Volatile Secretions in Three Species of *Dufourea* (Hymenoptera: Halictidae) Bees: Chemical Composition and Phylogeny

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Volatile secretions from Dufour's glands in three species of *Dufourea* bees, *Dufourea (Halictoides) dentriventris* (Nylander), *D.(H.) inermis* (Nylander) and *D.(Dufourea) minuta* Lepelletier have been studied by gas chromatography and mass spectrometry. It was found that the secretions are composed of complex blends of straight chain saturated and unsaturated 2- and 3-ketones and series of hexanoic and octanoic esters. Cephalic secretions from males and females of *D.(H.) inermis* and *D.(D.) minuta* contain sex- and species-specific blends of methylcarbinols and corresponding long chain carboxylic esters. Mass spectrometric fragmentation patterns of esters are described.

In phylogenetic studies of bees, the group Dufoureinae has commonly been accepted as a subunit of the family Halictidae. Michener [1] found it to be more closely related to the halictines than to any other group of bees. In our studies on the chemistry of volatile gland secretions from bees we have, however, found that representatives of Dufoureinae diverge from the general pattern of the rest of the family. The present study describes the composition of volatile secretions of three species of the genus Dufourea representing two subgenera.

Chemical investigations were performed by combined gaschromatography and mass spectrometry using a LKB 2091 instrument. During the period from 1978–1984 ca. 40 samples were analysed. Gaschromatographic separations of compounds from pentane extracts of Dufour's glands or heads were carried out on 50 m glass capillary columns under temperature programme from 50–200 °C at a rate of 5 °C per minute. Mostly WG 11 was used as stationary phase; in some cases mixtures of FFAP and OV 17 were preferred. Identifications of natural compounds were based on comparison of gaschromatographic retention times and mass spectra with those of synthetic reference samples.

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Heads

Volatile secretions of heads of males and females of *Dufourea (Dufourea) minuta* Lepelletier and *Dufourea (Halictoides) inermis* (Nylander) form species- and sex-specific blends. Characteristic series of bishomologue methylcarbinols ranging from 2-nonanol to 2-nonadecanol and several corresponding esters are consistently present, while in some female head extracts traces of Dufour's gland constituents (see below) have also been found. Among typical metabolites of β-ketoacyl derivatives straight chain uneven numbered methylcarbinols containing 7 and more carbon atoms are widespread in nature. They are important volatile signals in chemical communication systems of Hymenoptera [2]. While females of *D.(D.) minuta* contain the higher boiling carbinols, in males the more volatile ones are prevailing. Particularly interesting is the occurrence of several esters produced from methylcarbinols and even numbered straight chain carboxylic acids which to our knowledge are the first identified from insects. While in *D.(D.) minuta* octanoates of 2-nonanol, 2-undecanol and 2-tridecanol are found, some corresponding butyrates and decanoates are present in *D.(H.) inermis*. In *D.(D.) minuta* males 1-methyldecyl octanoate (2-undecyl octanoate) is the major component, whereas in *D.(H.) inermis* 1-methyldecyl decanoate (2-undecyl decanoate) dominates the males' cephalic secretion. Females contain smaller amounts of esters than males. Esterified long chain

alkan-2-ols are known as plant lipids [3]; the biogenesis of these chiral compounds in barley spike epicuticular wax has been extensively studied [4].

Mass spectra of esters

EI-mass spectra of esters with long alkylchains at either side of the carboxyl group show the following characteristics:

A) a small signal of the molecular ion (better detected by CI);
B) a strong acylium ion;
C) a signal of the "free acid";
D) a signal caused by the "protonated acid";
E) signals at $m/z = 60$ and 61 corresponding to $C_2H_4O_2$ and $C_2H_5O_2$;
F) alkene fragments as products of a Mc Lafferty rearrangement at the alcohol side;
G) oxygen containing ions caused by α-cleavage of the alcohol chain.

In unbranched saturated esters of primary alcohols ion **B** is less abundant than ion **D**; in esters with secondary alcohols relations are reserve. Ion **F** characterises the length of the alcohol chain and yields the highest numbered fragment observed in the spectrum when the alcohol contains at least 3 carbon atoms more than the acid. If the alcohol is shorter, ion **C** becomes the highest even numbered fragment. The small signals **G** give a specific information about the position of the oxygen function along the carbon chain. **G** is almost absent in esters with straight chain primary alcohols. Fragmentation caused by "classical" Mc Lafferty rearrangements at the acid side (like $m/z = 88$ in ethylesters) do not seem to play a role in the type of esters which is discussed here. For a better illustration, plotted mass spectra of octyl octanoate, 1-methylheptyl octanoate and 1-ethylhexyl octanoate are given in Fig. 1 in which ions **A**–**G** have been marked.

Dufour's glands

Results of the analyses of Dufour's gland secretions of *D.(D.) minuta, D.(H.) inermis* and *D.(H.) dentriventris* (Nylander) are compiled in Table I. Unbranched open chain compounds forming series of bishomologues make up characteristic blends of typical acetogenins. Carboxylic acids, alcohols, esters and ketones could be identified together with hydrocarbons C_{19}–C_{29}. The latter are sometimes found in relatively large amounts; they may in parts represent constituents of the cuticular lipids and are not listed in the table.

Several straight chain ketones which carry the carbonyl group in positions 2 or 3 are found in all three species. While uneven numbered methylketones are common among the volatiles from Hymenoptera [2], the even numbered vinylketones are seldomly found in arthropods. They have been identified from several termite species [5–7] and very recently from Dufour's glands of *D. novaeangliae* [8]. Vinylketones showing an additional terminal double bond have also been reported from termites [6, 7]. The esters occurring in the secretion represent combinations of

Table I. Volatile constituents in the Dufour gland secretion of *Dufourea (H.) dentriventris, D.(H.) inermis* and *D. (Dufourea) minuta.*

Compound	*D.d.*	*D.i.*	*D.m.*
Nonan-2-one	x	x	x
Undecan-2-one	x	x	x
10-Undecen-2-one	x	x	x
Tridecan-2-one		x	x
1-Decen-3-one	x	x	
Dodecan-3-one	x	x	
1-Dodecen-3-one	L	L	L
1,11-Dodecadien-3-one	x	L	L
1-Tetradecen-3-one	x	x	L
1,13-Tetradecadien-3-one	x	x	L
Tetradecatrien-3-one			x
Hexanoic acid	x	x	L
Octanoic acid	x	x	x
Octanol	x		
Hexadecanol		x	
Octadecanol			x
Octyl hexanoate	L	x	
Decyl hexanoate	x	x	
Decenyl hexanoate	x	x	
Dodecyl hexanoate	x	x	x
Dodecenyl hexanoate	x	x	x
Tetradecyl hexanoate	x	x	x
Tetradecenyl hexanoate		x	
Hexadecyl hexanoate		L	
Hexadecenyl hexanoate		x	x
Octadecyl hexanoate	x	L	L
Octadecenyl hexanoate			x
Eicosyl hexanoate			x
Octyl octanoate	x	x	
Octenyl octanoate	x	x	
Decyl octanoate	x	x	
Decenyl octanoate	x		
Dodecyl octanoate	x		
Octadecyl octanoate			x
Octyl decanoate	x		
Dodecyl decanoate			x

x, compound present; L, present in large amounts.

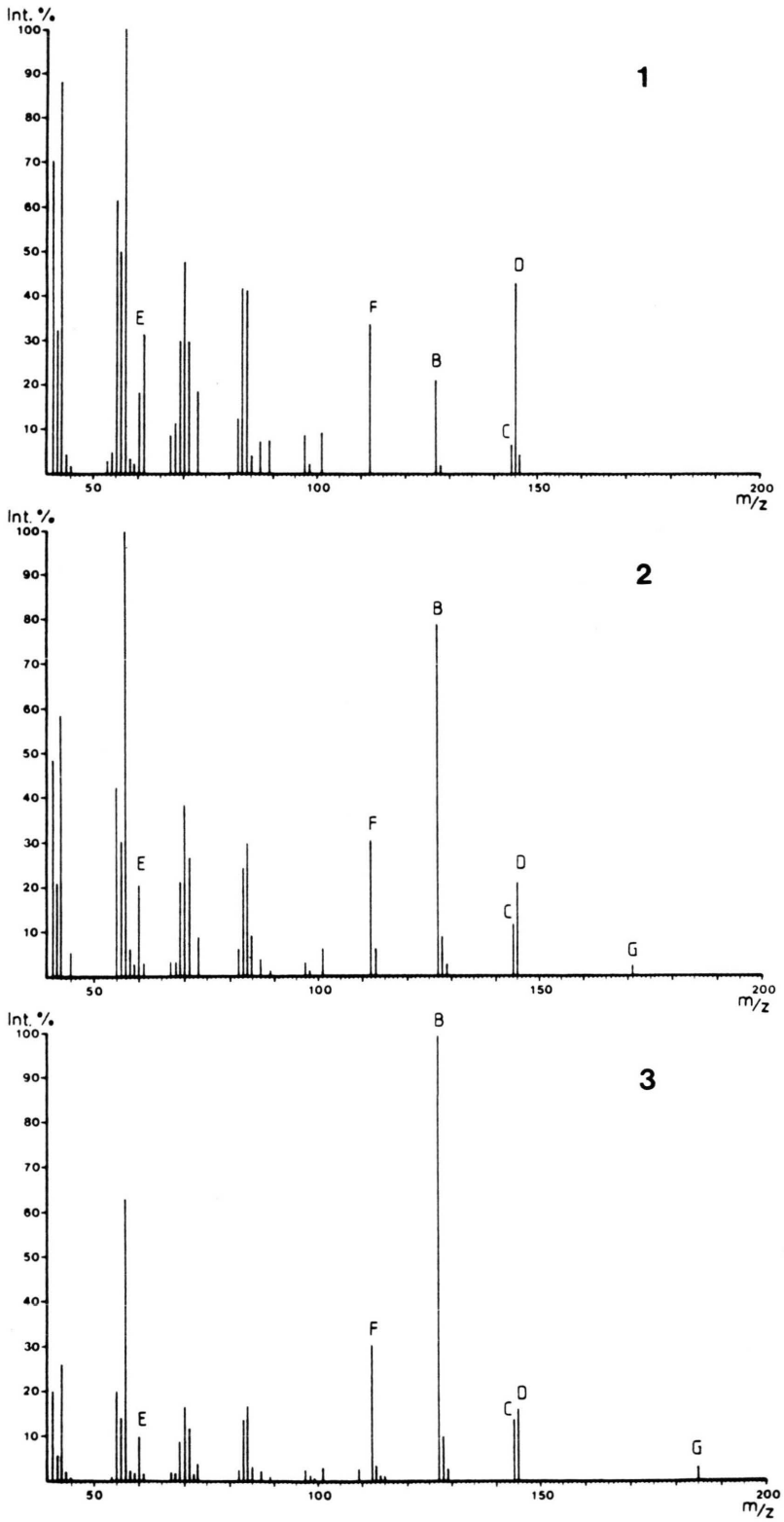

Fig. 1. 70 eV Mass spectra of esters of octanoic acid with: 1 = 1-octanol; 2 = 2-octanol; 3 = 3-octanol.

hexanoic and octanoic acids with a series of bis-homologue primary alcohols ranging from octanol to eicosanol, respectively. The esters are found in all three species; not all of them, however, are present in all species. Esters constitute one of the most common group of natural products. In insects they occur frequently either as volatile signals or as wax constituents, covering a wide range of different biological functions [9]. Esters of hexanoic and octanoic acid with long chain alcohols, compounds containing up to 30 carbon atoms have also been identified from *D. marginata* [10] and *D. novaeangliae* [8].

The composition of the Dufour's gland secretions in the three *Dufourea* species reported here show basically large qualitative similarities. The differences between them lay partly in the occurrence of individual compounds, but mainly in the quantitative overall pattern of the constituents. The "ester pattern" in *D.(H.) dentriventris* and *D.(H.) inermis* points to a stronger chemical relationship between these two species in comparison to *D.(D.) minuta*, as is further supported by the co-occurrence of some of the other compounds. Morphological characteristics [11] and the new data presented here, as well as by Cane [10] and Wheeler *et al.* [8], support the position of the subfamily Dufoureinae as well being separated from the other two subfamilies of Halictidae. The chemical composition of the Dufour's gland volatile secretion from those *Dufourea* bees studied so far even indicate a possible phylogenetic relationship to melittid (aliphatic esters [13]) and andrenid (terpenoid esters [14]) bees as discussed by Cane [12]. In the palaearctic region there are four genera within the subfamily Dufoureinae [15]. Because of the phylogenetic position of this group analyses of cephalic secretions and of Dufour's glands constituents of the remaining 3 genera might well lead to a better understanding of relationships within the primitive bees.

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