The Exocrinology of the Queen Bumble Bee *Bombus terrestris* (Hymenoptera: Apidae, Bombini)

Abraham Hefetz[a], Timo Taghizadeh[b] and Wittko Francke[b]

[a] Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv 69978, Israel
[b] Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Bundesrepublik Deutschland

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Chemical analyses are presented of prominent exocrine glands of queen *Bombus terrestris* including mandibular, labial and hypopharyngeal glands in the head, Dufour's gland and tarsal glands. A plethora of about 500 substances were identified belonging to various aliphatic compounds including hydrocarbons, various classes of esters, alcohols, methyl ketones and fatty acids. A group of chiral hydroxy acids and their butanoic acid esters are reported for the first time in bees.

Introduction

Bumble bees have attracted much attention in recent years because of the extensive commercialization of several species as pollinators of greenhouse crops. They are considered as primitively eusocial bees since, unlike the highly social bees and ants overt aggression and competition between the queen and her workers are regular events during colony development (Röseler *et al.*, 1990). *Bombus terrestris*, a popular cultivated species for pollination, is one of the best studied bumble bee species with respect to its sociobiology.

Colony development in *B. terrestris* is characterized by two social phases (Duchateau and Velthuis, 1988). At the first phase a clear division of labor occurs: workers perform all the nest duties whereas the queen is the primary egg layer. During this time the queen has clear control over worker reproduction, and interactions between the two castes are cooperative, or at least not overtly hostile. As the colony's worker population increases it enters the next social phase, that of "competition". Some of the workers begin to develop ovaries and lay haploid (male) eggs, despite the presence of a queen (van Doorn and Heringa, 1986). They also become progressively more aggressive towards the queen and towards other workers and sometimes evict the queen from her nest. A hallmark of the process of competition is the mutual destruction of queen and worker egg cells and the devouring of their contents (van Doorn, 1988). It has been postulated that queen control of worker reproduction is mediated pheromonally, and that the decline in the putative queen pheromone results in worker reproduction.

The first experimental evidence supporting this hypothesis was presented by Honk *et al.* (1980), who showed that queens from which the mandibular glands were extirpated were not able to delay ovarian development in groups of workers. In a later study Röseler *et al.* demonstrated that queen mandibular gland extracts were able to inhibit the production of JH (juvenile hormone) by the CA (corpora allata) of queenless workers (Röseler *et al.*, 1981). Body washes were as active as the mandibular gland extracts, which suggests that the queen applies the pheromone over her body while grooming. However, queens without mandibular glands were still able to inhibit ovarian development to some extent. This result suggests that queen control is either achieved by both behavioral and pheromonal mechanisms, or by pheromones that are not produced exclusively by the mandibular glands. In order to understand the pheromonal mechanisms underlying social behavior in *B. terrestris* it is important to unravel the chemical nature of the exocrine secretions that may be involved.

Reprint requests to Prof. Dr. W. Francke.

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In his pioneering work on the exocrinology of bumble bees, Bergström has reported extensively on the chemistry and function of the labial gland secretion of male bumble bees (Bergström *et al.*, 1973, 1981, 1981a). Later studies have concentrated on the chemical analysis of exocrine secretions of queens and workers. Genin *et al.*, (1984) compared the composition of extracts obtained from the cuticle and from the labial glands of males, queens and workers of *B. hypnorum*. Investigations on the volatile constituents of the tarsal glands of *B. terrestris* revealed a complex mixture of hydrocarbons with which the bees seem to mark food sites that have been visited (Schmitt *et al.*, 1991). In a comparative study Tengö *et al.*, (1991) demonstrated that Dufour's gland secretion contains species specific multicomponent mixtures covering a wide range of volatility. The function of this glandular secretion in *B. hypnorum* is to mark a trail leading from the nest entrance to the inner parts of the nest (Hefetz *et al.*, 1993). Chemical analysis of the secretion in this species also showed interesting intra-nidal differences between workers which are correlated with size polymorphism (Tengö *et al.*, 1991). A recent comparative study of several species of bumble bees showed a congruency in the hydrocarbon constituents between cuticular washes and Dufour's gland secretions (Oldham *et al.*, 1994).

The studies on bumble bee pheromones to date demonstrated that they constitute complex blends of chemicals. It is therefore imperative to gain a thorough knowledge of the chemical composition of the various exocrine secretions in order to understand their possible biological function. In this study we present a comprehensive inventory of the volatile constituents of several of the prominent exocrine glands of queen *B. terrestris*.

Material and Methods

Bees and sample preparation

Colonies of *B. terrestris* were either obtained from Bio-Bee Sde-Eliahu Industry (colonies were obtained a few days after the first worker ecclosed and contained a small group of first batch workers), or were founded from virgin queens that were mated in the laboratory and treated for hibernation prevention according to the established procedure (Röseler, 1985). Colonies were confined in nesting boxes (18x27x12 cm) made of Styrofoam and lined with a cardboard base. Commercial honey water (BeeHappy) and fresh pollen (collected by honey bees) were supplied to all colonies. The colonies were kept in a climatic room under a constant temperature of 30°C in constant darkness.

For glandular extraction queens were dissected under chilled water, and the glands were transferred into 2 ml pentane (Merck, Uvasol). The following queen exocrine glands were analysed: mandibular, labial, hypopharyngeal, tarsal, and Dufour's glands. Cephalic glands including mandibular glands, hypopharyngeal glands and labial glands were removed in this sequence in order to avoid as far as possible cross contamination during the dissections.

Chemical analyses

As described earlier (Klimetzek *et al.*, 1989), crude extracts were concentrated in microvials at 40°C to 30 µl. Analyses of volatiles were carried out by combined capillary gas chromatography / mass spectrometry (Fisons GC8000 equipped with a 30m 0.25mm DB-5 capillary column temperature programmed from 60°C for 3 min. to 300°C at a rate of 5°C/min. For separation of enantiomers, a 2,6-heptakis-(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin capillary column was used, temperature programmed from 50°C for 5 min. to 170°C at 5°C/min. and linked to a Fisons MD800 mass spectrometer, 70eV). The compounds were identified by their fragmentation patterns and by comparision with authentic samples (The Wiley, 1989). Hydroxylated compounds present in the crude extracts were either methylated with diazomethane or silylated with bis-(trimethylsilyl)-acetamide (Mielniczuk, 1992), followed by GC/MS. For methylation, 10 µl of a solution of diazomethane in pentane were added to 10 µl of the crude extract, concentrated (Klimetzek *et al.*, 1989), and submitted to GC/MS analysis. Silylation was performed on a part of the methylated extract (5 µl) that was treated with 3 µl bis-(trimethylsilyl)-acetamide at room temperature for 30 min. Subsequently, the mixture was concentrated and analysed by GC/MS.

Position of double bonds was elucidated by alkylthiolation using dimethyl disulfide (DMDS)

(Vincenti, 1987). Double bond positions in unsaturated fatty acids were determined by methylthiolation of the methylated extracts. For alkylthiolation, 10 µl of the extract were mixed with 50µl of carbon disulfide, 50 µl DMDS, and 5 µl of iodine solution (60mg in 1ml ether) and heated to 60°C for 12h. The excess of iodine was destroyed with 50–100 µl aqueous sodium thiosulfate solution (0.5g in 10 ml H_2O). After addition of 300 µl pentane and 50 mg sodiumchloride the organic layer was separated, concentrated, according to the known procedure (Klimetzek *et al.*, 1989), and submitted to GC/MS analysis.

Synthetic compounds

Reference compounds were either commercially available or prepared by standard procedures. Double bonds were produced from the corresponding triple bonds. For the synthesis of the optically active 3-hydroxy carboxylic acids, readily available 3-keto acids were stereoselectively hydrogenated with [S-(-)-2,2'-bis-(diphenylphosphino)-1,1'-binaphthalene]chloro (p-cymene) ruthenium chloride as a catalyst (Kitamura *et al.*, 1992; Gênet *et al.*, 1994, 1995). Esterification of the alcohol moiety in the hydroxy acids was carried out with butanoyl chloride according to the standard procedure.

Results

Five prominent exocrine glands of *B. terrestris* were chemically analysed revealing a wealth of compounds including hydrocarbons, alcohols, carbonyl compounds, carboxylic acids, various types of esters and oxygenated acids.

Hydrocarbons

A total number of 188 hydrocarbons could be identified (Table I). Straight chain hydrocarbons ranging from dodecane to heptatriacontane formed a backbone in almost all secretions. As usual for hydrocarbon patterns from biological material, uneven numbered homologues always showed higher concentrations than the adjacent even numbered ones. Maximum concentrations were found in C_{23}, C_{25}, C_{27}, C_{29} and C_{31}. A large number of unbranched alkenes forming clusters of

Table I. List of hydrocarbons identified in exocrine glands of *Bombus terrestris*. M: Mandibular gland; H: hypopharyngeal gland; L: labial gland; T: tarsal gland; D: Dufour's gland; ****: major; ***: medium; **: minor; *: trace.

	M	H	L	T	D
A. Alkanes					
Dodecane					*
Tridecane					*
Tetradecane		*			*
Pentadecane		*	*		*
Hexadecane	*	*			*
Heptadecane		*	*		*
Octadecane	*	*	*	*	*
Nonadecane	*	*		*	*
Eicosane	*	*	**	*	*
Heneicosane	***	**	**	***	***
Docosane	**	***	**	**	**
Tricosane	***	***	***	****	***
Tetracosane	**	***	**	**	**
Pentacosane	***	***	**	****	***
Hexacosane	**	**	**	***	**
Heptacosane	***	***	***	****	****
Octacosane	**	**	**	***	**
Nonacosane	***	***	***	****	****
Triacontane	**	**	**	**	**
Hentriacontane	*	***	***	***	***
Dotriacontane	*	*	**	**	
Tritriacontane	*	*	*	*	
Tetratriacontane	*	*	*	*	
Pentatriacontane		*	*	*	
Hexatriacontane		*	*	*	
Heptatriacontane		*			
B. Alkenes					
1-Decene	*	*	*		
2-Decene	*	*	*		
1-Tridecene		*			
4-Tridecene		*			
Tetradecene		*			
Pentadecene					*
5-Pentadecene			*		
4-Hexadecene					*
5-Hexadecene			*		*
6-Hexadecene					*
7-Heptadecene					*
8-Heptadecene					*
8-Nonadecene			*		*
9-Nonadecene		*	*		*
8-Eicosene					*
9-Eicosene					*
10-Eicosene					*
1-Heneicosene					*
7-Heneicosene	*	*	*	*	*
8-Heneicosene				*	*
9-Heneicosene	*		*	*	*
10-Heneicosene			*	*	*
8-Docosene					*
9-Docosene				*	*
10-Docosene					*
11-Docosene					*
5-Tricosene		*		*	*

Table I (continued).

	M	H	L	T	D
7-Tricosene	*	*	**	*	***
8-Tricosene		*			*
9-Tricosene	**	**	**	**	***
10-Tricosene		*	*	*	**
11-Tricosene		*	*	*	**
7-Tetracosene					**
9-Tetracosene				*	**
10-Tetracosene				*	*
11-Tetracosene				*	*
12-Tetracosene					*
5-Pentacosene	*	*		*	*
7-Pentacosene	**	**	**	**	***
8-Pentacosene	*	*	*	*	*
9-Pentacosene	**	**	**	**	***
10-Pentacosene	**	**	**	*	***
11-Pentacosene	**	**	**	*	***
12-Pentacosene	**	**	**	*	***
5-Hexacosene		*	*	*	*
7-Hexacosene	*	*	*	**	***
9-Hexacosene	*	*	*	**	***
10-Hexacosene		*	*	**	**
11-Hexacosene	*	*	*	**	***
12-Hexacosene	*	*	*	*	**
13-Hexacosene		*	*	**	***
5-Heptacosene	**	*	**	**	**
7-Heptacosene	***	***	***	****	****
8-Heptacosene	**	**	**		***
9-Heptacosene	***	***	***	****	****
10-Heptacosene	**	***	***	***	****
11-Heptacosene	**	**	**	***	***
12-Heptacosene	***	***	***	****	****
13-Heptacosene	***	***	***	****	****
5-Octacosene					**
7-Octacosene		*	**	**	****
8-Octacosene			*	*	
9-Octacosene	*	*	**	**	****
10-Octacosene	*	*	*	*	**
11-Octacosene	*	**	*	*	***
12-Octacosene	*	*	**	*	**
13-Octacosene	*	**	**	*	***
14-Octacosene	*	*	**	*	**
5-Nonacosene	*	**	**	***	***
7-Nonacosene	***	***	***	****	****
8-Nonacosene			**	***	***
9-Nonacosene	***	***	***	****	****
10-Nonacosene	**	**	**	***	****
11-Nonacosene	***	**	**	***	****
12-Nonacosene	**	**	**	***	***
13-Nonacosene	***	**	**	***	****
14-Nonacosene	**	**	**	***	****
7-Triacontene			*	**	
9-Triacontene			*	**	**
10-Triacontene					*
11-Triacontene					*
12-Triacontene					*
13-Triacontene					*
14-Triacontene					*
15-Triacontene					*
5-Hentriacontene			**	**	
7-Hentriacontene		*	**	***	***
8-Hentriacontene			***		

Table I (continued).

	M	H	L	T	D
9-Hentriacontene	*	**	**	***	****
10-Hentriacontene	*	*	**	**	**
11-Hentriacontene	*	**	***	***	***
12-Hentriacontene	*	*	**	**	**
13-Hentriacontene	*	*	**	**	**
14-Hentriacontene	*	*	**	**	**
15-Hentriacontene	*	*	**	**	**
Dotriacontene	*				
Tritriacontene					*
9-Tritriacontene			*	*	
10-Tritriacontene			*	*	
Pentatriacontene					*
C. Alkadienes					
Heneicosadiene				*	
Tricosadiene				*	*
Pentacosadiene				*	
7,17-Pentacosadiene					*
8,16-Pentacosadiene					**
7,15-Hexacosadiene			*		*
7,17-Hexacosadiene				**	*
Heptacosadiene		**			
5,13-Heptacosadiene					*
5,19-Heptacosadiene					*
7,15-Heptacosadiene	*			**	**
7,17-Heptacosadiene			**		***
7,19-Heptacosadiene				**	***
8,16-Heptacosadiene	*		**	**	***
8,18-Heptacosadiene	*			**	**
9,15-Heptacosadiene	*				
9,17-Heptacosadiene	*		**	**	***
Octacosadiene			*		
7,19-Octacosadiene				*	**
9,15-Octacosadiene					*
9,17-Octacosadiene				*	***
9,19-Octacosadiene				*	**
Nonacosadiene		**			
5,19-Nonacosadiene			*		*
5,21-Nonacosadiene					*
7,15-Nonacosadiene				*	*
7,17-Nonacosadiene					**
7,19-Nonacosadiene	***		***	***	****
7,21-Nonacosadiene			**	**	
8,16-Nonacosadiene					**
8,18-Nonacosadiene	***		***	**	***
8,20-Nonacosadiene				***	***
9,15-Nonacosadiene			***		
9,17-Nonacosadiene			***	***	****
9,19-Nonacosadiene	***		***	***	****
10,16-Nonacosadiene			***		
10,18-Nonacosadiene				**	***
9,17-Triacontadiene					*
9,19-Triacontadiene					*
11,17-Triacontadiene					*
11,19-Triacontadiene					*
Hentriacontadiene		*			
7,19-Hentriacontadiene					**
8,20-Hentriacontadiene					**
8,22-Hentriacontadiene				**	**
9,17-Hentriacontadiene				*	**

Table I (continued).

	M	H	L	T	D
9,19-Hentriacontadiene					**
9,21-Hentriacontadiene				**	**
10,18-Hentriacontadiene				*	**
10,20-Hentriacontadiene			***		***
11,19-Hentriacontadiene			***	**	***
Tritriacontadiene			*		*
D. Methylalkanes					
3-Methyl pentadecane		*			
4-Methyl pentadecane		*			
4-Methyl heptadecane					*
5-Methyl heptadecane					*
3-Methyl nonadecane					*
9-Methyl heneicosane	***	*			
11-Methyl heneicosane	***	*			
9-Methyl tricosane					*
11-Methyl tricosane					*
11-Methyl pentacosane					*
13-Methyl pentacosane			*		*
3-Methyl heptacosane				*	
11-Methyl heptacosane	**			*	**
13-Methyl heptacosane	**			*	**
11-Methyl nonacosane					**
13-Methyl nonacosane	**				**
15-Methyl nonacosane	**				

up to 10 positional isomers (hentriacontene) showed maximum concentrations at the same chain lengths as the corresponding alkanes. In a series of alkadienes, including positional isomers, the double bonds were interrupted by even numbers of methylene groups. The closest distance between double bonds was found in 9,15-heptacosadiene and 9,15-nonacosadiene (4 methylene groups), while double bonds were most remotedly positioned in 5,21-nonacosadiene (19 methylene groups). Alkadienes, with nonacosadiene being most abundant, were predominantly found in labial, tarsal and Dufour's glands. The extreme complexities of the mixtures prevented the assignment of the double bond geometry in any of the unsaturated hydrocarbons.

Apart from straight chain hydrocarbons a multitude of methyl branched alkanes could be identified according to their mass spectra (Pomonis *et al.*, 1978, 1980). While considerable amounts of 9-methyl heneicosane and 11-methyl heneicosane were found almost exclusively in the mandibular glands, 11-methyl and 13-methyl heptacosane as well as 13-methyl nonacosane could be identified in the secretions of mandibular and Dufour's glands. Gas chromatographic separation of enantiomeres of the chiral methyl alkanes was impossible due to the similarities of chain lengths on both sides of the branching points.

Alcohols

Of the 18 alcohols identified, 4 represented chiral methyl carbinols, present in the mandibular glands (Table II). Unsaturated primary alcohols, 9-decenol and 9-dodecenol were only found in the

Table II. List of alcohols and carbonyl compounds found in exocrine glands of *Bombus terrestris*. M: Mandibular gland; H: hypopharyngeal gland; L: labial gland; T: tarsal gland; D: Dufour's gland; ****: major; ***: medium; **: minor; *: trace.

	M	H	L	T	D
Alcohols					
9-Decenol			*		
Decanol			*		*
9-Dodecenol			**		
Dodecanol		*	**		
Tetradecanol		*	*	*	
Hexadecanol		*		*	
Octadecanol		*		*	*
Eicosanol			*	*	
Docosanol				*	
Tetracosanol				*	
Hexacosanol				*	
Octacosanol				*	
Triacontanol				*	
Dotriacontanol				*	
Nonan-2-ol	*	*			
Decan-2-ol	*	*			
Undecan-2-ol	*				
Tetradecan-2-ol	*				
Ketones					
2-Heptanone	*				
2-Nonanone	**	*			
2-Decanone	*				
2-Undecanone	*	*			
2-Tridecanone	*				*
2-Tricosanone			*	*	
2-Pentacosanone			*	*	
2-Heptacosanone			*	*	
Aldehydes					
Nonanal		*			
Decanal		*			
Dodecanal			*		
Tetradecanal	*		*		
Octadecanal					*

labial glands. A series of 10 bishomologue fatty alcohols occurred in the tarsal glands.

Carbonyl compounds

Among the carbonyl compounds, 6 methylketones of medium chain length occurred predominantly in the mandibular glands, accompanied by the corresponding secondary alcohols (Table II). Long chain methylketones were found in the labial and tarsal gland only. Trace amounts of five aldehydes were more or less randomly distributed in several glands. In contrast, the spiroacetal 2-ethyl-7-methyl-1,6-dioxaspiro-[4.5]-decane, which is a cryptic ketodiol, is present in the mandibular glands only (Table IV).

Carboxylic acids

A total number of 67 carboxylic acids could be identified (Table III). The "classical" fatty acids proved to represent the most prominent components among this group. Methyl branched carboxylic acids were exclusively found in the mandibular glands. The absolute configuration of these chiral compounds remains to be determined. Similar to the straight chain alkenes, unsaturated carboxylic acids formed clusters of up to 5 positional isomers, the stereochemistry of which could not be assigned. Unsaturated acids with shorter chains were found in the labial glands, and those showing 16 and 18 carbon atoms were found in all glands investigated. The occurrence of acids with more than 20 carbon atoms was restricted to the mandibular and tarsal glands. Concentrations in Dufour's glands secretions reached trace amounts per component only.

Table III. List of acids and esters found in exocrine glands of *Bombus terrestris*. M: Mandibular gland; H: hypopharyngeal gland; L: labial gland; T: tarsal gland; D: Dufour's gland; ****: major; ***: medium; **: minor; *: trace.

	M	H	L	T	D
Acids					
Hexanoic acid	*	*	*	*	
Heptanoic acid				*	
7-Octenoic acid			*		
Octanoic acid	*	*	*	*	*
Nonanoic acid		*		*	*
Decenoic acid					*
7-Decenoic acid			*		
9-Decenoic acid			*		
Decanoic acid	*	*	*	*	*
Undecenoic acid					*
Undecanoic acid				*	*
7-Dodecenoic acid					*
9-Dodecenoic acid			**		
11-Dodecenoic acid			*		
Dodecanoic acid	**	*	**	*	*
Tridecanoic acid	*			*	*
5-Tetradecenoic acid					*
6-Tetradecenoic acid	*				*
7-Tetradecenoic acid					*
9-Tetradecenoic acid		*		*	*
11-Tetradecenoic acid					*
Tetradecanoic acid	***	*	*	**	*
Pentadecenoic acid	*				
Pentadecanoic acid	*	*	*	*	*
6-Hexadecenoic acid	**				*
7-Hexadecenoic acid	**	*	**		*
9-Hexadecenoic acid	**	**	**	**	*
11-Hexadecenoic acid	**	*	**	**	*
13-Hexadecenoic acid	*				
Hexadecanoic acid	***	**	*	***	*
6-Heptadecenoic acid	*				
8-Heptadecenoic acid	*				
Heptadecanoic acid	*		*	*	*
Octadecatrienoic acid	*	*			*
9,12-Octadecadienoic acid	***	*		***	*
8-Octadecenoic acid	*				
9-Octadecenoic acid	****	****	****	****	*
11-Octadecenoic acid	***	****	****		*
13-Octadecenoic acid	*				*
Octadecanoic acid	****	**	****	***	*
Nonadecanoic acid	*			*	
9-Eicosenoic acid					*
11-Eicosenoic acid	*			**	*
13-Eicosenoic acid				*	*
Eicosanoic acid	*		*	**	*
Heneicosanoic acid	*			*	
13-Docosenoic acid				**	*
Docosanoic acid	*			*	*
Tricosenoic acid	*				
Tricosanoic acid	*			*	
Tetracosanoic acid	*			*	
Pentacosanoic acid				*	
Hexacosanoic acid	*			*	
Heptacosanoic acid	*			*	
Octacosanoic acid	*			*	*
Nonacosanoic acid	*			*	
Triacontanoic acid	*			*	
Dotriacontanoic acid				*	
4-Me-tetradecanoic acid	*				
8-Me-tetradecanoic acid	*				
12-Me-tetradecanoic acid	*				
10-Me-pentadecenoic acid	*				
10-Me-pentadecanoic acid	*				
12-Me-pentadecanoic acid	*				
4-Me-hexadecanoic acid	*				
8-Me-hexadecanoic acid	*				
8-Me-octadecanoic acid	*				
Methyl esters					
Methyl octanoate		*	*		
Methyl dec-9-enoate			*		
Methyl decanoate			**		*

Table III (continued).

	M	H	L	T	D
Methyl dodec-9-enoate			*		
Methyl dodecanoate		*	**		*
Methyl tetradec-5-enoate					*
Methyl tetradec-6-enoate					*
Methyl tetradec-7-enoate					*
Methyl tetradec-9-enoate		*			*
Methyl tetradec-11-enoate			*		
Methyl tetradecanoate		*	**	*	*
Methyl hexadec-6-enoate	*				*
Methyl hedadec-7-enoate	*	*	**		*
Methyl hexadec-9-enoate	*	*	*		*
Methyl hexadec-11 enoate	*	*	***	*	*
Methyl hexadec-13-enoate	*		**	*	
Methyl hexadecanoate	*	*	***	*	**
Methyl octadecatrienoate			*		*
Methyl linoleate		*	*		*
Methyl octadec-9-enoate	*	*	****	*	****
Methyl octadec-11-enoate	*	*	****	*	****
Methyl octadec-13-enoate	*		**	*	
Methyl octadecanoate			***	*	***
Methyl eicosanoate					*
Ethyl esters					
Ethyl octanoate	*		*		
Ethyl decanoate			*		
Ethyl decanoate	*		*		*
Ethyl dodecadienoate			*		
Ethyl dodecenoate			*		
Ethyl dodecanoate			**		*
Ethyl tetradec-11-enaote			**		
Ethyl tetradecanoate			**		*
Ethyl hexadec-7-enoate			**		*
Ethyl hexadec-9-enoate			**		*
Ethyl hexadec-11-enoate	*		***		*
Ethyl hexadec-13-enoate			**		
Ethyl hexadecanoate	*		***	*	*
Ethyl octadecatrienoate					*
Ethyl octadeca-9,12-dienoate					*
Ethyl octadec-9-enoate		*	***		***
Ethyl octadec-11-enoate	*	*	***	*	**
Ethyl octadec-13-enoate			*		*
Ethyl octadec-15-enoate			*		
Ethyl octadecanoate			**		**
Ethyl eicosanoate					*
2-Propyl esters					
2-Propyl dodecanoate			*		*
2-Propyl tetradecanoate	**	**	*	*	*
2-Propyl hexadec-11-enoate			*		
2-Propyl hexadecanoate	*		*		*
2-Propyl octadec-9-noate			*		*
2-Propyl octadecanoate					*
Acetates					
Dodecyl acetate			*		
Octadecyl acetate					*
Docosyl acetate				*	
Tetracosyl acetate				***	
Hexacosyl acetate				***	
Octacosyl acetate				*	
Triacontyl acetate				*	
Nonadec-10-en-2-yl acetate					*

Table III (continued).

	M	H	L	T	D
Nonadec-12-en-2-yl acetate					*
Heneicosen-2-yl acetate					*
Heneicos-2-yl acetate					*
Tricosen-2-yl acetate					*
Tricos-2-yl acetate					*
Pentacosen-2-yl acetate					*
Pentacos-2-yl acetate					*
Heptacos-9-en-2-yl acetate			*	*	*
Heptacos-2-yl acetate					*
Nonacosadien-2-yl acetate					*
Nonacos-9-en-2-yl acetate			*	*	**
Nonacos-2-yl acetate					*
Butyrates					
Decyl butyrate			*		*
Dodec-9-enyl butyrate			*		
Dodecyl butyrate			***	*	*
Teradecyl butyrate			*		
Hexanoates					
Dec-9-enyl hexanoate			*		
Decyl hexenoate			*		
Decyl hexanoate	*		*	*	*
Dodecenyl hexanoate					*
Dodec-9-enyl hexanoate			*		
Dodec-11-enyl hexanoate			*		
Dodecyl hexanoate	*	*	***	*	*
Tetradecyl hexanoate			*		
Wax type esters					
Octyl dodecanoate			*		
Octyl octadec-9-noate					*
Octyl octadec-11-noate			*		*
Dec-9-enyl decanoate			*		
Decenyl hexadecanoate	*				
Decyl octanoate	*		**		*
Decyl dec-9-enoate			*		
Decyl decanoate	*		**		
Decyl dodecanoate	*		**		**
Decyl tetradecanoate	***		**		*
Decyl hexadec-11-noate			*		
Decyl hexadecanoate	*				
Decyl octadec-9-enoate	**	*	*	*	***
Decyl octadec-11-enoate			*		**
Dodec-9-enyl octanoate			*		
Dodec-9-enyl decanoate			*		
Dodec-9-enyl dodecanoate			**		
Dodec-9-enyl tetradecenaote			*		
Dodec-9-enyl tetradecanoate			*		
Dodecenyl hexadecadienoate			*		
Dodecenyl hexadecenoate	*				
Dodecenyl octadecadienoate	*		***		*
Dodecenyl octadecenoate			*		

Table III (continued).

	M	H	L	T	D
Dodecyl octanoate	*		***	*	*
Dodecyl decenoate				*	**
Dodecyl dec-7-noate			*		
Dodecyl dec-9-noate			*		
Dodecyl decanoate	*		***		**
Dodecyl dodec-9-noate			*		
Dodecyl dodec-11-noate			*		
Dodecyl dodecanoate	***		****	*	*
Dodecyl tetradec-9-enoate			*		
Dodecyl tetradec-11-enoate	*		***	*	
Dodecyl tetradecanoate	*		****	*	*
Dodecyl hexadeca-dienoate	*		***		*
Dodecyl hexadecenoate					**
Dodecyl hexadec-7-enoate			*		
Dodecyl hexadec-9-enoate			*		
Dodecyl hexadec-11-enoate	***	**	****	**	
Dodecyl hexadec-13-enoate			*		
Dodecyl hexadecanoate			****	*	**
Dodecyl octadeca-trienoate	*		***		
Dodecyl octadeca-dienoate	*		***		*
Dodecyl octadec-9-enoate	**	**	***	**	**
Dodecyl octadec-11-enoate	***	**	****	**	**
Dodecyl octadec-13-enoate			**		
Dodecyl octadecanoate					*
Dodecyl eicosenoate	*				*
Dodecyl eicos-13-enoate			**		
Tetradecyl octadece-noate	*				
Tetradecyl octadec-9-enoate			*		*
Tetradecyl octadec-11-enoate			*		*
Tetradecyl octadec-13-enoate			*		
Tetradecyl octadecanoate					*
Hexadecenyl octadecen-oate			*		*
Hexadecyl dodecanoate					*
Hexadecyl hexadecen-oate	*				
Hexadecyl hexadecanoate	*		*	*	*
Hexadecyl octadeca-dienoate					*
Hexadecyl octadecen-oate				*	
Hexadecyl octadec-9-enoate			*		**
Hexadecyl octadec-11-enoate			*		**
Hexadecyl octadecan-oate			*		*
Octadecenyl octadecatri-enoate					*
Octadecenyl octadecen-oate					*
Octadecyl dodecanoate					*
Octadecyl tetradecan-oate					*
Octadecyl hexadecan-oate				*	*
Octadecyl octadec-9-enoate					*
Octadecyl octadec-11-enoate					*
Octadecyl octadecanoate					*
Eicosyl tetradecanoate				*	
Eicosyl octadecenoate				*	*
Eicosyl octadec-9-enoate			*		

Carboxylic acid esters

Esters form the second largest group (157 aliphatic esters listed in Table III) of volatiles present in the various glands. Three types of aliphatic esters could be distinguished: Esters with a short alcohol component, esters with a short acid component, and wax type esters with similar chain lengths on either side of the functional group. In addition, a small amount of fatty acid esters of the diterpene geranylcitronellol were found predominantly in Dufour's and labial glands (Table IV).

Esters with a short alcohol component prevailed in labial and Dufour's glands. Dufour's glands were also characterized by many acetates, albeit in small amounts. Among the acetates, tetracosyl acetate and hexacosyl acetate were found in fairly large amounts in tarsal glands. Among the medium volatiles, dodecyl hexanoate formed a major component in labial glands. With respect to the composition of wax type esters, labial and Dufour's glands secretions were most complex. These included a series of even numbered alcohols (ranging from C_8 to C_{20}) and a series of even numbered acids (between C_8-C_{18}). Decyl and dodecyl esters having a total of 24,26,28 and 30 carbon atoms were most abundant. While at the alcohol moiety desaturations were exclusively found in position 9, the acid moieties showed double bond positions at 7,9,11 and 13, again forming clusters of

Table IV. List of terpenes and esters as well as miscellaneous compounds found in exocrine glands of *Bombus terrestris*. M: Mandibular gland; H: hypopharyngeal gland; L: labial gland; T: tarsal gland; D: Dufour's gland; ****: major; ***: medium; **: minor; *: trace.

	M	H	L	T	D
Terpene esters					
Geranylcitronellyl decanoate					*
Geranylcitronellyl dodecanoate	*		*		*
Geranylcitronellyl tetradecenoate			*		*
Geranylcitronellyl tetradecanoate			*		
Geranylcitronellyl hexadecenoate			*		*
Geranylcitronellyl hexadecanoate			*		*
Geranylcitronellyl octadecatrienoate					*
Geranylcitronellyl octdecenoate					*
Geranylcitronellyl octadecanoate					*
Miscl. Compounds					
Limonene		*	*		
Squalene	***	***			**
Geranylcitronellol					*
2-Ethyl-7-methyl-1,6-dioxaspiro-[4.5]-decane	*				
mass/base peak					
398/314 (Steroide?)	***				*
400/43 (Steroide?)	*				
400/95 (Steroide?)	*				
412/55 (Steroide?)	*				
414/43 (Steroide?)	*				
414/382 (Steroide?)	*				
428/396 (Steroide?)	*				

Table V. List of oxygenated carboxylic acids found in the exocrine glands of *Bombus terrestris*. M: Mandibular gland; H: hypopharyngeal gland; T: tarsal gland; ****: major; ***: medium; **: minor; *: trace.

	M	H	T
Oxygenated Acids			
2-Hydroxypropionoic acid	*		
3-Hydroxyhexanoic acid	*		
3-Hydroxyoctanoic acid	***		
3-Hydroxydecanoic acid	****	*	
3-Hydroxydodecenoic acid	*		
3-Hydroxydodecanoic acid	***		
3-Hydroxytetradecenoic acid	*		
3-Hydroxytetradecanoic acid	*		
11-Hydroxydodecanoic acid			*
12-Hydroxytetradecanoic acid			*
13-Hydroxytetradecanoic acid			*
13-Hydroxytetradec-5-enoic acid			*
13-Hydroxytetradec-7-enoic acid			*
14-Hydroxyhexadecanoic acid			*
15-Hydroxyhexadecanoic acid			*
15-Hydroxyhexadec-7-enoic acid			*
15-Hydroxyhexadec-9-enoic acid			*
17-Hydroxyoctadec-9-enoic acid			*
3-Butyroxyoctanoic acid	**		
3-Butyroxydecanoic acid	***		
3-Butyroxydodecanoic acid	*		

positional isomers like the dodecyl hexadecenoates in the labial glands. Double bond position in unsaturated esters including positional isomers could be distinguished by the mass spectra of their DMDS-derivatives. Isomeric esters such as formed by shifting of the ester group, as in the pair dodecyl dodecanoate and decyl tetradecanoate, can be easily identified even when they are not resolved by gas chromatography since they produce pronounced signals of the protonated acids in the EI-mass spectra (Tengö *et al.*, 1985).

A special class of higher oxygenated compounds is represented by the hydroxylated acids, some of which are esterified with butanoic acid. 3-Hydroxydecanoic acid and its butyrate form major components of the mandibular glands secretion. The EI spectra of the corresponding methyl esters are shown in Fig. 1. In the mass spectrum of methyl 3-hydroxydecanoic acid (Fig. 1A) the base peak at *m/z* 103 is formed by α cleavage of the 3-hydroxy group giving rise to *m/z* 71 upon loss of methanol. The doublet at *m/z* 152/153 is the result of elimination of water and methanol or a methoxy group, respectively. The signal at *m/z* 74 represents the

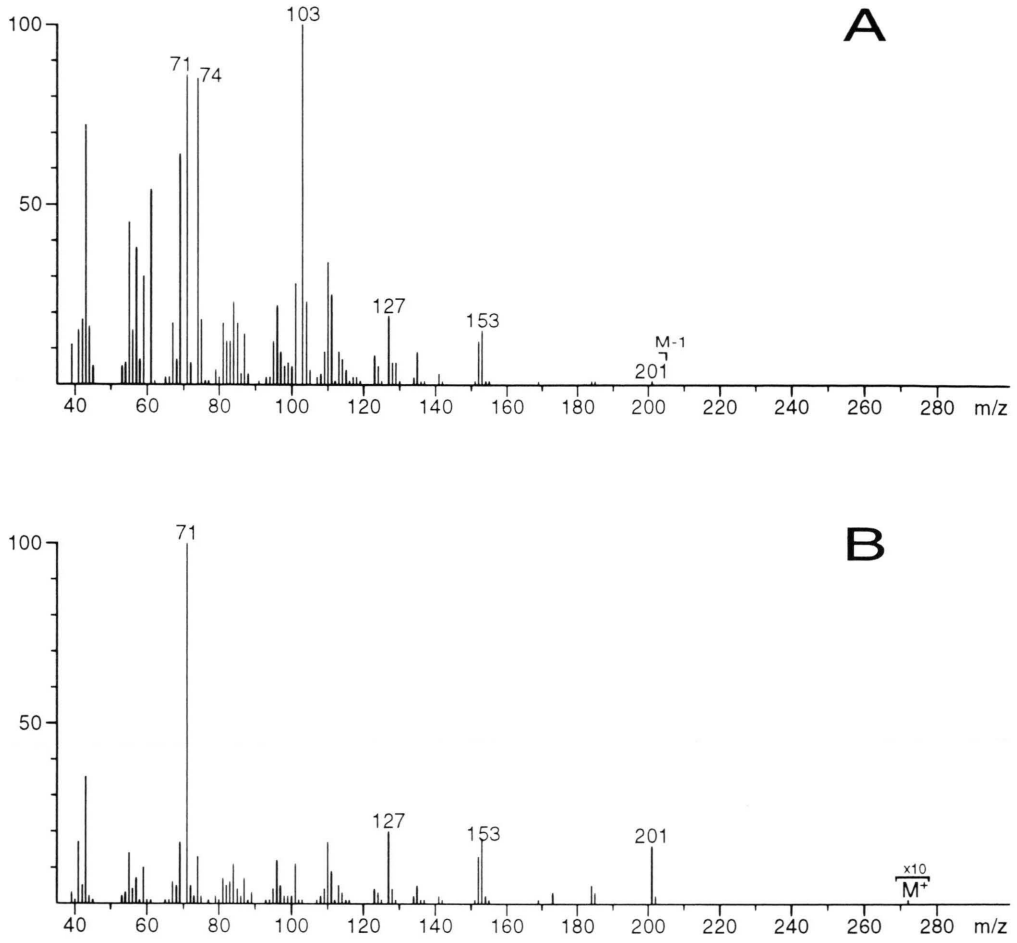

Fig. 1. Mass spectra of A) methyl-3-hydroxydecanoic acid and B) methyl 3-butyroxy decanoate.

characteristic Mc Lafferty fragment of methyl esters, while *m/z* 127 may be regarded as its complement (minus a proton). The mass spectrum of methyl 3-butyroxy decanoate (Fig. 1B) is highly dominated by *m/z* 71 which is represented by the acylium ion of the side chain. Other characteristic ions in this spectrum are the same as in the parent ester.

Discussion

As in many social hymenoptera queens of *B. terrestris* proved to be a rich source of a multitude of volatile compounds that may serve in chemical communication or have other biological functions. The extensive study of these products showed that glands cannot be characterized by possessing certain groups of chemicals but, on the contrary typically have a plethora of volatile substances.

The compounds listed in Tabs. I–V show the almost complete pattern of volatiles present in queens of *B.terrestris*. Apart from a group of higher boiling compounds only some trace components remained unknown. The mass spectra of these polar substances suggested the structures of steroides; their masses and base peaks of 70 eV spectra are shown in Tab. IV.

Among the compounds found in the glands of *B. terrestris*, aliphatic hydrocarbons were most abundant. Hydrocarbons are omnipresent in insects' excocrine secretion. They form components of the sex pheromone blends in many dipteran species (Howard and Blomquist, 1982; Blomquist

et al., 1987), in the rove beetle *Aleochara curtula* (Peschke and Metzler, 1987), in the leafminers (Francke *et al.*, 1988), and in geometrid moths (Li *et al.*, 1993). Although they are very abundant in the social Hymenoptera, there is little experimental evidence as to their function. In ants, female's Dufour's gland produces undecane that functions as a sex pheromone (Walter *et al.*, 1993). The solitary bee, *Xylocopa virginica*, produces alkanes in its Dufour's gland and uses them for marking flowers that have been depleted of their resources (Vinson *et al.*, 1978). In *B. terrestris*, flower marking is also accomplished by using hydrocarbons, but in this species they are products of the tarsal glands and deposited on flowers that are rewarding. In ants, they comprise the bulk of the postpharyngeal gland secretion and are apparently applied to the cuticular surface, explaining the congruency between these two secretions (Bagneres and Morgan, 1991; Hefetz, 1992; Soroker *et al.*, 1995). The report suggesting the postpharyngeal secretion as a source of recognition cues (Soroker *et al.*, 1994), renders hydrocarbons as the natural candidates for forming such a signal. The same congruency in hydrocarbons was also found in several species of *Bombus* (Oldham *et al.*, 1994), lending credence to the recognition hypothesis. However, in bumble bees, in contrast to ants, there is no evidence that the glandular secretion is actively applied onto the cuticular surface. The quantities of hydrocarbons present in the glandular exudates of *B. terrestris* renders unlikely the possibility that they are simply lining the cuticular intima of the glands. It is also unlikely that these complex mixtures merely serve as a solvent for the other glandular components: for that function a simple mixture or even a single compound would suffice, as was shown for the defensive secretion of *Bledius* species (Dettner, 1987). Moreover, the glands possess not only straight chain alkanes, but also many alkenes, alkadienes, and branched alkanes, suggesting a specific function for these compounds. Whether hydrocarbons serve as integral components of the secretion in *B. terrestris* queens, or form a neutral group of compounds, awaits experimental evidence.

Apart from the hydrocarbons, fatty acids and their esters with straight chain alcohols form two other large groups of compounds identified from *B. terrestris*. Although both groups of chemicals are widespread among the various exocrine glands, some glandular specificity can be found. The mandibular gland secretion shows the most complex acid pattern, whereas the labial and Dufour's glands secretions show the broadest spectra of esters, although each gland possesses a very different composition. Among the acids "classical" saturated representatives like myristic, palmitic and stearic acids as well as oleic acid are always dominant, while vaccenic acid is an additional major product in the hypopharyngeal and in the labial glands. Similar to the alkanes, unsaturated acid form clusters of up to five geometric isomers. Only the mandibular glands produce chiral methyl branched acids, among which at least 12-methyl tetradecanoic acid is known to exhibit antibiotic properties (Hattori *et al.*, 1987).

Esters are one of the most widespread classes of aliphatic compounds and they are particularly abundant in the exocrine secretion of bees (Wheeler and Duffield, 1988; Francke *et al.*, 1983). They are the major components in Dufour's gland secretion of *Andrena* species (Tengö and Bergström, 1975; Cane, 1981) where they serve as a brood cell lining. In the anthophorid bee *Eucera palestinae* esters are also abundant and, in addition to the brood cell lining also serve to mark the nest entrance (Shimron *et al.*, 1985). Ethyl and methyl esters are male specific components of the cephalic secretion of *Scaptotrigona postica* (Engels *et al.*, 1990). They are also found on the cuticular surface of honey bee larvae and are reported to act as an attractant for the bee mite *Varroa jacobsoni* (Le Conte *et al.*, 1989). The small group of 2-propylesters, in particular 2-propyl tetradecanoate are typical surfactants and may be used to spread the glandular content after its secretion. Esters of unsaturated fatty acids and 2-propanol are pheromones of *Dermestes* species (Francke *et al.*, 1979).

While wax type esters appear to be almost ubiquitous to bees, no specific function could be attributed to esters with less than 40 carbon atoms. They may be used, due to their high hydrophobicity, as lubricants, but the remarkably complex mixture suggests a communicative function. Octadecyl (Z)-9-tetracosanoate, for example, was identified as a pheromone in the cockroach *Nauphoeta cinerea* (Takahashi and Fukui, 1983).

As a constituent of the marking pheromone of male bumble bees, the diterpene geranylcitronellol is present in the labial gland of *Bombus* species

(Bergström *et al.*, 1973, 1981, 1981a), and of *Psithyrus rupestris* (Svensson and Bergström, 1977). Heads of queens and workers of *B. hypnorum* contain rather high amounts of esters of geranylcitronellol with unsaturated fatty acids, but the biological function of these esters is still unknown (Ayasse *et al.*, 1995).

The carbonyl compounds identified, just like most of the compounds described from the queens, clearly originate from the acetate pool. The methyl ketones, methyl carbinols and the corresponding esters show distinct distribution among the glands. The ketones represent decarboxylated β-keto carboxylic acids which are formed either during anabolism or metabolism of common fatty acids. The majority of these methyl ketones are therefore composed of an uneven number of carbon atoms. It is interesting to note that two groups of ketones showing a gap of 10 carbon atoms were found. Three high boiling compounds which are seldom found in Hymenoptera were found in the labial and the tarsal glands of *B. terrestris*. The low boiling ketones, on the other hand, occur predominantly in the mandibular glands. These ketones are especially widespread among the Hymenoptera as constituents of volatile secretion in ants, and solitary as well as social bees. The corresponding methyl carbinols are also frequently found in bees. In the stingless bee *Scaptotrigona postica* they are produced by the queen and serve as attractants for workers. Interestingly, a group of acetates of long chain methyl carbinols occurs almost exclusively in Dufour's gland. The spiroacetal 2-Ethyl-7-methyl-1,6-dioxaspiro-[4.5]-decane, which also occurs in social wasps (Francke *et al.*, 1979a), solitary bees (Francke *et al.*, 1981), and in fruit flies (Fletcher and Kitching, 1995) represents a higher oxygenated degradation product of fatty acids, namely 2,9,-dihydroxy-undecane-5-one. Spiroacetals of this type are known to be pheromones of bark beetles and fruit flies (Francke *et al.*, 1977; Birgersson *et al.*, 1995).

The most interesting compounds identified are oxygenated fatty acids and their derivatives (Table V). A group of 10 hydroxy acids showing the hydroxyl group towards the end of the chain occurs exclusively in the tarsal glands. These compounds form a link to the chemistry of lactone producing halictine and colletid bees (Hefetz *et al.*, 1978, 1979), since these lactones are formed from ω-hydroxy and ω-1-hydroxy acids. The mandibular glands are especially rich in 3-hydroxy acids. The structure of these acids may be linked to the above mentioned methyl ketones and methyl carbinols. They clearly represent products of β-oxidation and are reported as a substructure of bacterial lipids (Imoto *et al.*, 1983). 3-Hydroxy decanoic acid and two lower homologues were also reported as antifungal agents from the metathoracic glands of the leaf cutting ant *Atta sexdens* (Schildknecht and Knoob, 1971). In methanol extracts of the cephalic secretion obtained from males and femals of the solitary wasp, *Campsoscotia ciliata* a series of methylesters of 3-hydroxy acids with methyl 3-hydroxydecanoate as the main component was identified (Borg-Karlson *et al.*, 1987).

Careful examination of the enantiomeric composition of the 3-hydroxy acids and their butyrates revealed that the hydroxy acids in *B. terrestris* do not show constantly the same purity: whereas 3-hydroxyhexanoic acid is represented by the pure (*S*)- enantiomer, 3-hydroxytetradecanoic acid is almost racemic. The main component in this group, 3-hydroxydecanoic acid shows an enantiomeric excess (ee) of more than 99% of the (*S*)-enantiomer. Corresponding, the butyric acid esters show at least this enantiomeric purity.

Especially intriguing in the finding of 3-hydroxy acids in the mandibular glands and 10-hydroxy acids in the tarsal gland is the analogy to the honey bee acids. In honey bees, 9–2-(*E*)-hydroxydecenoic acid and the corresponding 9-oxo compound are components of the queen pheromone (Slessor *et al.*, 1988). In view of the indication that in *B. terrestris* a putative queen pheromone is present in the mandibular glands (van Honk *et al.*, 1980), one may speculate that this function resides with the hydroxylated acid derivatives present in the gland. Whether these compounds with or without the association of the hydroxy acids from the tarsal glands are involved in queen control over the workers' behavior, is currently being investigated.

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Ayasse M., Marlovits T., Tengö J., Taghizadeh T. and Francke W. (1995), Are there pheromonal dominance signals in the bumble bee *Bombus hypnorum* L (Hymenoptera, Apidae) ? Apidologie **26,** 163–180.

Bagneres A. N. and Morgan E. D. (1991), The postpharyngeal glands and the cuticle of Formicidae contain the same characteristic hydrocarbons. Experientia **47,** 106–111.

Bergström G. and Svensson B.G. (1973), Characteristic marking secretion of the forms *lapponicus* and *scandinavicus* of *Bombus lapponicus* Fabr. (Hymenoptera, Apidae). Chem. Scripta **4,** 231–238.

Bergström G. (1981), Chemical aspects of insect exocrine signals as a means for systematic and phylogenetic discussions in aculeate Hymenoptera. Entomol. Scand. **15** Suppl. 173–184.

Bergström G., Svensson B.G., Appelgren M. and Groth J. (1981a), Complexity of bumble bee marking pheromones: Biochemical, ecological, and systematical interpretations. In: Biosystematics of Social Insects (P.E. Howse and J.C. Clement ed.) 175–183.

Birgersson G., Debarr G.L., De Groot P., Dalusky M.J., Pierce H.D., Borden J., Meyer H., Francke W., Espelie K. and Berisford C. W. (1995), Pheromones in white pine cone beetle, *Conophthorus coniperda* (Schwarz) (Coleoptera: Scolytidae). J. Chem. Ecol. **21,** 143–167.

Blomquist G. J., Nelson D. R. and de Renobles V. (1987), Chemistry, biochemistry, and physiology of insect cuticular lipids. Arch. Insect. Biochem. Physiol. **6,** 227–265.

Borg-Karlson A.-K., Bergström G. and Kullenberg B. (1987), Chemical basis for the relationship between *Ophrys* orchids and their pollinators. Chemica Scripta **27,** 303–311.

Cane J. H. J. (1981), Dufour's gland secretion in the cell linings of bees (Hymenoptera: Apoidae). J. Chem. Ecol. **7,** 403–410.

Conte J. Le, Arnold G., Trouiller J., Masson C., Chappe B. and Ourisson G. (1989), Attraction of the parasitic mite *Varroa* to the drone Larvae of the honey bees by simple aliphatic esters. Science **245,** 638–639.

Dettner K. (1987), Chemosystematics and evolution of beetle chemical defenses. Ann. Rev. Entomol. **32,** 17–48.

Doorn A. van and Heringa J. (1986), The ontogeny of a dominance hierarchy in colonies of the bumble bee *Bombus terrestris* (Hymenoptera, Apidae). Insectes Sociaux **33,** 3–25.

Doorn A. van (1988), Reproductive dominance in bumblebees: an ethophysiological study. Ph.D dissertation University of Utrecht.

Duchateau M.J. and Velthuis H.H.W. (1988), Development and reproductive strategies in *Bombus terrestris* colonies. Behaviour **107,** 186–207.

Engels W., Engels E., Lübke G., Schröder W. and Francke W. (1990), Volatile cephalic secretions of drones, queen and workers in relation to reproduction in the stingless bee, *Scaptotrigona postica* (Hymenoptera: Apidae: Trigonini). Entomol. Gen. **15,** 91–101.

Fletcher M.T., and Kitching W. (1995), Chemistry of fruit flies. Chem. Rev. **95,** 789–828.

Francke W., Heeman V., Gerken B., Renwick J.A.A. and Vité J.P. (1977), Methyl-1,6-dioxaspiro[4.4]nonane, principal aggregation pheromone of *Pityogenes chalcographus* (L.). Naturwissenschaften **64,** 590.

Francke W., Levinson A.R., Jen T.L. and Levinson H.Z. (1979), Carbonsäureisopropylester – Eine neue Klasse von Insektenpheromonen. Angew. Chem. **91,** 843.

Francke W., Hindorf G. and Reith W. (1979a), Mass-spectrometric fragmentation of alkyl-1,6-dioxaspiro-[4.5]decanes. Naturwissenschaften **66,** 618.

Francke W., Reith W., Bergström G. and Tengö J. (1981), Pheromone bouquet of the mandibular glands in *Andrena haemorrhoa* F. (Hym. Apoidea). Z. Naturforsch. **36c,** 928–932.

Francke W., Schröder W., Bergström G. and Tengö J. (1983), Esters in volatile secretions of bees. Nova Acta Reg. Soc. Scient. Upsaliensis. Ser. V: C **3,** 127–136.

Francke W., Toth V., Szöcs G., Krieg W., Ernst H. and Buschmann E. (1988), Identifizierung und Synthese von Dimethylalkanen als Sexuallockstoffe weiblicher Miniermotten (Lyonetiidae). Z. Naturforsch. **43c,** 787–789.

Gênet J.P., Pind C., Ratovelomanana-Vidal C., Mallart S., Pfister X., Galopin C. and Laffitte J.A. (1994), Enantioselective hydrogenation reactions with a full set of performed and prepared in *situ* chiral diphosphineruthenium (II) catalysts. Tetrahedron: Asymmetry **5,** 675–690.

Gênet J.P. (1995), General synthesis of chiral Ru^{II} catalysts $(P^*P)RuX_2$ using CODRu-(2-methylallyl)$_2$. Efficient catalysts for asymmetric hydrogenations. Acros Organics Acta **1**(1), 4–9.

Genin E., Jullien R., Perez F., Fonta C. and Masson C. (1984), Preliminary result on the chemical mediators of the bumblebee *Bombus hypnorum* (Hymenoptera, Apoidae, Bombini). C.R. Acad. Sci. Paris, **299** Ser III n° 8, 297–302.

Hattori M., Miayachi K., Hadda S., Kakiuchi N., Kiuchi F., Tsuda Y. and Namba T. (1987), Effects of long-chain fatty acids and fatty alcohols on the growth of *Streptococcus mutans*. Chem. Pharm. Bull **35,** 3507–3510.

Hefetz A., Blum M.S., Eickwort G. C. and Wheeler J. W. (1978). Chemistry of the Dufour's gland secretion of halictine bees. Comp. Biochem. Physiol. **61B,** 129–132.

Hefetz A., Fales H. M. and Batra S. W. T. (1979), Natural polyesters: Dufour's gland macrocyclic lactones from brood cell laminesters in *Colletes* bees. Science **204,** 415–417.

Hefetz A., Errard C. and Cojocaru M. (1992), Heterospecific substances in the postpharyngeal gland of ants reared in mix groups. Naturwissenschaften **79,** 417–420.

Hefetz A., Tengö J., Lübke G. and Francke W. (1993), Inter-Colonial and intra-Colonial variation in Dufour's gland secretion in the bumble bee species *Bombus hypnorum* (Hymenoptera: Apidae). In: Sensory Systems of Arthropods (Weise K., Gribakin F.G. and Renninger G., eds), Birkhäuser Verlag, Basel, pp. 469–480.

Honk C.G.J. van, Velthuis H.H.W., Röseler P.F. and Malotaux M.E. (1980), The mandibular glands of *Bombus terrestris* queens as a source of queen pheromones. Ent. Exp. Appl. **28,** 191–198.

Howard R. W. and Blomquist G. J. (1982), Chemical ecology and biochemistry of insect hydrocarbons. Ann. Rev. Entomol. **27**,149–172.

Imoto M., Kusumoto S., Shiba T., Naoki H., Iwashita T., Reitschel E.T., Wollenweber H-W., Galanos C. and Lüderitz O. (1983), Chemical structure of *E. coli* lipid A: linkage site of acyl groups in the disaccharide backbone. Tetrahedron Lett. **24,** 4017–4020.

Li J., Gries G., Gries R., Bikic J. and Slessor K. (1993), Chirality of synergistic sex pheromone of the western hemlock looper *Lambdina fiscellaria lugubrosa* (Hulst) (Lepidoptera: Geometridae). J. Chem. Ecol. **19,** 2547–2561.

Kitamura M., Tokunaga M. and Noyori R. (1992), Practical synthesis of BINAP-ruthenium (II) dicarboxylate-complex. J. Org. Chem. **57,** 4053–4054.

Klimetzek D., Köhler J., Krohn S. and Francke W. (1989), Das Pheromon-System des Waldreben-Borkenkäfers, *Xylocleptes bispinus* Duft (Col., Scolytidae). J. Appl. Ent. **107,** 304–309.

Oldham N.J., Billen J. and Morgan E.D. (1994), On the similarity of Dufour's gland secretion and cuticular hydrocarbons of some bumble bees. Physiol. Entomol. **19,**115–123.

Mielniczuk Z., Alugupalli S., Mielniczuk E. and Larrson L. (1992), Gas chromatography-mass spectrometry of liposaccharide 3-hydroxy-fatty acids: Comparison of pentaflourobenzoyl and trimethylsilyl ester derivatives. J. Chromatogr. **623,** 115–122.

Peschke K. and Metzler M. (1986), Cuticular hydrocarbons and female sex pheromones of the rove beetle, *Aleochara curtula* (Coleoptera, Staphylinidae). Insect Biochem. **17,** 167–178.

Pomonis J.G., Fatland C.F., Nelson D.R. and Zacylskie R.G. (1978), Insect hydrocarbons: Corroboration of the structure by synthesis and mass spectrometry of mono- and dimethylalkanes. J. Chem. Ecol. **4,** 27–39.

Pomonis J.G., Nelson D.R. and Fatland C.F. (1980), Insect hydrocarbons 2. Mass spectra of dimethylalkanes and the effect of the number of methylene units between methyl groups on fragmentation. J. Chem. Ecol. **6,** 965.

Röseler P.F., Röseler I. and Honk C.G.J. van (1981), Evidence for inhibition of corpora allata activity in workers of *Bombus terrestris* by a pheromone from queen's mandibular glands. Experientia **37,** 348–351.

Röseler P.F. (1985), A technique for year-round rearing of *Bombus terrestris* (Apidae, Bombini) colonies in captivity. Apidologie **16,**165–170.

Röseler P.F., Röseler I. and Honk C.G.J. van (1990), Castes and reproduction in bumblebees. In: Social insects, an evolutionary approach to castes and reproduction, Engels W. (ed). pp. 147–166. Springer-Verlag, Berlin.

Schildknecht H. and Knoob K. (1971), Myrmicacin, das erste Insekten-Herbicid. Angew. Chem. **83,** 110.

Schmitt U., Lübke G. and Francke W. (1991), Tarsal secretion marks food sources in bumblebees (Hymenoptera: Apidae). Chemoecology **2,** 35–40.

Shimron O., Hefetz A. and Tengö J. (1985), Structural and communicative functions of Dufour's gland secretion in *Eucera palestinae* (Hymenoptera: Anthophoridae). Insect Biochem. **15,** 635–8.

Slessor K. N., Kaminski L., King G. G. S., Borden, J. H. and Winston, M. L. (1988), Semiochemical basis of the retinue response to queen honey bees. Nature **332,** 354–356.

Soroker V., Vienne C., Hefetz A. and Nowbahari E. (1994), The postpharyngeal gland as a "Gestalt" organ for nestmate recognition in ant *Cataglyphis niger*. Naturwissenschaften **81,** 510–513.

Soroker V., Vienne C., and Hefetz A. (1995), Hydrocarbon dynamics within and between nestmates in the *Cataglyphis niger* (Hymenoptera: Formicidae). J. Chem. Ecol. **21,** 365–378.

Svensson B. and Bergström G. (1977), Volatile marking secretions from the labial gland of North European Pyrobombus D. T. males (Hymenoptera, Apidae). Insectes Soc. **24,** 213–224.

Takahashi S. and Fukui M. (1983), Studies on the mating behavior of the cockroach, *Nauphoeta cinerea* (Olivier) (Dictyoptera: Blaberidae). Appl. Entomol. Zool. **18** 357–360.

Tengö J. and Bergström G. (1975), All-trans farnesyl hexanoate and geranyl octanoate in the Dufour's gland of *Andrena* (Hymenoptera: Apidae). J. Chem. Ecol. **1,** 253–268.

Tengö J., Groth I., Bergström G., Schröder W., Krohn S. and Francke W. (1985), Volatile secretions in three species of *Dufourea* (Hymenoptera: Halictidae). Z. Naturforsch. **40 c,** 657–660.

Tengö J., Hefetz A., Bertsch A., Schmitt U., Lübke G. and Francke W. (1991), Species specificity and complexity of Dufour's gland secretion of bumble bees. Comp. Biochem. Physiol., **99B,** 641–646.

The Wiley/NBS Registry of Mass Spectral Data (1989), (Mc Lafferty F.W. and Stauffer D.B., eds.). Wiley Interscience New York.

Vincenti M., Guglielmetti G., Cassani G. and Tonini C. (1987), Determination of double bond position in diunsaturated compounds by mass spectrometry of dimethyl disulfide derivatives. Anal. Chem. **59,** 694–699.

Vinson S. B., Frankie G. W., Blum M. S. and Wheeler J. W. (1978), Isolation, identification and function of the Dufour's gland secretion of *Xylocopa virginica texana* (Hymenoptera: Anthophoridae). J. Chem. Ecol. **4,** 315–323.

Walter F., Fletcher D. J. C., Chautems D., Cherix D., Keller L., Francke W., Fortelius W., Rosengren R. and Vargo E. L. (1993), Identification of the sex pheromone of an ant, *Formica lugubris* (Hymenoptera, Forrmicidae). Naturwissenschaften **80,** 30–34.

Wheeler J. W. and Duffield R. M. (1988), Pheromones of Hymenoptera and Isoptera. In: CRC Handbook of Natural Pesticides IV part B (E. D. Morgan and N. Bhushan Mandava), CRC Press Boca Raton Florida, pp. 59–206.