Natural Waxes Investigated by Soft Ionization Mass Spectrometry*

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Field ionization (FI) and field desorption (FD) mass spectra of 35 aliphatic long-chain and branched long-chain compounds, representing the major six classes of constituents of natural waxes, are examined. In the FI mode the molecular ions are usually formed at comparatively low levels, while in FD such species are almost exclusively generated. In addition, some fast atom bombardment mass spectra, in the positive and negative ion mode, of selected lipids are recorded for comparison. In general, field ionization and field desorption techniques are found to be superior for mass spectrometric studies of low-polarity compounds.

Examination of several natural waxes, such as *Jojoba* wax, preen gland wax of goose, beeswax and epicuticular wax of Norway spruce, shows that field desorption mass spectrometry has a most promising role in the characterization of wax components. The intense molecular ions allow the identification of the constituents of complex lipid mixtures without the need for derivatization. Thus, for the first time, the carbon number distribution, especially of the high-mass wax constituents can be established for the natural waxes investigated. For identification of high-mass wax constituents the present results suggest that field desorption mass spectra could be best employed in conjunction with class and individual separation of constituents by chromatographic procedures.

The analysis of natural waxes is nowadays of major concern because of its importance in the problem of forest damage.

Introduction

The chemical investigation of the natural waxes has presented a formidable challenge to the chemist because of their complexity and the relative high molecular weight of many of their components [1]. Most waxes contain an appreciable proportion of high molecular weight esters and consequently it has been necessary, as a preliminary step, to hydrolyse them or submit them to methanolysis. The resultant unsaponifiable or neutral fraction and the acid fraction are then separated into their component classes by column or thin layer chromatography [2]. After derivatization, when necessary, the single class fractions are examined by gas chromatography (GC)-mass spectrometry (MS) to identify the constituents [3].

For complete characterization of the lipid constituents, however, it is necessary to establish their carbon number distribution in the unhydrolyzed wax and in recent years GC at high temperature has been successfully applied to esters of up to 68 carbons [4, 5]. In some of the waxes examined, a substantial part of the material was found to be insufficiently volatile for GC analysis [6]. This problem can be overcome by direct probe mass spectrometric analysis and recently compositional analysis of natural wax ester mixtures has been carried out by tandem mass spectrometry [7]. Furthermore non-volatile, high-mass plant constituents such as glycolipids can be analyzed by soft ionization MS in the mass range up to 4000 mass units and above [8−13].

For soft ionization modes it was previously shown that field desorption (FD)MS [14] with its unique ability to record almost exclusively the molecular ion group of thermally stable molecules of low volatility, had a valuable role to play in the analyses of *Carnauba* wax and the epicuticular waxes of the Australian Cape River Fan Palm [15] and several European co-

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nifers [16]. In particular, the carbon number distribution of high-molecular weight constituents of these waxes could be readily established.

Besides FD-MS, other soft ionization modes, such as field ionization (FI) [17] and fast atom bombardment (FAB) [18], are applied in the present work to the compositional, mass spectrometric analysis of natural waxes. The aim of this study is to explore the possibilities for the characterization of waxes by the examination of the soft ionization mass spectra of firstly, a number of several long-chain model compounds, secondly several representative pure wax components and thirdly, several natural waxes, such as *Jojoba* wax, preen gland wax of goose, yellow beeswax and epicuticular wax of Norway spruce.

Results and Discussion

Lipid components

FD-MS, FI-MS and partly FAB-MS have been used in this investigation of 35 representative compounds of the major six substance classes of the constituents of natural waxes. The molecular species and typical fragment ions of the positive ion mode with their relative abundances are listed in Table I for the hydrocarbons, monomeric esters, fatty acids, alcohols, ethers, ketones and glycerides examined and their mass spectrometric behaviour is discussed below.

Hydrocarbons

Under the experimental conditions employed all *n*-hydrocarbons analyzed show the molecular ion group at 0–20 mA emitter heating current (e.h.c.) during FD-MS and at a temperature below 250 °C during FI-MS (Table Ia). The only significant change with increase in e.h.c. is an increase of the prominent $[M-2]^{+\cdot}$ ion from 10% at 5 mA to 30% at 10 mA e.h.c.

Methyl-branched hydrocarbons show in addition dimeric molecular $[2M]^{+\cdot}$ ions and, at low abundance, multiply-charged molecular ions $[M]^{2+}$ as shown for squalene in Fig. 1. Its saturated analogue squalane also shows no fragmentation. However, at 5 mA e.h.c. the relative abundance of the $[M-2]^{+\cdot}$ ion is much increased to 38% compared to squalene (2%). Furthermore, in the FI mode at temperatures above 250 °C elimination of hydrogen molecules is observed with the saturated squalane giving a base peak $[M-2]^{+\cdot}$ at *m/z* 420. By contrast the highly unsaturated squalene shows mainly the formation of the molecular ion.
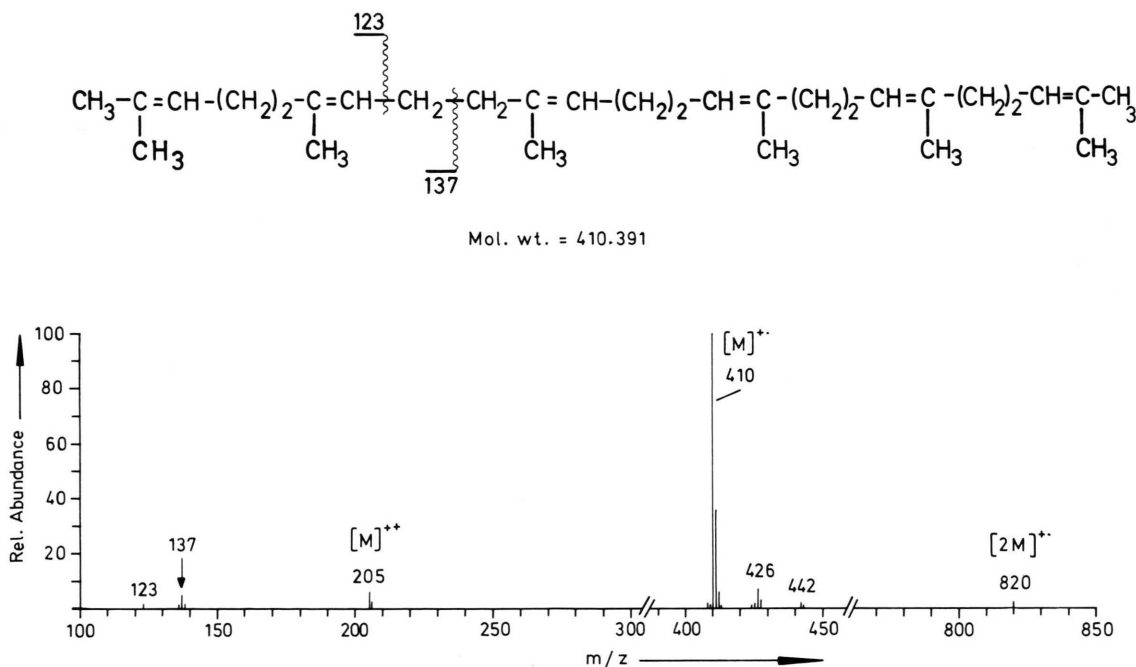

Fig. 1. FD mass spectrum of squalene.

Table I. Behaviour of long-chain lipids and wax constituents in FI- and FD-MS.

Compound	Elemental composition	m/z	Molecular species	Rel. abund. FD/FI(%)	m/z	Typical fragments	Rel. abund. FD/FI(%)
a) Hydrocarbons							
n-tricosane	$C_{23}H_{48}$	324	$[M]^{+\cdot}$	100/100			
n-tetracosane	$C_{24}H_{50}$	338	$[M]^{+\cdot}$	100/100			
n-pentacosane	$C_{25}H_{52}$	352	$[M]^{+\cdot}$				
n-hexacosane	$C_{26}H_{54}$	366	$[M]^{+\cdot}$	100/100			
n-heptacosane	$C_{27}H_{56}$	380	$[M]^{+\cdot}$	100/100			
n-octacosane	$C_{28}H_{58}$	394	$[M]^{+\cdot}$	100/100			
n-nonacosane	$C_{29}H_{60}$	408	$[M]^{+\cdot}$	100/100			
n-triacontane	$C_{30}H_{62}$	422	$[M]^{+\cdot}$	100/100			
n-hentriacontane	$C_{31}H_{64}$	436	$[M]^{+\cdot}$	100/100			
n-hexatriacontane	$C_{36}H_{74}$	506	$[M]^{+\cdot}$	100/100			
squalene	$C_{30}H_{50}$	205	$[M]^{2+}$	6/ 0			
		410	$[M]^{+\cdot}$	100/100			
		820	$[2M]^{+}$	2/ 0			
squalane	$C_{30}H_{62}$	420	$[M-2]^{+\cdot}$	38/100			
		422	$[M]^{+\cdot}$	100/ 80			
		844	$[2M]^{+}$	2			
b) Esters R−COO−R′							
dodecyl tetradecanoate	$C_{26}H_{52}O_2$	396	$[M]^{+\cdot}$	100/100	229	$(RCOOH_2)^{+}$	0/ 90
		793	$[2M+H]^{+}$	9/−			
hexadecyl hexa-decanoate	$C_{32}H_{64}O_2$	240.5	$[M+H]^{2+}$	1/ 0			
		480	$[M]^{+\cdot}$	100/100	257	$(RCOOH_2)^{+}$	0/ 30
		961	$[2M+H]^{+}$	12/−			
c) Acids							
n-tetracosanoic acid	$C_{24}H_{48}O_2$	185	$[M+2H]^{2+}$	5/ 0	60	acetic acid	0/ 2
		368	$[M]^{+\cdot}$	100/100	324	$[(M+H)-COOH]^{+}$	9/ 0
		737	$[2M+H]^{+}$	18/−	351	$[(M+H)-H_2O]^{+}$	8/ 1
n-octacosanoic acid	$C_{28}H_{56}O_2$	213	$[M+2H]^{2+}$	4/ 0	60	acetic acid	1/ 1
		424	$[M]^{+\cdot}$	100/100	380	$[(M+H)-COOH]^{+}$	7/ 1
		849	$[2M+H]^{+}$	15/ 0	407	$[(M+H)-H_2O]^{+}$	7/ 1
n-triacontanoic acid	$C_{30}H_{60}O_2$	227	$[M+2H]^{2+}$	3/ 0	60	acetic acid	0/ 2
		452	$[M]^{+\cdot}$	100/100	408	$[(M+H)-COOH]^{+}$	6/ 0
					435	$[(M+H)-H_2O]^{+}$	9/ 0
C_{27}-phthienoic acid	$C_{27}H_{52}O_2$	408	$[M]^{+\cdot}$	100/100	363	$[M-COOH]^{+\cdot}$	11/ 0
					391	$[(M+H)-H_2O]^{+}$	7/ 1
24-methyl-hexa-cosanoic acid	$C_{27}H_{54}O_2$	410	$[M]^{+\cdot}$	100/100	365	$[M-COOH]^{+\cdot}$	5/ 0
					393	$[(M+H)-H_2O]^{+}$	2/ 1
oleic acid	$C_{18}H_{32}O_2$	282	$[M]^{+\cdot}$	100/100	264	$[M-H_2O]^{+\cdot}$	1/ 50
		565	$[2M+H]^{+}$	7/ 0	238	$[(M+H)-COOH]^{+}$	4/ 0
		847	$[3M+H]^{+}$	5/ 0			
linoleic acid	$C_{18}H_{32}O_2$	280	$[M]^{+\cdot}$	100/100	236	$[(M+H)-COOH]^{+}$	4/ 0
		561	$[2M+H]^{+}$	7/ 0			
		841	$[3M+H]^{+}$	6/ 0			
ω-hydroxy-hen-cosanoic acid	$C_{21}H_{42}O_3$	365	$[M+Na]^{+}$	65/ 0	306	$[M-2H_2O]^{+\cdot}$	0/ 8
		343	$[M+H]^{+}$	100/ 12	325	$[(M+H)-H_2O]^{+}$	4/100
1,24-tetracosan-dioic acid	$C_{24}H_{64}O_4$	421	$[M+Na]^{+}$	30/ 0	191	$[(M+2H)-H_2O]^{2+}$	0/ 20
		399	$[M+H]^{+}$	100/ 10	354	$[(M+H)-COOH]^{+\cdot}$	10/ 0
		200	$[M+2H]^{2+}$	0/ 5	362	$[M-2H_2O]^{+}$	0/ 16
					381	$[(M+H)-H_2O]^{+}$	18/100
d) Alcohols							
n-dotriacontanol	$C_{32}H_{66}O$	466	$[M]^{+\cdot}$	100/ 6	448	$[M-H_2O]^{+}$	0/100
					449	$[(M+H)-H_2O]^{+}$	20/ 60
1,28-octacosandiol	$C_{28}H_{58}O_2$	427	$[M+H]^{+}$	100/100	409	$[(M+H)-H_2O]^{+}$	5/ 2
1,2-hexadecandiol	$C_{16}H_{34}O_2$	259	$[M+H]^{+}$	30/ 1	61	$[HOCH_2COH]^{+\cdot}$	32/ 28
		517	$[2M+H]^{+}$	20/ 0	227	$[H(CH_2)_{14}CHOH]^{+\cdot}$	100/100
		776	$[3M+H]^{+}$	10/ 0	241	$[(M+H)-H_2O]^{+}$	22/ 1
10-nonacosanol	$C_{29}H_{60}O$	425	$[M+H]^{+}$	2/ 1	157	$[H(CH_2)_9CHOH]^{+\cdot}$	41/100
		849	$[2M+H]^{+}$	4/ 0	297	$[H(CH_2)_{19}CHOH]^{+\cdot}$	43/ 96
		1274	$[3M+H]^{+}$	1/ 0	406	$[M-H_2O]^{+}$	100/ 60

Compound	Elemental composition	*m/z*	Molecular species	Rel. abund. FD/FI(%)	*m/z* Typical fragments	Rel. abund. FD/FI(%)
e) Ethers R−O−R						
dihexadecylether	$C_{32}H_{66}O$	467	$[M+H]^+$	100/100	225 $[R]^{+\cdot}$	1/ 17
					255 $[ROCH_2]^{+\cdot}$	1/ 20
dioctadecylether	$C_{36}H_{74}O$	523	$[M+H]^+$	100/100	253 $[R]^{+\cdot}$	4/ 10
					283 $[ROCH_2]^{+\cdot}$	2/ 34
f) Ketones R−CO−R						
13-pentacosanone	$C_{25}H_{50}O$	183	$[M]^{2+}$	3/ 9	197 $[RCO]^{+\cdot}$	8/ 75
		366	$[M]^{+\cdot}$	100/100		
		733	$[2M+H]^+$	1/−		
16-hentriacontanone	$C_{31}H_{62}O$	225	$[M]^{2+}$	4/−	239 $[RCO]^{+\cdot}$	5/−
		450	$[M]^{+\cdot}$	100/−		
22-tritetra-contanone	$C_{43}H_{86}O$	309	$[M]^{2+\cdot}$	3/ 2	323 $[RCO]^{+\cdot}$	26/ 20
		618	$[M]^{+\cdot}$	100/100		
g) Glycerides R = $C_{15}H_{31}$						
1-monopalmitin	$C_{19}H_{38}O_4$	331	$[M+H]^+$	100/ 14	211 $[R]^{+\cdot}$	1/ 0
					239 $[RCO]^{+\cdot}$	6/ 18
					256 $[RCOOH]^{+\cdot}$	2/ 48
					299 $[M−H_2COH]^{+\cdot}$	37/100
					313 $[(M+H)−H_2O]^+$	4/ 1
1,2-dipalmitin	$C_{35}H_{68}O_5$	284	$[M+H]^{2+}$	3/ 0	239 $[RCO]^{+\cdot}$	21/ 6
		569	$[M+H]^+$	100/ 1	256 $[RCOOH]^{+\cdot}$	5/ 40
					313 $[(M+H)−RCOOH]^+$	6/ 4
					537 $[M−H_2COH]^{+\cdot}$	8/ 0
					550 $[M−H_2O]^{+\cdot}$	1/100
1,3-dipalmitin	$C_{35}H_{68}O_5$	569	$[M+H]^+$	100/ 1	239 $[RCO]^{+\cdot}$	31/ 4
					256 $[RCOOH_2]^+$	5/ 8
					313 $[(M+H)−RCOOH]^+$	7/ 4
					550 $[(M)−H_2O]^{+\cdot}$	9/100
tripalmitin	$C_{51}H_{98}O_6$	806	$[M]^{+\cdot}$	100/ 16	239 $[RCO]^{+\cdot}$	0/ 31
					551 $[(M+H)−RCOOH]^+$	1/100
					789 $[(M+H)−H_2O]^+$	0/ 4

A mixture of paraffinic hydrocarbons was isolated by column chromatography in the course of an investigation of the cuticle wax of "Granny Smith" apples [19]. The FD spectrum of this material shows only molecular ion groups of *n*-hydrocarbons with $[M]^{+\cdot}$ signals at *m/z* 324 (C_{23}, 3%), 338 (C_{24}, 2%), 352 (C_{25}, 7%), 366 (C_{26}, 2%), 380 (C_{27}, 28%), 394 (C_{28}, 6%), 408 (C_{29}, 100%), 422 (C_{30}, 1%) and 436 (C_{31}, 2%). The corresponding samples from pear, plum, Scotch pine and Norway spruce epicuticular waxes show a similar chain length distribution. It is noteworthy that the most abundant chain length always consists of 29 carbon atoms, as measured from the relative abundance of the molecular ions in each of the above samples.

Esters

Both of the pure synthetic, straight-chain esters analyzed (Table I b) produce no detectable fragmen-

tation in FD-MS. Low level ions are attributed to $[M]^{2+}$ and $[2M+H]^+$ molecular species. The base peaks in the corresponding FI mass spectra are also due to the molecular ions, but fragmentation occurs and produces ions which represent protonated *n*-fatty acids derived from the esters.

Acids

Higher *n*-alkanoic acids are especially common in plant waxes. Besides the dominant molecular ion, their FD and in particular FI spectra show some fragmentation caused by the loss of water and the loss of the carboxyl group from the protonated molecule. The FI spectra also show low level ions at *m/z* 60 indicating the formation of acetic acid (Table I c).

An example of a single methyl-branched acid, with the branching remote from the carboxyl group, is 24-methylhexacosanoic acid isolated from wool wax [20]. Its spectrum shows a similar low level fragmen-

tation pattern as with the unbranched acids discussed above.

Multi-branched acids with branches in the 2,4,6 and 2,4,6,8 positions commonly occur as esters in the waxes of bird preen glands and of certain bacterial waxes [1]. 2,4,6-Trimethyltetracos-2-enoic (C_{27}-phthienoic) acid, isolated from the lipids of *Mycobacterium tuberculosis* [21] was analyzed as a representative compound. The fragmentation pattern with its [M-17]$^{+·}$ and [M-45]$^{+·}$ ions is different from that of the straight-chain acids. A specimen of C_{32}-mycocerosic (2,4,6,8-tetramethyloctacosanoic) acid from tubercle wax was prepared and gives a spectrum with the molecular ion as base peak and a similar fragmentation level to that of C_{27}-phthienoic acid. In both samples the fragmentation could be clearly differentiated from ions representing the molecular ion groups of impurities of related acids.

Unsaturated fatty acids form dimers and trimers at temperatures between 100 and 300 °C [22, 23]. However, the rapid transfer to the gas phase during FI and the extremely fast desorption from the FD-emitter occur already at temperatures up to 100 °C and hence only mass spectrometric formation of dimeric and trimeric, protonated FD cluster ions are observed with very low relative abundances. Again the base peak is occupied by the molecular ions of the unsaturated acids investigated.

FD-MS of ω-hydroxyhencosanoic acid yields the protonated molecule as the most abundant ion, whereas in the FI spectrum the base peak is the [M-17]$^{+·}$ ion. Thus, fragmentation occurs only during FI-MS caused by the elimination of one and two water molecules (Table I c). Similar behaviour is found for the 1,24-tetracosandioic acid. Besides the dominant [M+H]$^{+}$ ion, however, the less abundant [M-45]$^{+·}$ ion is produced during FDMS. Noteworthy is the strong [M+Na]$^{+}$ ion of the hydroxy acid and the α−ω acid. The FI spectra of these both acids show low level [M+2H]$^{2+}$ ions besides the prominent [M-17]$^{+·}$ ion. In the FD spectrum of the corresponding acetylated and methylated derivative of the above-hydroxy acid, AcO(CH$_2$)$_{20}$COOMe, the [M+H]$^{+}$ ion is still the base peak with some loss of acetic acid.

No significant, positive and negative ions of *n*-triacontanoic acid were obtained by FAB, whereas [M+H]$^{+}$ and [(M+glyc)+H]$^{+}$ ions in the positive ion mode and [M−H]$^{−}$ and [(M+glyc)−H]$^{−}$ ions in the negative ion mode were detected during the analysis of 2,3-dimethylhencosanoic acid, 1,22-docosandioc acid and a mixture of C_{12}-, C_{14}- and C_{16}-ω-hydroxy acids. The ω-hydroxy acids also produce abundant [(M+H)−H$_2$O]$^{+}$ ions.

Alcohols

The higher alkan-1-ols are widespread particularly in plant waxes. Dotriacontan-1-ol, the principal alcohol of *Carnauba* wax [24] was chosen as representative compound. The most abundant ion in the FD mass spectrum (Table I d) is the molecular ion followed by the [M-17]$^{+·}$ ion while, in contrast, the most abundant ion in the FI mass spectrum is the [M-18]$^{+}$ ion, indicating that thermal elimination of water and the formation of a terminally unsaturated hydrocarbon is the dominant mechanism.

Alkane α−ω diols show different behaviour as seen for 1,28-octacosandiol. During FI and FD, protonation of the molecule gives rise to the base peak at *m/z* 427, while only a slight loss of water is observed. For FAB, no characteristic positive or negative ions were observed for 1-dotriacontanol and 1,28-octacosandiol.

Another interesting result is found in the analysis of compounds with secondary hydroxy-groups. The EI and FI spectra of 10-nonacosanol in Fig. 2a and Fig. 2b respectively, show the same major fragmentation by α-cleavage [25]. The FD spectrum of this secondary alcohol has the same fragment ions as the FI-spectrum, although, during FD-MS the [M-18]$^{+·}$ ion is the prominent ion while during FI-MS the two fragment ions at *m/z* 157 and at *m/z* 297 are the most abundant species. Analogous α-cleavage also occurs during FI-MS and FD-MS of 1,2-hexadecandiol leading to the abundant fragment ions at *m/z* 61, 227 and 241. In FD, both compounds also form protonated cluster ions.

Ethers

Although not wax constituents [1], it was of interest to record the FI and FD behaviour of compounds of comparable chain length as an indication of the relative stability of the ether-linkage in relation to that of the centrally situated ester-linkage discussed above. During FI-MS around 400 °C and FD-MS above 15 mA e.h.c. the symmetrical, synthetic ether compounds investigated show the same fragmentation pattern leading to [R]$^{+·}$ and [ROCH$_2$]$^{+·}$ ions (Table I e). In general, the relative abundances of
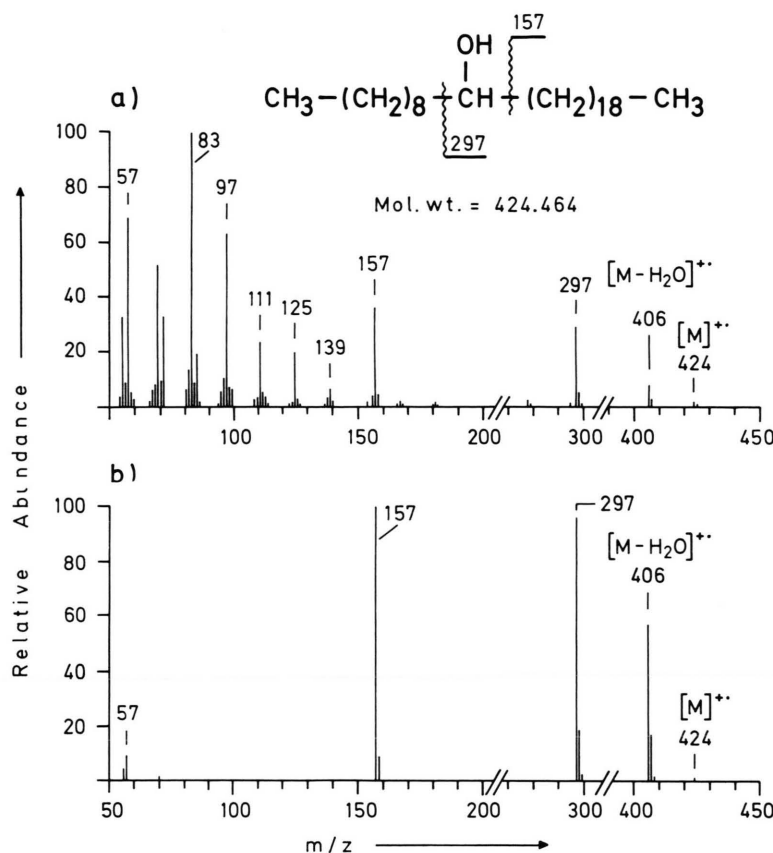
Fig. 2. EI (a) and FI (b) mass spectra of 10-nonacosanol.

these fragment ions are higher during FI than during FD. Furthermore, protonation of the molecules leads to the prominent mass signals in the FI and the FD spectra.

Ketones

The molecular ions of the three symmetrical, synthetic ketones were the prominent signals during FI-MS and FD-MS (Table I f). However, in the FI mass spectra a very abundant $[RCO]^{+\cdot}$ fragment ion can be seen in addition to low level $[M]^{2+}$ doubly-charged molecular ions. Furthermore, the FD molecular ion group shows evidence of considerable hydrogen transfer which is also observed for all other compounds of higher polarity investigated.

Glycerides

The FD mass spectra of mono- and dipalmitin, which possess one and two hydroxyl groups respec-

tively, show the $[M+H]^+$ ion as the prominent molecular species, whereas the $[M]^{+\cdot}$ ion is the most abundant ion for tripalmitin with no hydroxyl group (Table I g). Some thermal fragmentation also occurs, leading to the common fragment ions $[C_{15}H_{31}CO]^+$ at m/z 239 and $[C_{15}H_{31}COOH]^{+\cdot}$ at m/z 256 (palmitic acid). Moreover decarboxylation and the loss of water from the protonated molecule can be observed leading to the fragment ions $[M-44]^+$ and $[M-17]^+$. Principally, FI-MS leads to the same fragmentation pattern for tripalmitin, although, in contrast to the FD mass spectrum, the fragment ions are more abundant due to enhanced thermal degradation. With the exception of monopalmitin with 32% relative abundance, the molecular ions appear only at very low levels between 2% and 3%. The most abundant FI signals are observed for the $[M-31]^+$ ion for mono-glycerides, the $[M-H_2O]^{+\cdot}$ ion for diglycerides and the $[(M+H)-RCOOH]^+$ ion for triglycerides. In general, the FI spectra of triglycerides are similar to

those obtained on chemical ionization [8]. For FAB, only $[C_{15}H_{31}COO]^-$ ions were recorded with high intensities.

Summarizing the above results it becomes clear that, except for the secondary hydroxy compounds, FD- and FIMS produce almost exclusively molecular ion species and reduce the mass spectrometric fragmentation of the compounds investigated. Above the best anode temperature in FD and the best probe temperature in FI, however, fragmentation can be thermally induced [14]. Usually protonation of the molecules occurs when nucleophilic functions such as hydroxyl or carboxyl groups are available. A higher degree of fragmentation is observed during FI-MS, and a general pattern consisting of $[M-17]^+$, $[M-18]^{+\cdot}$, $[M-44]^+$ and $[M-45]^+$ ions is typical. From the results presented in Table I and the comparison with EIMS (Fig. 2) it is clear that the degree of FD/FI-fragmentation is not strong enough to seriously interfere with the detection of compounds as their molecular ions or protonated molecules when mixtures of homologues derived from waxes are being examined. With the exception of acids or other polar derivatives, FAB seems to be unsuitable for the investigation of aliphatic, long-chain compounds and hence cannot be used for the bulk analysis of natural waxes.

Natural waxes

In order to establish whether FI/FD-MS is suitable for the compositional analysis of complex organic mixtures, well-known natural waxes have been examined.

Jojoba

The FD mass spectrum of wax isolated from *Simmondsia chinensis (Jojoba)* is shown in Fig. 3. According to Miwa [26], *Jojoba* wax consists mainly of $C_{36}-C_{46}$ monomeric esters formed by unsaturated $C_{18:1}$, $C_{20:1}$, $C_{22:1}$ and $C_{24:1}$ *n*-fatty acids and alcohols. Actually the molecular ions of these unsaturated esters can be seen at m/z 532 (C_{36}), 560 (C_{38}), 588 (C_{40}), 616 (C_{42}) and 644 (C_{44}). The molecular ion of a monounsaturated C_{42}-ester is seen at m/z 618 and the base peak of the FD mass spectra is due to docosenyl eicosenoate [7, 26]. No fragmentation is observed, but another homologues series of ions appears at m/z 1122, 1150, 1178, 1206, 1234, 1262 and 1290. Although esters can form $[2M+H]^+$ cluster ions (Table I b), it is unlikely that these very abundant mass signals are due to such dimer molecular species. Hence *Jojoba* wax also contains a fraction of high-mass constituents similarily as reported for *Carnauba* wax [15] and the epicuticular wax of conifer needles [16].
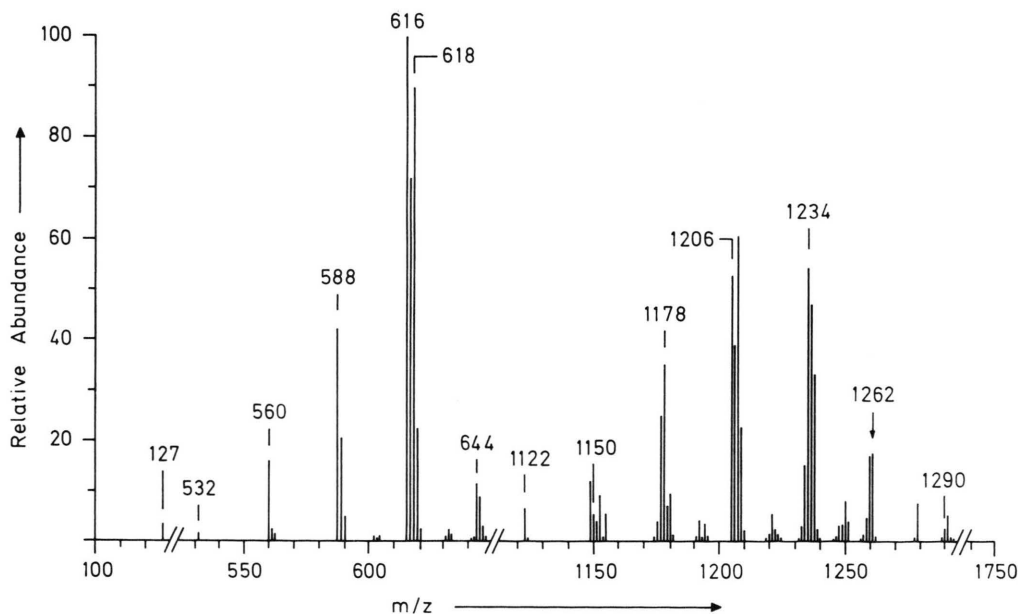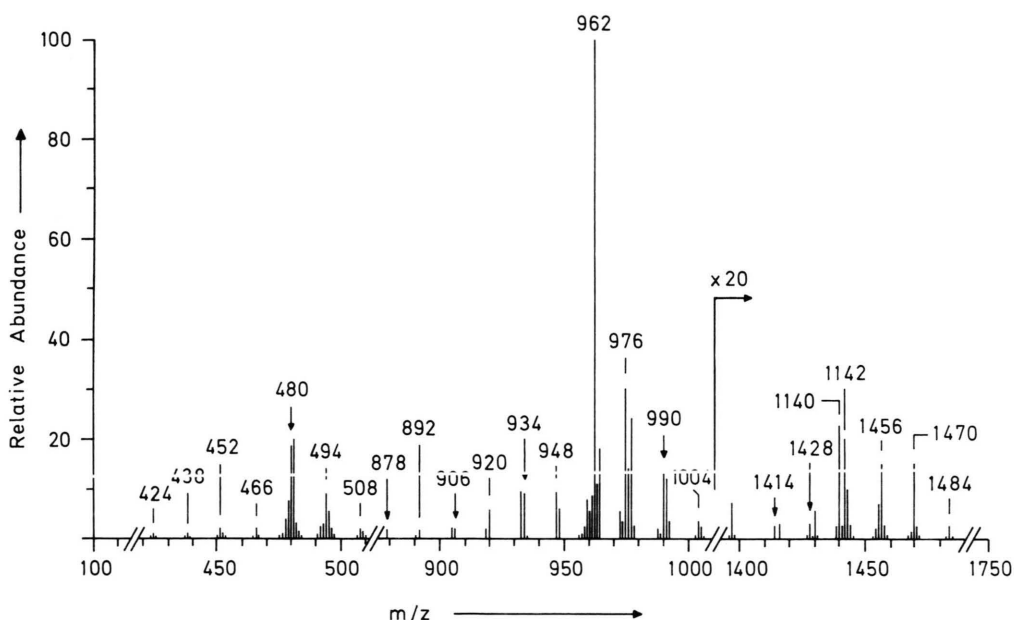

Fig. 3. FD mass spectrum of *Jojoba* wax.

Fig. 4. FD mass spectrum of preen gland wax of goose.

Goose

In Fig. 4 the FD mass spectrum of the preen gland wax of the goose is shown. This wax consists mainly of monomeric esters formed by 2,4,6,8-tetramethyl-decanoic acid and its C_{15}-homologue esterified with C_{16}, C_{18} and C_{20} n-alcohols. To a minor degree these alcohols are also esterified with C_{16}, C_{18} and $C_{18:1}$ n-fatty acids [27]. Therefore the homologous group of molecular ions centered around m/z 480 represents the following monomeric esters: C_{32} at m/z 424, C_{33} at m/z 438, C_{34} at m/z 452, C_{35} at m/z 466, C_{36} at m/z 480, C_{37} at m/z 494 and C_{38} at m/z 508. The major signal at m/z 480 is due to octadecyl ester of 2,4,6,8-tetra-methyldecanoic acid. Furthermore, the most abundant homologous group of mass signals appears at m/z 878, 892, 906, 920, 934, 948, 962, 976, 990 and 1004. Another minor homologous series of molecular ions can be seen at m/z 1414, 1428, 1442, 1456, 1470 and 1484. While the latter series could be due to molecular ions of trimers, the other homologous group of mass signals can be explained by molecular ions of dimers.

Thus it appears that new, as yet unknown high-mass constituents have been detected in the *Jojoba* wax and the preen gland wax of goose. Although no elemental composition or structure is known, it is already possible to give the molecular weight distribution of these constituents.

Bee

The same is true for the yellow beeswax from *Apis mellifera*. Its FD mass spectrum is shown in Fig. 5. Beeswax contains 14% $C_{23}-C_{31}$ hydrocarbons, 35% $C_{38}-C_{52}$ monoesters, 14% $C_{56}-C_{66}$ diesters, 3% triesters, 4% $C_{24}-C_{34}$ hydroxymonoesters, 8% hydroxypolyesters, 12% $C_{14}-C_{36}$ free acids, 1% $C_{16}-C_{20}$ acid monoesters, 2% acid polyesters and 7% unidentified material [28, 29]. In Fig. 5 the molecular ions of the paraffins are seen at m/z 324 (C_{23}), 352 (C_{25}), 380 (C_{27}), 408 (C_{29}) and 436 (C_{31}) with heptacosane as their major component. The typical $[M-18]^{+ \cdot}$ signals of free alcohols are observed at m/z 378 (C_{27}), 406 (C_{29}), 434 (C_{31}), 462 (C_{33}) and 490 (C_{35}). The major free fatty acids can be seen with the molecular ions for palmitic acid at m/z 256 and n-tetracosanoic acid at m/z 368 [30]. The homologous series of molecular ions centered around m/z 676 represents monomeric esters with the following carbon number distribution: C_{40} at m/z 592, C_{42} at m/z 620, C_{44} at m/z 648, C_{46} at m/z 676, C_{48} at m/z 704, C_{50} at m/z 732 and C_{52} at m/z 760. In contrast to the minor acid monoesters, the $[M-18]^{+ \cdot}$ ions of the hydroxy-
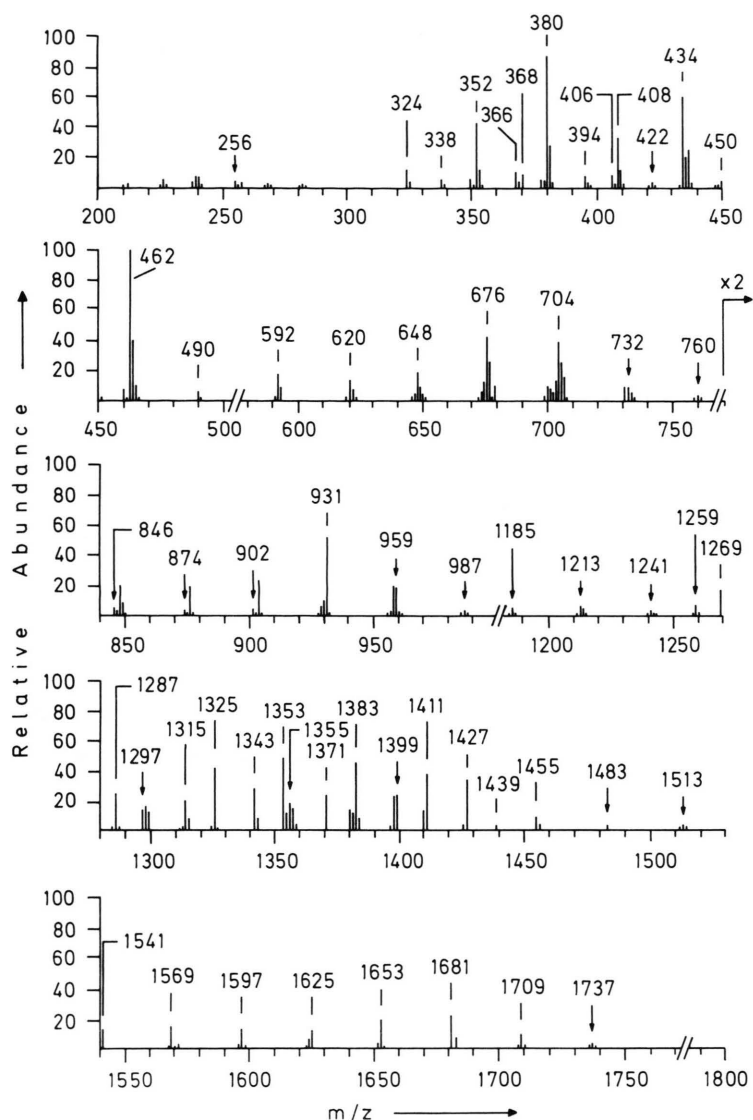
Fig. 5. FD mass spectrum of yellow beeswax.

esters can be seen at m/z 338 (C_{22}), 366 (C_{24}), 394 (C_{26}), 422 (C_{28}) and 450 (C_{28}). Molecular ions of diesters occupy the mass signals at m/z 846 (C_{56}), 874 (C_{58}), 902 (C_{60}), 931 (C_{62}), 959 (C_{64}) and 987 (C_{66}). The neutral triesters of the beeswax also only occur as molecular ions at m/z 1185 (C_{78}), 1213 (C_{80}), 1241 (C_{82}), 1269 (C_{84}), 1297 (C_{86}), 1325 (C_{88}), 1353 (C_{90}) and 1381 (C_{92}). A similar homologous group of molecular ions is seen for the monoacid triesters at

m/z 1355 (C_{88}), 1383 (C_{90}), 1411 (C_{92}) and 1439 (C_{94}). Hydroxy-monoacid triesters can be seen as molecular ions at m/z 1259 (C_{80}), 1287 (C_{82}), 1315 (C_{84}), 1343 (C_{86}), 1371 (C_{88}), 1399 (C_{90}), 1427 (C_{92}), 1455 (C_{94}), 1483 (C_{96}) and 1511 (C_{98}). Furthermore the same type of tetraester composed of hydroxy-acids is detected at m/z 1513 (C_{96}), 1541 (C_{98}), 1569 (C_{100}), 1597 (C_{102}), 1625 (C_{104}), 1653 (C_{106}), 1681 (C_{108}), 1709 (C_{110}) and 1737 (C_{112}).

Table II. Ester composition of yellow beeswax derived from the FD mass spectrum in Fig. 5.

Substance class	Components	Major compound	Mol. wt.
Hydroxy-monoester	$C_{22}-C_{30}$	$C_{24}H_{48}O_3$	384.360
Monoester	$C_{40}-C_{52}$	$C_{46}H_{92}O_2$	676.710
Diesters	$C_{56}-C_{66}$	$C_{62}H_{122}O_4$	930.934
Triesters	$C_{78}-C_{92}$	$C_{90}H_{176}O_6$	1353.347
Monoacid triesters	$C_{88}-C_{94}$	$C_{90}H_{174}O_8$	1383.321
Hydroxy-monoacid triesters	$C_{80}-C_{98}$	$C_{92}H_{178}O_9$	1427.347
Hydroxy-monoacid tetraesters	$C_{96}-C_{112}$	$C_{108}H_{208}O_{11}$	1681.572

The above results are summarized in Table II and the major compounds of the respective ester classes are listed. According to Downing et al. [30], the detected polyesters are probably composed of esterified hydroxy-C_{16}-acids, C_{16}- and C_{24}-acids, C_{28}-diols and $C_{24}-C_{32}$ alcohols. The FD mass spectrum of beeswax demonstrates that there is no interference between the various molecular species. The same is true for FI-MS, as the FI mass spectrum of beeswax is very similar to the FD spectrum. The only difference is due to the very abundant FI mass signal of protonated palmitic acid at m/z 257 which is derived from the thermal degradation of the esters and polyesters.

Spruce

The FI mass spectrum of epicuticular wax isolated from Norway spruce *(Picea abies)* by short-term extraction with chloroform is shown in Fig. 6. Recently the FD and FAB mass spectrum of this wax have
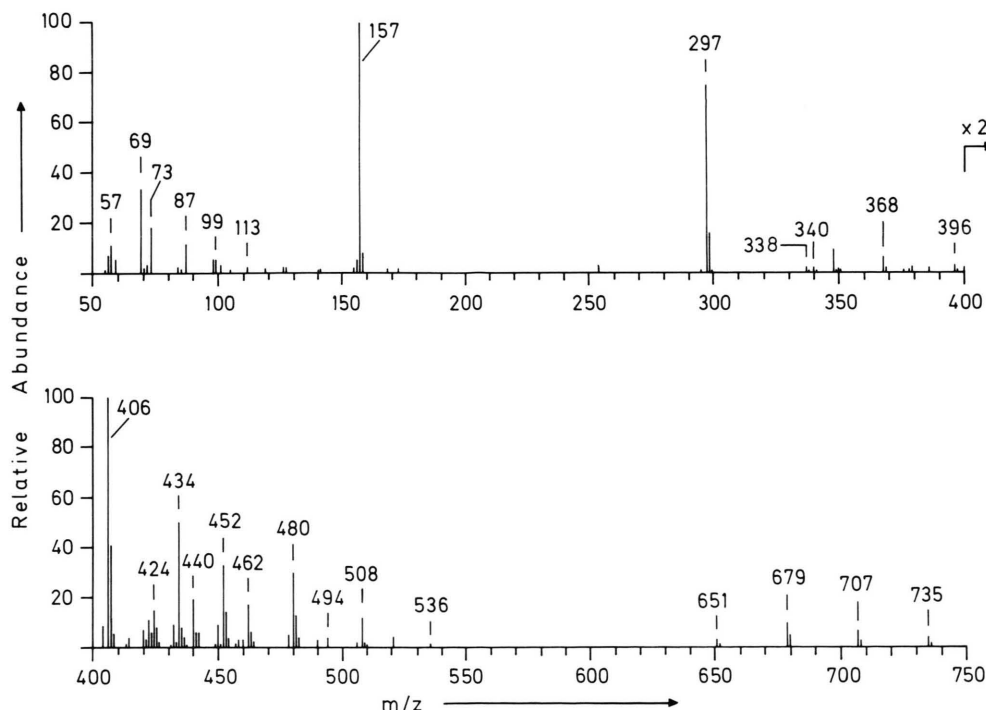

Fig. 6. FI mass spectrum of epicuticular wax of Norway spruce.

been discussed [16]. It could be shown that it consists mainly of estolides (polyesters) up to a molecular weight of 1753. The FD as well as the FI spectrum in Fig. 6 are dominated by typical fragment signals of 10-nonacosanol at m/z 157, 297 and 406 (Fig. 2). This compound is very common in coniferous, epicuticular waxes [31] and its occurrence could be also confirmed by GC-EI/FIMS. Furthermore, the following compounds which have also been identified by this combined technique can be seen as molecular ions in the FI mass spectrum: tetracosane at m/z 338, *n*-docosanoic acid at m/z 340, pentacosane at m/z 352, *n*-tetracosanoic acid at m/z 368, heptacosane at m/z 380, *n*-hexacosanoic acid at m/z 396, nonacosane at m/z 408, 10-nonacosanol at m/z 424, 24-methylene-9,19,-cyclolanostan-3-ol at m/z 440, octyl docosanoate at m/z 452 and octyl tetracosanoate at m/z 480. In addition, the mass signals at m/z 508 and at m/z 536 presumably indicate a C_{34}- and a C_{36}-monoester. The homologous group of ions at m/z 406, 434 and 462, already known from the beeswax is due to $[M-18]^{+\cdot}$ ions of C_{29}, C_{31} and C_{33} alcohols. The estolidic high-mass constituents of conifer waxes are composed mainly of C_{12}-, C_{14}- and C_{16}-ω-hydroxy acids, *n*-fatty acids and *n*-alcohols [32]. Hence the protonated molecular ion of the diesters can be seen at m/z 651 (C_{46}), 679 (C_{48}), 707 (C_{50}) and 735 (C_{52}).

The above examples show that FD and FI mass spectra of bulk samples can be used to characterize natural waxes and give information about the carbon-number distribution of its constituents, especially the high-mass compounds. Although polar compounds are preferred for FI/FD-MS it was also shown that the unpolar, long-chain polyesters can be detected without interference from other constituents.

Materials and Methods

Lipids and waxes

The pure standards and lipid model compounds and waxes were for the most part chosen from the collection of one of us (K. E. M.). The individual compounds were of high purity and were produced either synthetically or isolated from various waxes. The beeswax was provided by W. Kopiske, Institut Fresenius (F.R.G.) and 10-nonacosanol was supplied by Dr. R. A. Franich, Forest Research Institute Rotorura (N.Z.). The extraction of the epicuticular

wax of Norway spruce has been described recently in detail [15].

Instrumentation

FD, FI and FAB spectra were taken with a Finnigan MAT 731 mass spectrometer equipped with a combined EI/FI/FD/FAB ion source. For FI-MS, about 100 μg sample were transferred into a commercially available aluminium crucible which is mounted orthogonal to the FI emitter. The ion source was kept at a pressure below 10^{-7} Pa and at a temperature of 250 °C. The samples were heated linearly from 50 to 500 °C at a rate of 1 °C/s. Between the magnetic scans the emitter was flashed-heated to 1500 °C in order to avoid condensation. In general, forty spectra of positive or negative ions were recorded electrically. The FI signals of all these spectra were integrated and plotted using the Finnigan MAT Spectro-System SS 200. For FD-MS, 10 μl of the sample dissolved in chloroform was applied to the emitter by the syringe technique, the concentration was approximately 1 μg/μL. The ion source was at a pressure below 10^{-8} Pa and at a temperature of about 50 °C. The emitter was heated directly in steps of 1 mA per scan up to 30 mA. Again the ions produced during all scans were integrated and the summed spectra evaluated. For FAB, the copper target was mounted on the pushrod introduction system. A saddle field ion gun and power supply (Ion Tech Ltd., Teddington, England) modified by Finnigan MAT (Bremen, F.R.G.) was used for neutral beam production. Xenon of a purity of better than 99.99 vol% (Messer Griesheim, Düsseldorf, F.R.G.). The Xe^+ current output on average was about 0.05 mA. The samples were supplied in chloroform/methanol solution (1:1) and mixed with glycerol (glyc) under optical control with a stereomicroscope. The recording of the FAB ions was as described for the FI and the FD mode.

In addition, the unhydrolyzed, chloroform-soluble epicuticular wax of *Picea abies* was analyzed by GC-EI/FIMS using a Varian gas chromatograph model 3700 with a 10 m capillary column DB 1, helium as carrier gas and the Finnigan MAT 212 mass spectrometer. The GC experimental conditions were: injector temperature 310 °C; column temperature program 180 °C, heating rate 23 °C/min, 250 °C, heating rate 3 °C/min, 320 °C, 15 min; split 20 ml/min. The mass spectra were recorded with a scanning speed of

1.1 s/mass decade in both ionization modes. The experimental conditions for FI-MS were: voltage and emitter potential 3 kV, counter electrode −8 kV and mass range *m/z* 100−650; for EI-MS: accelerating voltage 3 kV, ionization energy 70 eV and mass range *m/z* 40−500.

Conclusion

Although the described selection of compounds does not represent all classes of lipid constituents occurring in waxes, it is sufficiently broad to indicate that FI/FD-MS can play a very useful role in the chemical investigation of natural wax composition. In particular the ability of FD to obtain spectra on materials of low volatility provides much information about high-mass constituents. It is therefore readily applicable to the very high molecular weight compounds present in waxes which are beyond the capability of chromatographic techniques. An example is the appreciable proportion of plant waxes which are often not volatile enough for GC analysis, and are considered to be composed of diesters or hydroxyacid polymers with estolidic bonds. Such high molecular weight material could make an appreciable contribution towards the physical properties of the wax.

It is suggested that FD-MS could profitably be used in investigations of wax composition in combination with other spectroscopic techniques (*e.g.* IR) and fractionation by some form of chromatography. It is visualized that such an investigation of an ester containing wax would be conducted in several stages.

1) A qualitative examination by FD-MS of the wax before submission it to any further change. This could take the form of scanning over a wide mass range at several levels of e.h.c. The more readily emitted components are more evident in early scans. Some degree of class separation is thus achieved and this is, in itself, of diagnostic value.

2) The wax is fractioned by chromatographic procedures. Hydrocarbons, esters, free *n*-alcohols and acids should be separable in this stage. FD spectra and IR spectra are recorded on all fractions.

3) The ester fractions from 2 are saponified and the alcoholic and acid fractions resolved by chromatography into mixtures of the component classes. Again IR spectra indicate the class component and the FD spectra establish the carbon chain length distribution of the homologous mixtures.

By the use of FDMS in this manner a great deal of information regarding wax composition can be amassed very easily. No practical problems have occurred with the compounds examined except that the more polar acids, hydroxy and dibases, could be methylated to avoid the formation of cationized molecular ions, $[M+Na]^+$, $[M+K]^+$ which could complicate the spectra of mixtures of such homologues. An alternative might offer direct FAB-MS.

It has been observed that when FD spectra are recorded of an homologous series over a relatively narrow range (100−150 masses) the distribution of carbon numbers as measured from the major ion of each compound $[M]^{+\cdot}$ or $[M+H]^+$, is in fair agreement with the results obtained by GC where this has been available. Although not specifically looked for in all the spectra taken, it appears from the evidence of this and other observations that the formation of a doubly charged molecular ion group in low abundance may be a general feature of many classes of long carbon chain compounds. However, when a mixture containing several classes of components is scanned, the FD spectrum must of necessities be qualitative as the emission of different classes of compound can vary widely.

Although FD-MS used in the manner proposed gives clear evidence of the molecular weights of wax components, as far as structural details are concerned, it is not as informative as GC-MS when this technique is applicable. For instance using FD-MS, it is difficult to differentiate between straight and branched-chain compounds of the same molecular weight whereas GC-MS can provide definitive evidence from GC retention values and EI mass spectra. However, GC-MS is limited in scope by the low volatility of the higher molecular weight components as well as by practical problems such as column phase bleed and others created by maintaining the interface at high temperatures. FD-MS could then well augment the results of GC-MS but it is seen to have a more universal role in obtaining, along the lines suggested earlier, a more complete insight into the composition of waxes than has hitherto been possible.

The present work indicates that under FD conditions no cleavage of the carbon chain takes place in straight or methyl-branched hydrocarbons, and fragmentation associated with ether, carbonyl and ester groups in the chain is minimal with fragment ions present at about 1% abundance of the molecular ion. Although this information has been based mainly on

compounds 30–33 carbons in length, the present FDMS examination of natural mixtures, especially of esters, suggests that this observation applies at least to esters and ketones over a wide range of carbon numbers.

Nowadays the analysis of natural waxes is again of major concern, because recent studies indicate that the epicuticular wax layer of plants could have a relevant function concerning the phytotoxic impact of air pollutants [15, 33]. Thus waxes could play an important role in the present forest damage and it seems that especially soft ionization MS is very suitable to investigate the complex structure and chemical changes on and in tree leaves [34, 35].

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