Multicomponent Mandibular Gland Secretions in Three Species of Andrena Bees (Hym., Apoidea)

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Andrena, Mandibular Gland, Pheromone, Spiroacetals

50 volatile constituents of the mandibular gland secretions in males and females of three species of Andrena bees, A. wilkella (Chr.), A. ovatula (K.) and A. ocreata (K.), have been identified. The secretions are made up of a series of spiroacetals of four different systems together with monoterpenes and unbranched acyclic compounds. Many of the components, particularly several of the dominant ones, occur in both sexes of all three species, so that the volatile secretions are qualitatively very similar. Females contain about 100 μg per individuum, which is 3 – 5 times the amount of the males’ secretions. Major spiroacetals are E,E- and Z,E-2,8-dimethyl-1,7-dioxaspiro[5.5]undecane and Z,E-2-ethyl-7-methyl-1,6-dioxaspiro[4.5]undecane, while prominent monoterpenes are geraniol and citronellol. Straight chain hydrocarbons C_{17}, C_{19}, C_{21} and C_{23} occur in fairly large amounts, mostly with increasing concentrations. With respect to the quantitative distribution of components of minor concentration, the bouquet of A. wilkella is clearly distinguishable from A. ovatula and A. ocreata which from the morphological and ethological point of view are more closely related. Behaviour tests with A. wilkella indicate high biological activity of the main spiroacetal.

Introduction

Bees of the species-rich genus Andrena (Hymenoptera, Apoidea) emit, when handled, a strong odour which is easily perceived by humans. As proved in the 11 species investigated so far, the compositions of the volatile multicomponent secretions are species specific [1 – 3], while the odour bouquets of males and females strongly resemble each other. Andrena males mark spots along their flight routes with their mandibular gland secretion when patrolling species specific mating flight areas. It has been shown [4] that this secretion acts as a pheromone for males, while the female behaviour has not been studied in this respect.

The present report concerns three Andrena species, A. ocreata (Chr.), A. ovatula (K.) and A. wilkella (K.) which all belong to the subgenus Taeniandrena Hed. [5]. From the morphological point of view they are very closely related. In the males, the copulatory apparatus and the segmentation of the antennae offer more definite characteristics for species determination. In the females, however, the differences are subtle, and the determination is based on the colour of different parts of the fur and the wings and also on the punctuation and the shagreening of the cuticle. The characteristics are relatively poorly defined, and the taxonomy of old worn females is usually very difficult. A. ocreata can often hardly be distinguished from A. ovatula. On the other hand A. wilkella, even in the female sex, may quite well be distinguished from the other two species, which Warncke (pers. comm.) regards as more closely related.

Phenology, habitat selection and behaviour of these three species are very similar. On the island of Öland, where this investigation was carried out, A. ocreata and A. ovatula appear typically at the same time in the first week of June and A. wilkella about a week later. The males of A. wilkella perform mating flight along the sides of shrubbery and hedges at a level of 1 – 3 m above the ground, while males of the other two species patrol in open areas horizontally and close to the ground. However Stöckhert states [6], referring to A. similis (=A. ocreata) [7] males: “Nicht selten sieht man sie auch wie die δ δ von lapponica Zett. und clarkella K. an von der Sonne beschienenen Baumstämmen auf und ab fliegen”, and on one occasion on Öland A. ovatula and A. wilkella males were observed to patrol together a flowering hawthorn (Crataegus oxyacantha L.).
Species belonging to the subgenus Taeniandrena forage commonly on flowers of the family Leguminosae. *A. ocreata* and *A. ovatula* on Öland seem to prefer especially *Oxytropis campestris*. Also their mating flights occur mostly among the flowers of this plant, which often dominates large areas of the biotope during the flight season. *A. ovatula* occurs sometimes nesting in large aggregations. It is the only species of those dealt with here that appears in a second generation in late July and August.

The purpose of this study was to search for possible species distinguishing characteristics through chemical analyses of the volatile mandibular gland secretion. It is a continuation of our analyses in the chemical language of solitary bees, forming a basis for ethological and ecological studies of chemical signalling.

### Materials and Methods

All bees used in this study were collected on Öland, an island in the Baltic near the mainland of southern Sweden, during the period 1974–1981. After preliminary studies in 1974 (20 samples were analysed, 14 of them by GC/MS) and 1975, the main isolations/identifications were carried out in 1980, with complementary analyses in 1981. After collection, the bees were kept alive in a refrigerator at 8–12 °C. Chemical analyses were carried out during the collection day or the day after. The cephalic odours of *Andrena* bees have been shown to emanate from the mandibular glands [1, 2]. In 1974 and 1975, whole heads, with the mandibles pulled out of their attachments to expose the mandibular glands, were put on a precolumn glass tube [8]. This was heated up to 150 °C for 10 minutes to drive off volatile compounds directly into the glass capillary column, OV-101 and SE-30 were used as stationary phases. For the analyses performed in 1980 and 1981, 1–5 heads were extracted in pentane. The concentrated extracts were directly submitted to GC/MS using a WG-11 glass capillar column [9]. In 1980 and 1981, 7 and 4 samples respectively, were analysed. In all series a LKB-2091 GC/MS was used. Voucher specimens are kept at the ecological station for taxonomic purposes.

### Results and Discussion

**A) Chemical analyses:** 50 volatile components were identified from the cephalic secretions of male and female *A. wilkella*, *A. ocreata* and *A. ovatula*. Fig. 1

![Fig. 1. Andrena wilkella, male cephalic secretion 50 m glass capillary column, WG 11 as stationary phase, programme 70–180 °C, 3 °C/min](image_url)
shows a representative region between tridecane and docosane in a chromatogram of the pentane extract from five *A. wilkella* males. In some cases a few volatile compounds were found to elute before tridecane; compounds higher boiling than docosane include tricosane, tetracosane, pentacosane and some unsaturated hydrocarbons of the same chain length. They are probably cuticular substances. Compounds are listed in Table I in order of increasing retention times on a glass capillary column coated with WG-11 as stationary phase. Identifications based on mass spectral data and – with the exception of com-

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<th>wi.¿</th>
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ponents 24, 37 and 38—on gas chromatographic retention times as compared to pure synthetic reference samples. It can be seen that the compositions are complex. The compounds found represent three major groups of substances: spiroacetals, unbranched acyclic compounds and isoprenoids. As shown in Table I the bouquets of the three species and sexes are basically very similar; differences are found in the relative amounts of several constituents, especially those of minor concentration. A few minor components are found to occur species-specifically.

The *Taeniandrena* spp. contain as was also shown for *A. haemorrhhoa* [9], a remarkable blend of spiroacetals. In the present study 19 spiroacetals were identified. Four different systems are present: two 1,6-dioxa[4.4]nonanes (I), three 1,6-dioxa[4.5]decanes (II), one 1,6-dioxa[4.6]-undecane (III) and four 1,7-dioxa[5.5]undecanes (IV) [10]. Most of the compounds occur as mixtures of *E,Z*-diastereomers in nature [11].

The spiroacetals 2/10 and 6/8 have been identified earlier from *A. wilkella* [3] while 3/5 and 11 have been found in common wasps [12] and *A. haemorrhhoa* respectively [9]. Components 12/17, 15/18, 19/21, 20, 38 and 41 are found for the first time as natural products. Compounds 38 and 41 represent trihydroxyketones which upon ringclosure form spiroacetals with an additional hydroxylgroup; similar compounds are found in *Dacus oleae* [15].

It is remarkable that all spiroacetals found in *Taeniandrena* show an unbranched carbon skeleton with 11 or 13 carbon atoms and, starting from one end of the sidechain, the spirocentre is always in position 6.

The two isomers of 2,8-dimethyl-1,7-dioxa[5.5]undecane (2/10) are present as major compounds in all males and females; the relative concentration of the *E,Z*-isomer (10) is higher in the extracts than in freshly synthetized mixtures of diastereomers. The *C₁₃*-spiroacetals 3/5 and 6/8 are present in all species and in both sexes. Five spiroacetals with 13 carbon atoms comprising 11 diastereomers are found. Compound 11 is present in all species and in both sexes, while components 13, 15, 17 occur both in *A. wilkella* and *A. ocreata*. All other *C₁₃*-spiroacetals are found exclusively in *A. wilkella* so that species-specific bouquets are formed. Hydroxyspiroacetals (38) and (41) may well be regarded as biogenetically closely related to 2,8-dimethyl-1,7-dioxa[5.5]undecane (2/10); (38) occurs in all species and in both sexes, (41) could not be detected in *A. ovatula*. The mass-spectrometric fragmentation of the new spiroacetals follows the general patterns [9, 13, 14]; plotted spectra are given in Fig. 2.

Compound (4) which is present in both sexes of all species occurs in some species in relatively large amounts. Though the actual structure of the substance is still unknown, we tentatively report it to be a rather unstable unsaturated spiroacetal.

The unbranched acyclic compounds identified, 22 in all, represent substances of different polarities. They obviously show a distribution which is different for different chemical subgroups and therefore interesting from a comparative point of view. Among the 14 hydrocarbons heptadecane (27) and nonadecane (36) are found in all species and in both sexes in major of medium amounts. Many hydrocarbons occur only in *A. wilkella*, especially in the males. In contrast to other *Andrena* species [1, 2], no straight chain primary alcohols could be identified from the *Taeniandrena* spp., but nonanal (9) and decanal (16) were present specifically in *A. ocreata*. The only secondary alcohol, 2-undecanol (23) was absent in *A. wilkella*. While the four carboxylic acids (39), (43), (45) and (48) were found in *A. wilkella* males only, tetradecylbutyrate (47) the only nonterpenoid ester, was exclusively demonstrated in *A. ocreata*.

As in the other classes of compounds, similarities and differences are also found in the distribution of terpenoids. Eight compounds could be identified: four alcohols, two aldehydes and two esters. Some of them occur in relatively large amounts. Geraniol
(34) is present in males and females of all three species as a major component but the occurrence of its trans-isomer nerol (33) was found to be restricted to *A. wilkella*. In contrast neral (26) and geranial (29) as well as citronellol and 2,6-dimethyl-5-hepten-1-ol (25) could be identified in all species and in both sexes. The latter norterpenoid may be a metabolite related to the monoterpenes present in the secretion. While citronellyl acetate (28) was not found in *A. ovatula*, *A. ocreata* was the only species containing citronellyl propanoate (32).

B) Behavioural Tests. Preliminary tests on the biological activity of certain mixtures of synthetic compounds were performed in the mating area of *Andrena wilkella* males. As *A. wilkella* belongs to a group of species that are strongly attracted to the orchid *Ophrys lutea* Cav. [16], which they pollinate (B. Kullenberg, pers. comm.), a hexane extract of this flower was presented to the males for comparison. The substances were offered evaporating from a 6 × 10 mm velvet dummy, soaked with defined amounts of extracts or pure compounds. Data are given in Table II. The high frequency of approaches of behaviour type 2, which represents high excitement of the males [4], indicates a strong behaviour-releasing power of especially the *A. wilkella* main spiroacetal (2/10). The total amount of approaches released by this compound during each test period is notable, too. It suggests that males are attracted from greater distances and/or that they are arrested in the immediate surroundings of the odour source. The terpenes did not elicit any significant behaviour; compound (4) might be of importance in the odour bouquet.

Table II. Field tests on the biological activity of some substances on *Andrena wilkella* males. Compounds mixed in natural proportions.

<table>
<thead>
<tr>
<th>Tested substances</th>
<th>Relative frequency of events releasing behaviour type [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 1 p No n</td>
</tr>
<tr>
<td>Compounds 2/10</td>
<td>82 9 9 159 3</td>
</tr>
<tr>
<td>Compounds 26, 29, 30, 34</td>
<td>23 8 69 14 2</td>
</tr>
<tr>
<td>Compounds 2/10, 3/5, 6/8, 11, 12/17, 26, 29, 30, 34</td>
<td>61 17 32 23 2</td>
</tr>
<tr>
<td><em>Ophrys lutea</em>, flower extract</td>
<td>22 20 58 148 8</td>
</tr>
</tbody>
</table>

2 = % male approaches of behaviour type 2 representing high excitement of the males [4].
1 = % male approaches of behaviour type 1 representing low excitement of the males [4].
p = % males passing within 0.1 m distance.
No = Number of events observed.
n = Number of 5-minute test periods.
Conclusions

The earlier results in the analyses of *Andrena* mandibular gland secretions comprise 11 species which represent 6 subgenera [1, 2, 9]. A comparison showed that the amounts of volatile material secreted are roughly the same. Chemical similarities between conspecific males and females are found also for the *Taeniandrena* species reported on here. The large number of spiroacetals found in the three species and the relatively high amounts of some of them are characteristic for this group, although small amounts of a variety of spiroacetals were found in *A. haemorrhhoa* [9]. The *Andrena* sensu strictu subgenus contained several short chain ketones as characteristic compounds whereas the *Hoplandrena*, the *Biareolina* and the *Melandrena* contained several alcohols, acetates and butyrates [1, 2]. As in the other subgenera, chemical similarities between conspecific males and females are found in the *Taeniandrena* group, too. Species specificity seems to be based more on quantitative compositions while the scarce qualitative differences are found merely in compounds of low concentration. In behaviour tests 2,8-dimethyl-1,6-dioxaspiro[5.5]undecane showed high biological activity as a pheromone of *A. wilkella*, while the role of the terpenes in the mandibular secretion is not yet completely understood.