclohexane fraction is then collected, and the cyclohexane removed from the volatile oil by vacuum distillation. Overall estimated costs for the

[1] The SEMIOCHEM project was led by IACR-Rothamsted (now Rothamsted Research) with three industrial partners: English Hop Products Limited (now Botanix Limited), pioneers in the extraction and purification of essential oils, Richard Wood Partnerships, who grew the *Nepeta* cultivars to develop necessary agronomic protocols, and Agrisense-BCS Limited, who have considerable experience with formulation and marketing of pheromone-based products. Later work involving cat studies was done collaboratively with the Institute of Anthrozoology at the University of Southampton. The SEMIOCHEM 2 project, funded under the same LINK initiative, and which will commence shortly, will be led by Rothamsted and includes three of the partners (Botanix, Agrisense and University of Southampton), along with a new partner company, Biological Crop Protection (BCP) Limited.

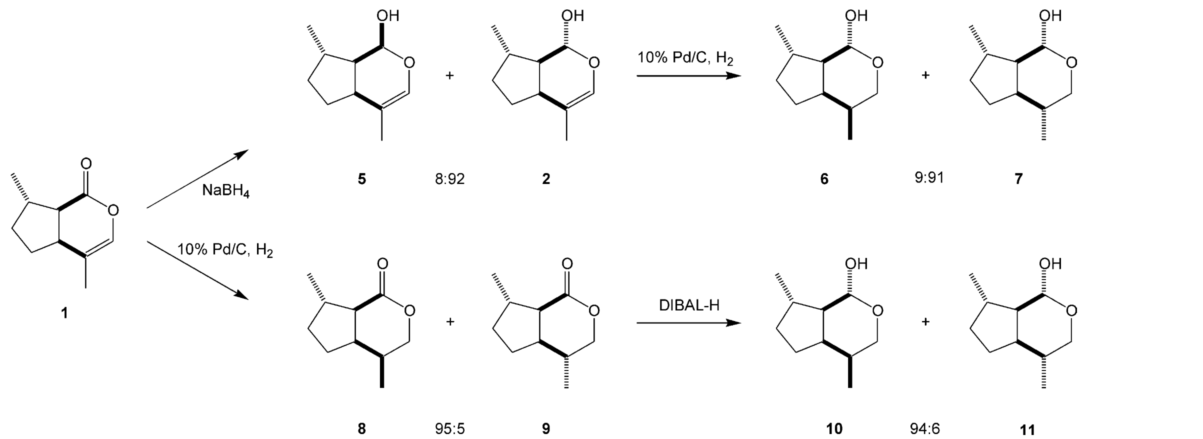
Fig. 4. Steam distillation of harvested *Nepeta cataria* (Lamiaceae) plant material.

final product **1**, including all costs associated with land preparation, module production, harvesting and extraction were approximately £1/g, which compares favourably with the costs of producing the material by conventional synthesis from citronellol of nearly £1000/g. Reduction of the **1** to obtain the nepetalactol **2** was initially attempted using enzymatic routes, for example, baker's yeast, but was not successful. Although a biological route was preferable, milder chemical methods were sought. Reductions were initially carried using diisobutyl aluminium hydride (DIBAL-H), but a route using a much safer reagent, sodium borohydride ($NaBH_4$), which can be used on a commercial scale, was explored. The crude oil containing **1** was reduced successfully in a methanolic solution of $NaBH_4$ at 0 °C, with the products comprising a 92:8 mixture of **2** and the nepetalactol **5**, with no significant other products (see Scheme 1). The reaction was subsequently developed to commercial scale with no complications.

In order to use the commercially available materials **1** and **2** against aphids and to attract aphid parasitoids, these compounds were formulated using polymer extrusion technology to give a slow but consistent release rate. Here, the material, in the form of a flexible PVC rope, can be cut easily into different lengths to provide the required release rates. Optimal release rates for these formulations were determined, and stable release profiles for these formulations over a period of 1 month have been achieved. Formulation has now been scaled up and 200 kg batches of formulated polymer lures are routinely prepared.

The biological activities of the formulations of **1** and **2** were determined for aphids and aphid parasitoids in a range of field trials. The sex pheromone for the aphids, *Sitobion fragariae* and *Rhopalosiphum padi* comprise **1** and **2** respectively. Using water-traps, plant-derived material was shown to be as effective in attracting males of *S. fragariae* and *R. padi* as was the 95% enantiomerically pure synthetic material prepared from citronellol. Similarly, no differences were observed in the response of aphid parasitoids to the plant-derived polymer formulation of **1** and the synthetic material.

In addition to the primary aim of producing agents for the control of aphid pests by trapping of male aphids and attraction of aphid parasitoids, another use of plant-derived nepetalactones in SEMIOCHEM was identified in collaboration with groups in Seoul, Korea, at the Silwood Park campus of Imperial College and the University of Manitoba, Canada. During trials conducted in Korea and Canada, later at Silwood Park, it was found that significant numbers of the aphid predator lacewing, *Chrysoperla carnea*, were caught in water traps baited with formulations of **1** and **2**. Literature reports have revealed that the lacewing, *Chrysopa septempunctata*, is attracted to the vine, *Actinidia polygama* (Actinidiaceae), a plant that biosynthesizes dihydronepetalactols (Hyeon et al., 1968). Therefore, in addition to **1** and **2**, dihydronepetalactols were prepared for investigating the response of lacewings in the field (Scheme 1; Hooper et al., 2002). The enantiomerically pure diastereoisomers (1*R*,4*R*,4a*R*,7*S*,7a*R*)- and (1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactol [(**7**) and (**10**) respectively] were synthesized diastereoselectively from **1** (see Scheme 1). Compound **7** was synthesized by sodium borohydride reduction followed by hydrogenation, and **10** by hydrogenation, followed by DIBAL-H reduction. The stereochemistry of the compounds and several derivatives was determined by NMR spectroscopy and X-ray crystallography, and were identified as isoneomatatabiol (**7**) and neomatatabiol (**10**), natural products from *A. polygama*, for which the lactol stereochemistry was previously incompletely defined. Compound **10** was found to catch significant numbers of three species of lacewing in the field, in Korea, more *C. cognata*, and in the UK *Nineta vittata*, and most notably *Peyerimhoffina gracilis*. All species caught in significant numbers were found more frequently in traps releasing **10** than **7**, whilst in Korea, more *C. cognata*, *C. formosa* and *C. phyllochroma* were found in traps releasing **2**. The catch of *P. gracilis* was of particular interest, as this lacewing had not been previously recorded in the UK (Donato et al., 2001). When sexed, the lacewings of all species were found to be male, implying a possible pheromonal role for these or structurally related compounds. Further studies conducted in alfalfa fields in Canada using formulations of **1** and **2** showed significant numbers of the lacewing *C. occulta* in pheromone-baited water traps, with only male lacewings being caught (N.J. Holliday, personal communication). In EAG experiments, all the cyclopentanoid monoterpenes tested on *P. gracilis* elicited some response, but the largest effect came from **10**, which correlated with the results of field catches.



Scheme 1. Reduction of (4a*S*,7*S*,7a*R*)-nepetalactone (**1**) to form (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**2**), and synthesis of lacewing semiochemicals iso-neomatatabiol (**7**) and neomatabiol (**10**) starting from **1**.

Whilst work is continuing to develop plant-derived nepetalactones as commercial products for pest management and beneficial insect manipulation, an interesting opportunity is developing in the companion animal sector. Thus, a specifically pure isomer of compound **1** has been commercialised in the form of a controlled release dispenser for inclusion into cat toys. Such toys remain attractive to cats for up to 6 months and over 500,000 such dispensers have already been manufactured and incorporated into cat toys.

4. Future prospects

Unexpected developments arising from SEMIOCHEM, including the attraction of important aphid predators such as lacewings, and the use of the nepetalactone **1** in new sophisticated cat toys, have provided more extensive and unexpected exploitation opportunities, which will be researched in a new programme, SEMIOCHEM 2. These opportunities relate to the exploitation of aphid parasitoids and predators in UK agricultural and horticultural crop protection systems, and also to broader aspects of the exploitation of semiochemicals for control of pests such as vectors of disease pathogens, for example, mosquitoes, mites and ticks, and those causing hygiene or nuisance problems, for example, houseflies, cockroaches and dust mites.

Plant-based alternative production and delivery routes for other insect semiochemicals are being developed by partners in the SEMIOCHEM 2 consortium for use against insect pests of human health and welfare, for example, mosquitoes and sandflies. These include the oviposition pheromone, (5*R*,6*S*)-6-acetoxy-5-hexadecanolide, for pathogen-vectoring *Culex* spp. mosquitoes, produced via conversion of a Δ^5-fatty acid in the seed oil of the summer cypress, *Kochia scoparia* (Chenopodiacaeae) (Pickett and Woodcock, 1996; Olagbemiro et al., 1999), and a male-produced sex pheromone, 9-methylgermacrene-B, for the sandfly, *Lutzomyia longipalpis*, which vectors urban visceral Leishmaniasis, from the cranesbill, *Geranium macrorrhizum* (Geraniaceae) (Hamilton et al., 1996; Hooper et al., unpublished data).

5. Conclusions

Pheromones, and other semiochemicals, can be used with great success as components of insect pest management strategies. However, difficulties in achieving cheap and efficient synthesis starting from fine chemicals have obstructed commercial production. The highly successful SEMIOCHEM programme has established the basis for production and formulation of nepetalactone **1** on a commercial scale from the catmint, *N. cataria*, grown as a non-food arable crop in the UK, and is the first example of a plant resource being developed as a feedstock for the production of a commercially valuable insect pheromone. The availability of **1** and closely related molecules is enabling the development of new aphid pest control strategies, mainly by optimising the manipulation of populations of aphid parasitoids, for example, a SAPPIO programme, "3D Farming—Making biodiversity work for the farmer", co-ordinated by Professor Wilf Powell of Rothamsted Research), and also through the manipulation of populations of aphid predators such as lacewings. This work highlights the potential that higher plants offer as cheap and renewable resources for the production of speciality chemicals such as insect semiochemicals, through the wide array of secondary metabolites that they can generate, as exemplified through the new and exciting studies on production of semiochemicals for use in controlling insect pests of human health and welfare.

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