Pheromone Bouquet of the Mandibular Glands in *Andrena haemorrhoa F.* (*Hym., Apoidea*)

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The complex volatile secretion from the mandibular glands of *Andrena haemorrhoa* (Hym., Apoidea) has been analysed by GC/MS. It is composed primarily of three groups of compounds:

1. Spiroacetals of five different systems: two 1,6-dioxyspiro[4.4]nonanes, four 1,6-dioxaspiro[4.5]decanes, two 1,7-dioxaspiro[5.5]undecanes, one 1,6-dioxaspiro[4.6]undecane and one 1,7-dioxaspiro[5.6]dodecane.
2. Straight chain fatty acid derivatives: methyl ketones, primary and secondary alcohols, acetates and hydrocarbons.
3. Isoprenoids: geraniol and geranyl acetate.

Mass spectral fragmentation patterns have been studied for several synthesized spiroacetals.

Introduction

Andrena F. is one of the most species-rich genera among the superfamily Apoidea. It occurs on all continents except South America and Australia. Being a wide-spread species, rich in individuals, *Andrena haemorrhoa* F. is an important pollinator of a large number of plant species, which includes for example apple, cherry, pear and plum. *A. haemorrhoa* females are the main pollinators of Lady's Slipper, *Cypripedium calceolus* L. (Orchidaceae) [1]. Under experimental conditions the males have been shown to be attracted to the odour of the orchid *Ophrys lutea*, which is known to be pollinated through pseudo-copulatory movements on the flower labellum by *Andrena* spp males [2].

Andrena males perform mating flight behaviour in species specific habitats, a section of which is marked with odours from the mandibular gland secretion. The scent marks are attractive to conspecific males and females [3]. The mandibular glands in the females (Fig. 1) also play an active secretory role. The composition of the secretion in 12 *Andrena* species has been reported [4–6]; it was shown, in most species studied, that the secretions from males and females corresponded well within each species. We now want to give an account of a detailed analysis of the volatile compounds that make up the mandibular gland secretion in *A. haemorrhoa* females.

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Materials and Methods

For each analysis 10 freshly caught females were decapitated and their heads were sectioned under 400 µl of pentane along the horizontal line as shown in Fig. 1. Subsequently the solution was carefully concentrated to 2 µl in a 3 mm wide glass tube at 40 °C. The whole sample was then directly trans-

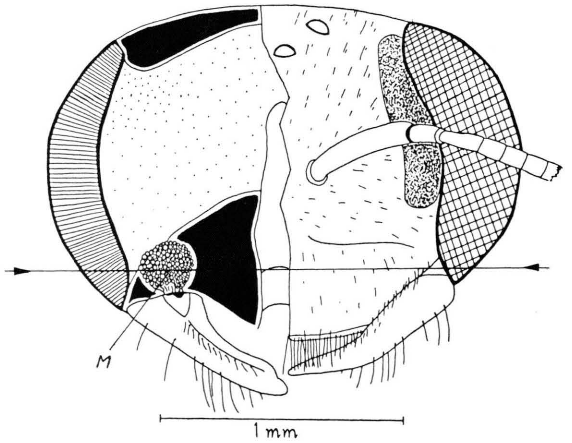


Fig. 1. Mandibular gland (M) in *Andrena* (After Nedel [7]). Extracted heads were sectioned along the horizontal line.

ferred to a LKB 2091 combined GC/MS-system. Columns were 50 m long glass capillary columns with WG 11 as stationary phase. Temperature programming was made from +70 °C to +180 °C, 3 °C per min.

The structures of all compounds identified have been confirmed by gas chromatographic retention values and mass spectra in comparison with those of authentic samples. Spiroacetals have been prepared by known procedures [8, 9]. The unsaturated secondary alcohols have been obtained by Lindlar hydrogenation from the corresponding acetylenic compounds, which were prepared according to the general procedure given by Brandsma [10].

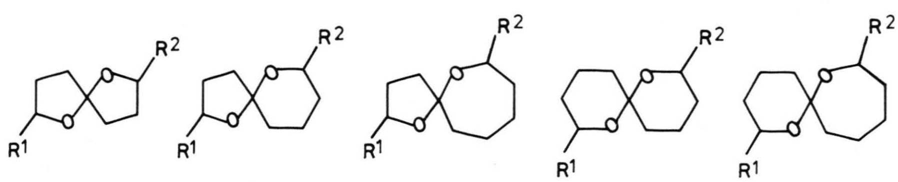
Results of the Chemical Analysis

A capillary gas chromatogram obtained from 10 female heads is shown in Fig. 2. Numbers given in the gas chromatogram refer to compounds listed in Table I. Later than eicosane (component no. 40) only hydrocarbons C_{21}–C_{27} (the uneven numbered ones predominating), tetradecyl acetate, and 2-pentadecanol elute. These compounds were also found in some of the earlier studied species [5]. Beside the monoterpenes geraniol and geranyl acetate, which have been found in other *Andrena* species too, only compounds with unbranched carbon skeletons were identified. These compounds could be divided in two groups. One contains wellknown hydrocarbons, primarily alcohols and corresponding acetates, also methyl ketones and corresponding secondary alcohols. The identified *Z*-4-undecene-2-ol and *Z*-4-tridecene-2-ol are as far as we know only now identified from insects. The other group of odor components is comprised of rather volatile alkyl spiroacetals, five different systems beeing present: two 1,6-dioxaspiro[4.4]nonanes (**I**), four 1,6-dioxaspiro[4.5] decanes (**II**), one 1,6-dioxaspiro[4.6]undecane (**III**), two 1,7-dioxaspiro-[5.5]undecanes (**IV**) and one 1,7-dioxaspiro[5.6]dodecane (**V**) [11].

Table 1. List of compounds found in *Andrena haemorrhoa* female heads. Numbers are according to the gas chromatogram given in Fig. 2.

1. E-2-Ethyl-E-7-methyl-1,6-dioxaspiro[4.5]decane
2. E-7-Ethyl-E-2-methyl-1,6-dioxaspiro[4.5]decane
3. Z-2-Ethyl-E-7-methyl-1,6-dioxaspiro[4.5]decane
4. E-7-Ethyl-Z-2-methyl-1,6-dioxaspiro[4.5]decane
5. 2,7-Dimethyl-1,6-dioxaspiro[4.6]undecane (1st isomer)
6. Nonanone-2
7. 2-Methyl-7-propyl-1,6-dioxaspiro[4.4]nonane (all isomers)
8. 2,7-Dimethyl-1,6-dioxaspiro[4.6]undecane (2nd isomer)
9. E-2-Methyl-1,7-dioxaspiro[5.6]dodecane
10. E-2-Methyl-E-8-propyl-1,7-dioxaspiro[5.5]undecane
11. Octyl acetate
12. E-7-Butyl-E-2-methyl-1,6-dioxaspiro[4.5]decane
13. Nonanol-2
14. E-7-Butyl-Z-2-methyl-1,6-dioxaspiro[4.5]decane
15. E,E-2,7-Dipropyl-1,6-dioxaspiro[4.4]nonane
16. E-2-Propyl-1,7-dioxaspiro[5.5]undecane
17. Undecanone-2
18. unknown
19. unknown
20. Decyl acetate
21. unknown
22. Heptadecane
23. Undecanol-2
24. Z-4-Undecene-2-ol
25. an Undecene-2-ol
26. Geranyl acetate
27. Decanol
28. unknown sulfur compound
29. Octadecane
30. Geraniol
31. unknown
32. Dodecyl acetate
33. Nonadecane
34. Tridecanol-2
35. Z-4-Tridecene-2-ol
36. a Tridecene-2-ol
37. a Tridecene-2-ol
38. Dodecanol
39. a Tridecadiene-2-ol
40. Eicosane

Most of the compounds occur as mixtures of *E-Z*-diastereomers [12] in nature; totally 16 spiroacetals are found in *A. haemorrhoa* heads. Compounds **1–4** and **16** have been previously identified from common wasps [13] and *A. haemorrhoa* respectively [6]. All other spiroacetals are found for the first time as natural products. While 1,7-dioxa-



I II III IV V

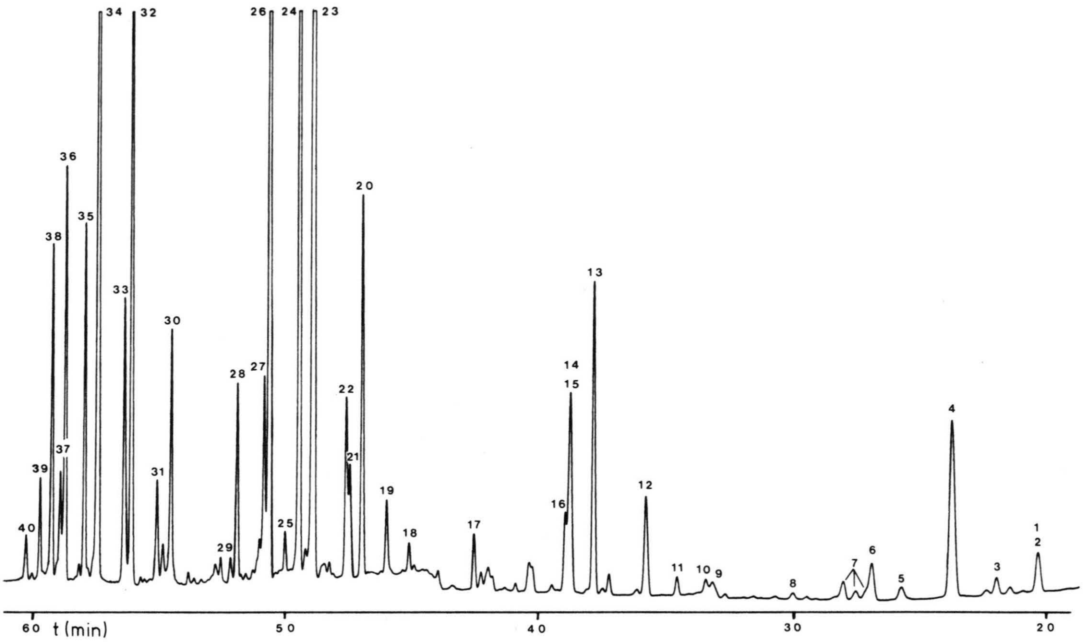


Fig. 2. Capillary gas chromatogram of volatile compounds in an extract of 10 female heads of *Andrena haemorrhoa*, females. Analytical conditions see the text.

spiro[5.6]dodecane, the parent compound of component **9** has been described as a synthetic substance by Stetter and Rauhut [14], 2,7-dimethyl-1,6-dioxaspiro[4.6]undecane (**5/7**) is the first of a completely new bicyclic acetal system.

Spectroscopic Properties of Spiroacetals

The mass-spectrometric fragmentation patterns of the 1,6-dioxaspiro[4.4]nonane-system and that of alkyl-1,6-dioxaspiro[4.5]decanes have been published [8, 15] and it has been shown that the spectra in the 1,6-dioxaspiro[5.5]undecane-system strongly resemble the latter. It now turns out that the new acetals, which contain a seven-membered ring, also furnish basically similar spectra. All compounds may well be distinguished by a couple of "diagnostic ions" (Fig. 3), which is illustrated by the plotted mass spectra of the new spiroacetals (Fig. 4).

As all spiroacetals identified up to now from insects show unbranched carbon skeletons (see below), at a given M^+ the ions A-D, which are present in all spectra, classify the spiroacetal system and determine the substitution pattern at the positions α to the oxygens. Additional information is obtained from the ions E and F; in the unsymmetrical 1,6-dioxaspiro[4.5]decane system, such as in 1,6-dioxaspiro[4.6]undecanes, the five membered ring is the more stable one yielding the most intense pairs of peaks in the series 84/87, 98/101 etc., depending on the substitution at C-2. In the 1,7-dioxaspiro[5.5]undecane-system the six-membered ring furnishes a corresponding doublet, as is the case in the 1,6-dioxaspiro[5.6]dodecane system. In contrast to the other spiroacetals the 1,6-dioxaspiro [4.4]nonanes show only small ions F, but extremely strong ions A/B when R^1/R^2 are at least an ethyl

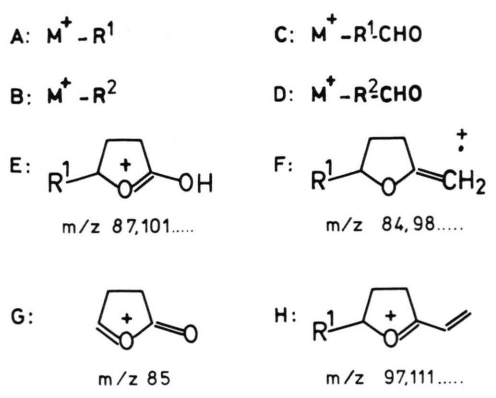


Fig. 3. Simplified fragmentation scheme of alkylspiroacetals.

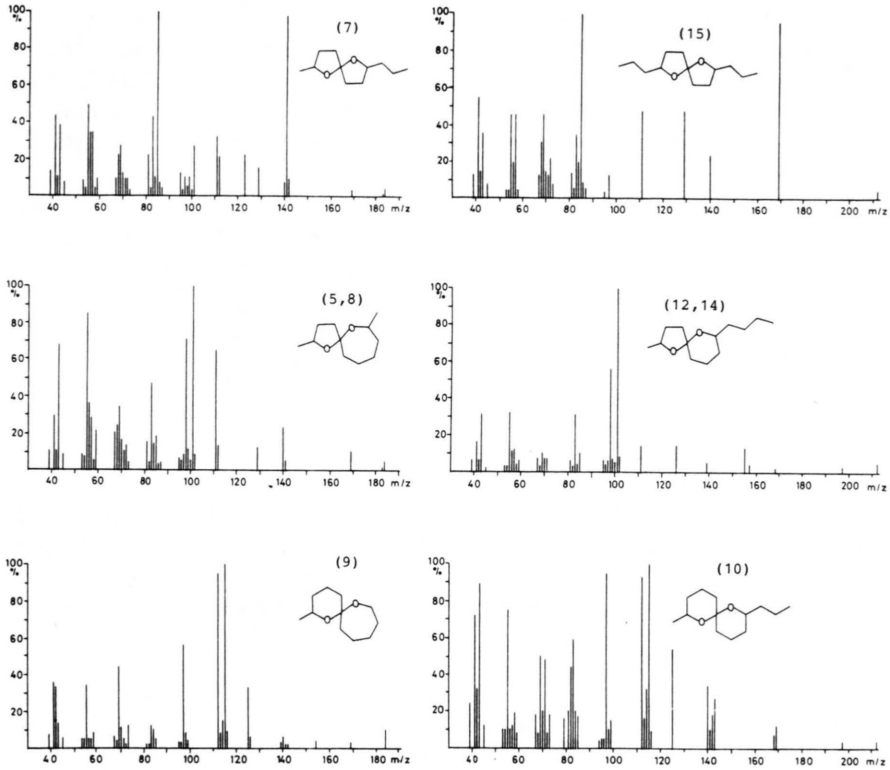


Fig. 4. 70 eV Mass spectra of new spiroacetals found in the mandibular gland secretion of female *Andrena haemorrhoa*. Numbers in brackets refer to compound numbers in table I.

group. Systems containing a five-membered ring show an intense ion G at $m/z = 85$ which may become the base peak when the alkyl substituent at C-2 is at least C_2H_5. Sprioacetals containing a seven-membered ring yield rather intense fragment ions H. Mass spectra of diastereomers usually do not differ significantly.

Determination of relative configuration of spiroacetals has been carried out on the basis of solvent depending shifts in ^{1}H-NMR spectra and γ-effects in ^{13}C-NMR spectra [16]. In all compounds containing an alkyl substituted six-membered ring the substituent is in the more stable equatorial position. The anomeric effect is responsible for the strongly preferred diaxial position of the oxygens at the spirocenter so that only one isomer of either **10** and **16** is formed in nature. These results have been confirmed recently by Schurig [17], Deslongchamps [18], and by Mori's synthesis of all energetically possible isomers of 2,8-dimethyl-1,7-dioxaspiro[5.5]-undecane [19], which is a main component in the *Andrena wilkella* cephalic secretion [6]. The stereochemistry of the isomers of 1,6-dioxaspiro[4.4]nonanes and 2,7-dimethyl-1,6-dioxaspiro[4.6]undecane is not yet clarified.

Discussion

Earlier bioassays with *A. haemorrhoa* showed a strong behaviour releasing activity of head extracts. The identified hydrocarbons have not yet been assayed but they are not regarded as pheromones. Though they may act as "solvents" for biologically active compounds it is even uncertain, whether they belong to the mandibular gland secretion at all: As whole heads have been analyzed the ubiquitious hydrocarbons – those with more than 20 carbon atoms are present in rather large amounts – may well originate from the cuticular wax layer as was shown for other *Andrena* spp. [4]. Several acyclic oxygen containing odour components when offered alone seemed to have only little excitation capacity;

in contrast the blend of compounds **13**, **20**, **23**, **26**, **30**, **32**, **34**, **38** together with tetradecylacetate mixed in naturally occuring proportions proved to induce significant response but to some lower degree than the natural material [3]. Furthermore preliminary tests with a mixture of terpens **26**, **30** and racemic compounds **1** – **4** and **16** indicate that spiroacetals may play an additional role in the chemical communication system of *A. haemorrhoa.* In this context it is interesting to note that spiroacetals have recently been found to act as potent pheromones in different insect species [13, 20, 21]. The now identified spiroacetals bouquet is by far the most complicated one published up to now. Detailed results on bioassays with a completed copy of the natural scent mark will be given elsewhere.

As already pointed out, all insect spiroacetals show an unbranched carbon skeleton and, with the exception of **16**, contain uneven numbers of carbon atoms. The structural similarities between the spiroacetals and straight chain pheromones are striking [22]. In contrast to other insect species investigated up to now, *A. haemorrhoa* contains both methyl substituted spiroacetals and unsaturated secondary alcohols of the same chain length. With the exception of **15** and **16**, all spiroacetals of *A. haemorrhoa* might well be biogenetically closely related to the alcohols **24** and **35**. As monoterpenes are also found in the now investigated pheromone bouquet, both the acetogenic and the mevalogenic pathways are represented in the mandibular gland secretion of *A. haemorrhoa.*

Acknowledgements

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