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ANALYSIS OF COMMERCIAL WAXES USING CAPILLARY SUPERCRITICAL FLUID CHROMATOGRAPHY–MASS SPECTROMETRY*

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SUMMARY

Supercritical fluid chromatography–mass spectrometric analyses of several commercial waxes including beeswax, bayberry wax, microcrystalline wax, Fischer-Tropsch wax, and montan wax have been performed using direct introduction of the capillary column effluent into the ion source of a Hewlett-Packard 5985B mass spectrometer. The individual wax components: high-molecular-weight esters, di- and triglycerides, carboxylic acids, alcohols, and alkanes, showed good chromatographic peak shape without requiring derivatization. Methane chemical ionization mass spectrometry yielded pseudomolecular ions and characteristic fragmentation patterns which allowed individual wax components ranging in molecular weight from *ca.* 300 to 1000 a.m.u. to be identified.

INTRODUCTION

The many sources and types of commercially-valuable waxes make a chemical definition of the term "wax" somewhat elusive[1]. Depending upon the wax being considered, major components may include saturated hydrocarbons in the range of *ca.* C_{20} to $>C_{50}$ (*e.g.*, paraffin and microcrystalline waxes), esters formed from even-numbered straight-chain C_{12}–C_{36} alcohols and carboxylic acids (*e.g.*, many natural waxes), triglycerides (*e.g.*, bayberry wax) and long-chain carboxylic acids (*e.g.*, acid montan wax). Other compound classes including alcohols and dicarboxylic acids (and their esters) may also be important components[1–5].

The chemical analysis of waxes dates back at least to the 1848 analysis of beeswax performed by J. Liebig in Giessen (see ref. 1). Recent methods to identify individual wax components are based on high-temperature gas chromatography (GC)[2–11]. Direct analysis of the wax yields the hydrocarbon and mono-ester patterns while the free fatty acids are determined as their methyl esters after treatment with diazomethane, and the alcohols are determined as their acetate derivatives after treatment with acetic anhydride[2]. The general applicability of high-temperature GC to the analysis of waxes is somewhat limited by the upper range of species that can be eluted and the need to derivatize the more polar components[2–11].

Capillary supercritical fluid chromatography (SFC) is a rapidly developing technique for the analysis of samples containing organic components that lack sufficient volatility and/or thermal stability to be separated by GC[12–15]. Capillary SFC has an additional advantage in that the low carrier flow-rates used allow for the direct coupling of SFC to mass spectrometry (SFC–MS), as well as to other GC detection systems, most notably flame ionization detection (SFC–FID)[12–17]. The ability of SFC to separate high-molecular-weight and/or polar compounds coupled with the qualitative data supplied by a mass spectrometer should result in a technique well-suited to the separation and identification of individual wax components without the need for chemical derivatization.

We have developed an SFC–MS methodology that allows for the separation and identification of individual wax components without any prior derivatization. Because of its compatibility with both FID and MS, carbon dioxide was used as the carrier fluid so that SFC–MS and SFC–FID chromatograms could be directly compared. The methane chemical ionization mass spectra of several high-molecular-weight alkanes, esters, di- and triglycerides, alcohols, and carboxylic acids obtained under SFC–MS conditions are reported. A variety of chemically different commercial waxes has been analyzed using SFC–FID and SFC–MS and the individual wax components have been identified based on their methane chemical ionization mass spectra.

EXPERIMENTAL

Wax samples

Various commercial waxes were selected for this study based on their different chemical compositions. Montan-derived Hoechst wax S, and the microcrystalline wax 195 were obtained from American Hoechst (Somerville, NJ, U.S.A.), the Fischer-Tropsch wax was obtained from Moore and Munger (Fairfield, CT, U.S.A.), and the bayberry wax was obtained from Aldrich (Milwaukee, WI, U.S.A.). The yellow beeswax was obtained from a local beekeeper. All waxes were used as received. Solutions of *ca.* 10 mg/ml in chloroform were typically prepared for analysis.

Instrumentation

SFC separations were performed using an SFT Model 250-TMP or a Model 501 B supercritical fluid pumping system supplied by Lee Scientific (Salt Lake City, UT, U.S.A.). Sample injections were performed using a Rheodyne Model 7410 valve fitted with a 0.5-μl sample loop. Split injections were performed in a manner similar to that previously described[18]. A 20-cm section of 1/16 in. O.D. stainless-steel tubing

was connected to the valve (held at room temperature) and to a 1/16 in. Swagelok stainless-steel tee (inside the chromatographic oven). The chromatographic column was inserted through the tee and tubing and butted up to the valve body. Appropriate lengths of either 10 or 25 μm I.D. fused-silica tubing were connected to the other arm of the tee to yield split ratios ranging from 1:10 to 1:100.

In order to maintain column pressure an outlet restrictor was fabricated in a manner similar to that reported by Guthrie and Schwartz[19]. The tip of the column (observed at 80 times magnification) was heated using a small oxygen–acetylene flame until the column end was just barely closed. The column was then pressurized to 200 atm with carbon dioxide and the closed tip was ground with 600 grit carborundum sandpaper until a gas flow-rate of 1–2 ml/min was achieved. With practice, a new column restrictor can be made in less than 30 min. When these restrictors were used for SFC–FID and SFC–MS the restrictor-associated problems such as restrictor plugging and detector "spiking" reported by other authors[20–22] were not observed.

SFC–FID was performed using a Hewlett-Packard Model 5890 gas chromatograph equipped with a conventional flame ionization detector. The chromatographic column was installed in the detector in a manner identical to that used for a GC column. Detector temperature was 380°C. A 10 m × 50 μm I.D. DB-5 (0.2 μm film thickness) chromatographic column supplied by J & W Scientific (Rancho Cordova, CA, U.S.A.) was used for all SFC–MS and SFC–FID separations. SFC–MS analyses were performed using a Hewlett-Packard Model 5985B GC/MS. The SFC column was inserted through the capillary direct interface (1/16 in. O.D. stainless-steel tubing) in a manner identical to that of a capillary GC column. This procedure placed the restrictor tip approximately 0.5 cm from the ion source of the mass spectrometer. The area of the transfer line surrounding the restrictor tip was heated to 100°C above the column temperature to aid cluster break-up prior to ionization[16].

Chemical ionization mass spectrometry (CI-MS) was performed using methane reagent gas introduced into the source region through the same 1/16 in. O.D. stainless-steel tube that was used for the chromatographic column guide. Total source pressure of the carrier carbon dioxide and the added reagent gas was typically 0.1 Torr. All standard spectra were obtained by injecting chloroform solutions containing approximately 2 mg/ml of each standard (resulting in *ca.* 50 ng of each species on-column). Chromatographic conditions were similar to those used for the sample waxes. The mass spectral scan range was typically 200 to 1000 a.m.u.

RESULTS AND DISCUSSION

SFC–CI-MS

Chemical ionization mass spectra were obtained under SFC–MS conditions with carbon dioxide carrier for the standard compounds listed in Table I. Representative spectra for each compound class are shown in Fig. 1. The spectra have been corrected for the hydrogen mass defect so that nominal masses are shown.

Similar to earlier reports on methane CI-MS of lower molecular weight species[23], all of the alkanes tested gave base peaks (relative intensity of 100%) for pseudomolecular ions at $(M - 1)^+$ resulting from hydride abstraction. Each alkane also showed characteristic methylene losses (*i.e.* at *m/z*, 211, 225, 239, 253 ...) with relative intensities ⩽25%. Each alcohol standard showed a base peak for the pseudomolec-

TABLE I

SFC–MS STANDARD COMPOUNDS

Species	*Formula*	*Nominal mol. wt.*
Alkanes		
Docosane	$C_{22}H_{46}$	310
Tetracosane	$C_{24}H_{50}$	338
Octacosane	$C_{28}H_{58}$	394
Dotriacontane	$C_{32}H_{66}$	450
Hexatriacontane	$C_{36}H_{74}$	506
Alcohols		
Hexadecanol	$C_{16}H_{33}OH$	242
Eicosanol	$C_{20}H_{41}OH$	298
Docosanol	$C_{22}H_{43}OH$	326
Carboxylic acids		
Teradecanoic (myristic)	$C_{13}H_{27}CO_2H$	228
Hexadecanoic (palmitic)	$C_{15}H_{31}CO_2H$	256
Octadecanoic (stearic)	$C_{17}H_{35}CO_2H$	284
Eicosanoic (arachidonic)	$C_{19}H_{39}CO_2H$	312
Docosanoic (behenic)	$C_{21}H_{43}CO_2H$	340
Tetracosanoic	$C_{23}H_{47}CO_2H$	368
Octacosanoic	$C_{27}H_{55}CO_2H$	424
Triacontanoic	$C_{29}H_{59}CO_2H$	452
Dotriacontanoic	$C_{31}H_{63}CO_2H$	480
Esters		
Palmityl stearate	$C_{17}H_{35}CO_2C_{16}H_{33}$	508
Stearyl stearate	$C_{17}H_{35}CO_2C_{18}H_{37}$	536
Stearyl behenate	$C_{21}H_{43}CO_2C_{18}H_{37}$	592
Arachidyl behenate	$C_{21}H_{43}CO_2C_{20}H_{41}$	620
Behenyl behenate	$C_{21}H_{43}CO_2C_{22}H_{43}$	648
Diglyceride esters		
Dimyristin	$(C_{13}H_{27}CO_2)_2\ C_3H_5OH$	512
Dipalmitin	$(C_{15}H_{31}CO_2)_2\ C_3H_5OH$	568
Distearin	$(C_{17}H_{35}CO_2)_2\ C_3H_5OH$	624
Triglyceride esters		
Tripalmitin	$(C_{15}H_{31}CO_2)_2\ C_3H_5$	806
Tristearin	$(C_{17}H_{35}CO_2)_3\ C_3H_5$	890
Triarachidin	$(C_{19}H_{39}CO_2)_3\ C_3H_5$	974

ular ion at $(M - 1)^+$ as well as ions at $(M - 17)^+$ resulting from loss of water from the protonated parent (39%, 41%, and 43% relative intensity for C_{16}, C_{20}, and C_{22} alcohols, respectively). Carboxylic acid standards all showed base peaks at $(M + 1)^+$ with a less intense peak at $(M - 1)^+$. The relative intensity of the $(M - 1)^+$ peak increased with the acid chain length from 25% to 95% for the C_{14} and C_{30} carboxylic acids, respectively.

The monoester standards all showed similar fragmentation patterns. As illustrated by the spectra of arachidyl behenate (Fig. 1) each standard ester displayed pseudomolecular ions at $(M + 1)^+$ (*ca.* 40% relative intensity), and at $(M - 1)^+$ (50–80% relative intensity). The base peak for each monoester was at $(A + H)^+$

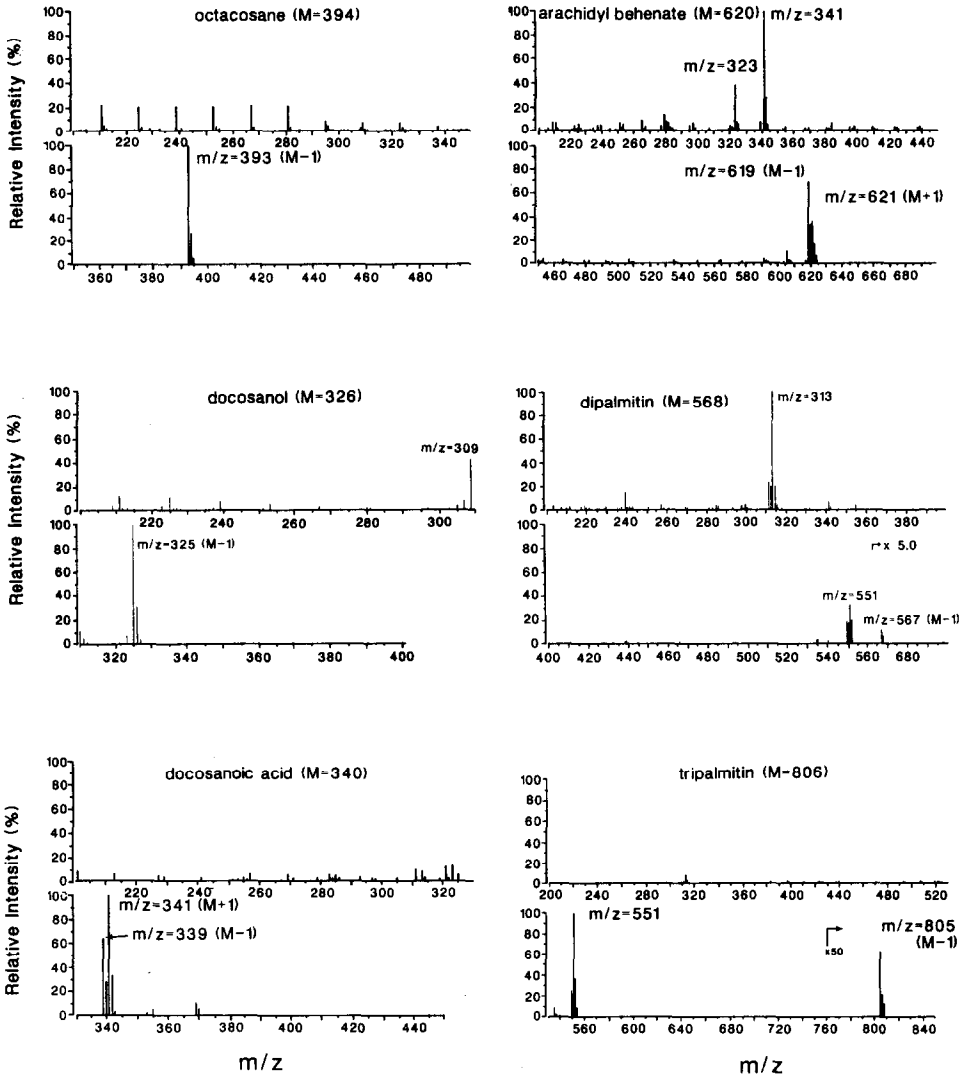

Fig. 1. SFC–MS methane chemical ionization mass spectra of representative standards. Experimental conditions and a discussion of the spectra are given in the text.

where A is the parent acid (*m/z* 341, Fig. 1). An additional fragment was observed for each monoester at $(MH - ROH)^+$ (*m/z* 323) where R is the attached alkyl group (relative intensity of *ca.* 25% for the stearate esters to *ca.* 40% for the behenate esters). Based on the $(A + H)^+$ and $(MH - ROH)^+$ fragment ions, the length of an unknown ester's acyl and alkyl groups may easily be determined[24]. These methane chemical ionization mass spectra obtained under SFC–MS conditions are similar to those obtained from wax esters introduced using a heated probe[25].

All of the di- and triglycerides showed low intensity (*ca.* 2% relative intensity) pseudomolecular ions at $(M - 1)^+$. Diglycerides showed $(M - 17)^+$ ions (*ca.* 31%

relative intensity) resulting from the loss of water from the protonated parent that is characteristic of alcohols (*m/z* 551, Fig. 1). The base peak ion is formed by the loss of one carboxylic acid from the protonated parent (*m/z* 313). Triglycerides also show a base peak ion formed from the loss of one carboxylic acid (*m/z* 551, Fig. 1). The observation of the carboxylic acid loss is valuable for determining the presence and identity of di- and triglycerides containing different carboxylic acid groups (discussed later in the text).

Wax sample analysis

The SFC separations of the individual components of five different commercial waxes are shown in Figs. 2–6. All of the standard and sample alkanes, alcohols, carboxylic acids, esters, diglycerides, and triglycerides showed good chromatographic behavior on the DB-5 column without the need for derivatization of the polar species and each of the these separations was performed at relatively low temperatures (*i.e.*, 125–135°C). Although analysis speed was not a primary focus of this work, good separation of individual components was achieved in less than 40 min for each of the waxes except the Fischer-Tropsch wax. The possible presence of later eluting species was investigated for each wax sample by programming the carbon dioxide pressure to 410 atm and holding for at least 30 min. No additional species could be detected using this procedure for any of the wax samples indicating that the elution of individual wax components was complete. However, since quantitative calibrations were not performed during this investigation, it is possible that some minor wax components were not eluted under these chromatographic conditions.

Microcrystalline wax. Microcrystalline waxes are a product of the crystalline residues left after distillation of petroleum waxes[2]. Further refinement yields a wax

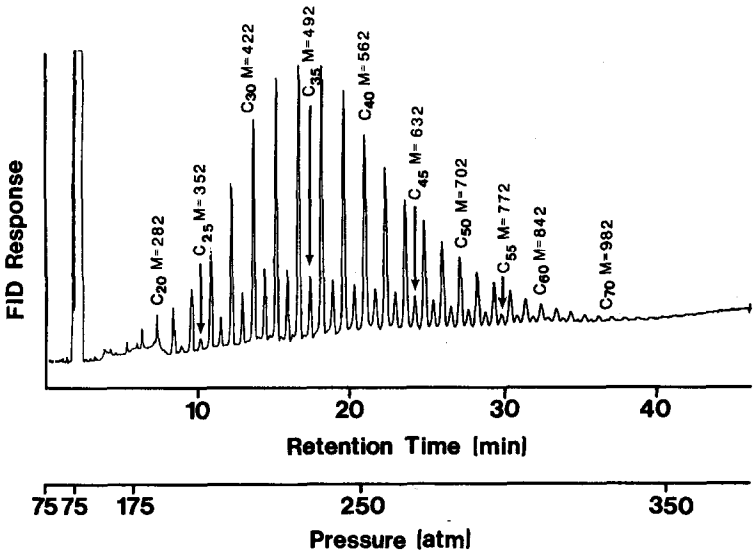

Fig. 2. SFC–FID chromatogram of a microcrystalline wax. The chain length and molecular weights of individual alkanes are shown on the chromatogram. Carbon dioxide pressure program was 75 atm (hold 2 min), a pressure ramp of 25 atm/min to 175 atm, then a pressure ramp of 5 atm/min. Column temperature was held at 135°C.

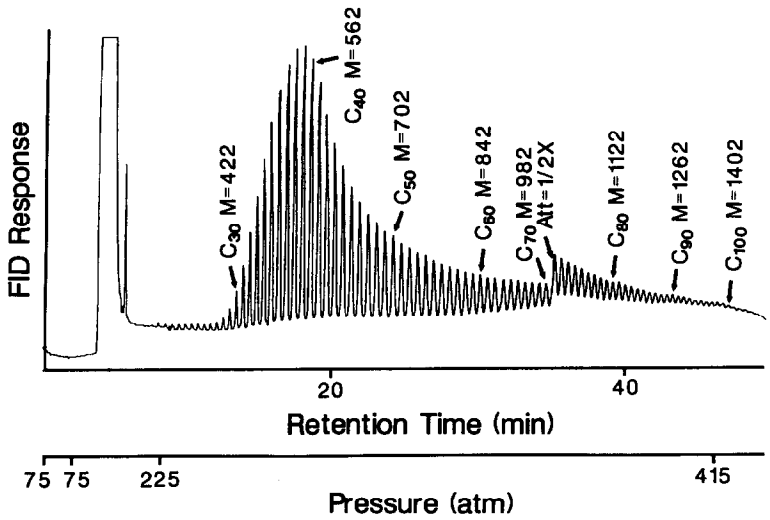

Fig. 3. SFC–FID chromatogram of a Fischer-Tropsch wax. The chain length and molecular weights of individual alkanes are shown on the chromatogram. Carbon dioxide pressure program was 75 atm (hold for 2 min), a pressure ramp of 25 atm/min to 225 atm, then a pressure ramp of 5 atm/min. Column temperature was held at 125°C. The recorder attenuation was reduced by one-half just after the elution of the C_{70} alkane.

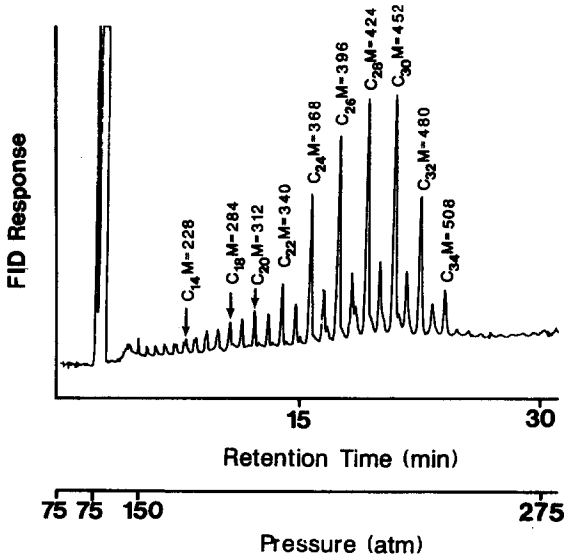

Fig. 4. SFC–FID chromatogram of an acid montan wax. The chain length and molecular weights of individual even-numbered carboxylic acids are shown on the chromatogram. The peaks between the even-numbered carboxylic acids were identified by SFC–MS to be odd-numbered carboxylic acids. Carbon dioxide pressure program was 75 atm (hold for 2 min), a pressure ramp of 25 atm/min to 150 atm, then a pressure ramp of 5 atm/min. Column temperature was held at 125°C.

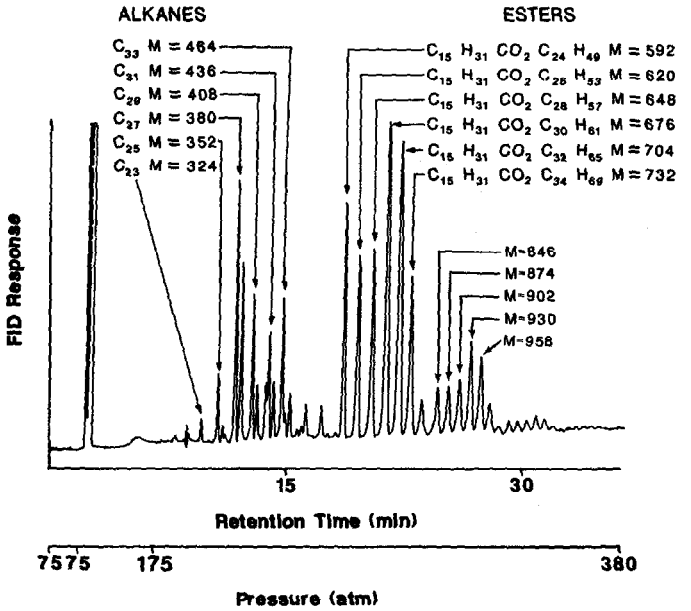

Fig. 5. SFC–FID chromatogram of yellow beeswax. The chain length and molecular weight of the alkane and ester components are shown on the chromatogram. Carbon dioxide pressure program was 75 atm (hold for 2 min), a pressure ramp of 25 atm/min to 175 atm, then a pressure ramp of 7 atm/min. Column temperature was held at 125°C.

containing almost exclusively *n*-alkanes. Fig. 2 shows the SFC separation of microcrystalline wax 195. Individual alkanes were identified based on their SFC–MS methane chemical ionization mass spectra and a comparison of their retention times with the standard *n*-alkanes listed in Table I. A strong even/odd preference was shown by this wax.

Fischer-Tropsch wax. Fischer-Tropsch waxes are produced from coal via the Fischer-Tropsch synthesis of normal alkanes. The Fischer-Tropsch wax shown in Fig. 3 is a product of the Sasol plant, South Africa, and contains normal alkanes ranging from *ca.* C_{20} to C_{100}. Individual components were easily identified using SFC–MS, except that the upper mass limit of our quadrapole mass spectrometer (1000 a.m.u.) precluded observation of molecular ions for species larger than C_{71} (M = 996). Unlike the microcrystalline wax, the Fischer-Tropsch wax has no even/odd carbon number preference as would be expected from a product of a Fischer-Tropsch synthesis.

The use of SFC–FID quantitations to determine the molecular weight distributions of the microcrystalline wax and the Fischer-Tropsch is shown in Fig. 7. Each individual alkane is separated and quantitated, allowing SFC–FID to give more detailed molecular weight distributions than physical methods or low-resolution chromatographic methods such as gel permeation chromatography. Since appropriate long-chain alkane calibration standards were not available for this study, quantitations were based on assuming equal FID response per unit weight for each alkane component. (The variation in FID response for lower molecular weight alkanes has been shown to be $\leqslant 3\%$[26].) The potential error in this assumption as well as the

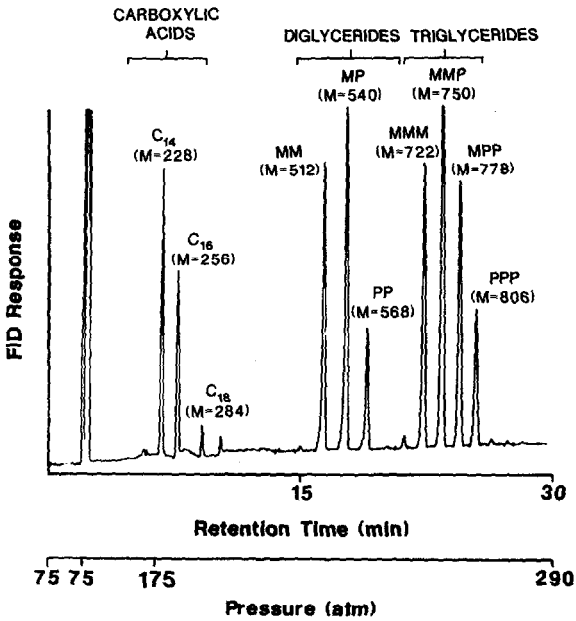

Fig. 6. SFC–FID chromatogram of bayberry wax. The individual di- and triglycerides are identified as myristate (M) and palmitate (P) esters of glycerol. Carbon dioxide pressure program was 75 atm (hold for 2 min), a pressure ramp of 25 atm/min to 175 atm, then a pressure ramp of 5 atm/min. Column temperature was 125°C.

possibility for splitter discrimination needs to be determined to ensure the accuracy of such quantitative data.

Acid montan wax. Montan waxes are obtained by the extraction of lignite coal, and consist primarily of C_{16}–C_{36} carboxylic acids esterified with C_{16}–C_{36} alcohols[2]. Selective oxidation is used to convert the esters to the straight-chain carboxylic acid components of an acid montan wax. As shown in Fig. 4, the acid montan wax consists primarily of even-numbered carboxylic acids from C_{22} to C_{34}, with smaller amounts of odd-numbered and lower molecular weight carboxylic acids. The identity of the even-numbered carboxylic acids was confirmed by comparing the chromatographic retention times and mass spectra of sample species with those of carboxylic acid standards listed in Table I.

Yellow beeswax. The most prevalent compound classes in yellow beeswax are long-chain esters and odd-numbered alkanes[2,3,5]. SFC–MS analysis showed that the most prevalent alkanes are C_{25}–C_{33} (Fig. 5). Esters ranging in molecular weight from 592 to 732 a.m.u. were also present. Each of these esters showed an intense ion at *m/z* 257 which allowed them to be identified as being formed from palmitic acid esterified with even-numbered alcohols ranging from C_{24} to C_{34}. The additional species eluting after the long chain esters appear to have molecular weights of 846, 874, 902, 930, and 958. The spectrum of the M = 846 species is shown in Fig. 8. Since no appropriate standards were available only tentative assignments can be made. The fragment ions at *m/z* 257 and *m/z* 239 are consistent with those shown by an ester formed with palmitic acid. The ion at *m/z* 591 can be assigned to the loss of

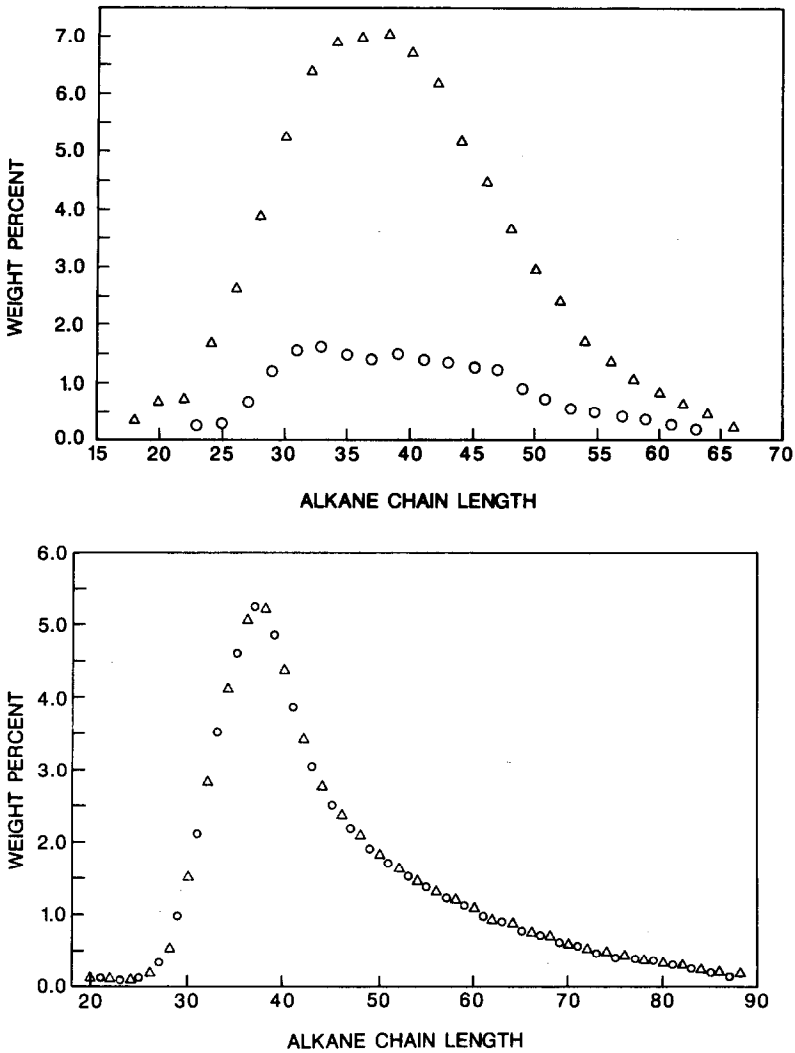

Fig. 7. Chain length distribution of normal alkanes from a microcrystalline wax (upper) and a Fischer-Tropsch wax (lower) as determined by SFC–FID. △ = Even-numbered alkanes; ○ = odd-numbered alkanes.

palmitic acid (256 a.m.u.) from the ionized parent, analogous to the same loss shown by tripalmitin (Fig. 1). A structure consistent with these ions would be a diester formed from two palmitic acids esterified to a C_{24} diol. The larger molecular weight species in this same cluster of peaks (*i.e.* M = 874, 902, ...).show similar ions corresponding to palmitic acid at m/z 257 and the loss of palmitic acid from the ionized parent indicating that these species are a series of diesters formed from two palmitic acids esterified to C_{24}, C_{26}, C_{28}, C_{30}, and C_{32} diols. The tentative assignment of diesters is also supported by earlier reports that yellow beeswax contains approximately 14% diesters[3,27].

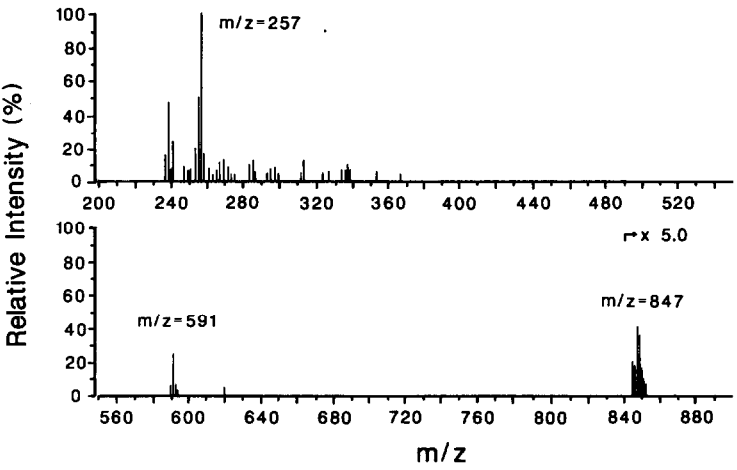

Fig. 8. SFC–MS methane chemical ionization mass spectrum of an unknown species from yellow beeswax. The species was tentatively identified as a diester formed from two palmitic acids esterified with a C_{24} diol. Tentative assignment of the fragment ions is discussed in the text.

Bayberry wax. As shown in Fig. 6, bayberry wax consists of free carboxylic acids (C_{14}, C_{16}, and C_{18}), diglycerides including dimyristin (MM on Fig. 6), a mixed diglyceride formed with one palmitate and one myristate group (MP), dipalmitin (PP), and triglycerides including trimyristin (MMM), a mixed triglyceride containing two myristate and one palmitate group (MMP), a mixed triglyceride containing one myristate and two palmitate groups (MPP), and tripalmitin (PPP). The identities of the three carboxylic acids, dimyristin, dipalmitin, and the tripalmitin were confirmed by comparison with the retention time and mass spectra of standard compounds.

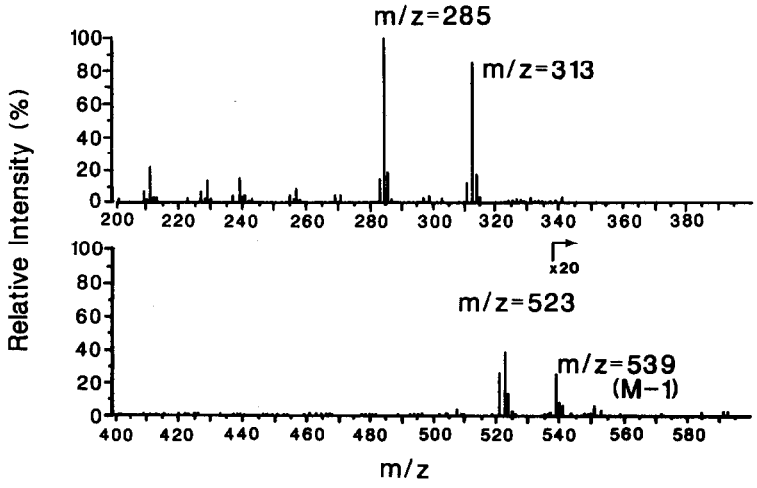

Fig. 9. SFC–MS methane chemical ionization mass spectrum of mixed diglyceride from bayberry wax formed with one palmitate and one myristate group esterified to glycerol. The identity of the major fragment ions is discussed in the text.

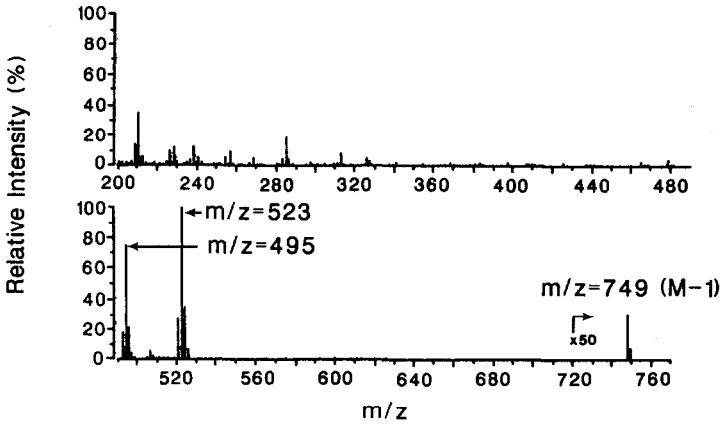

Fig. 10. SFC–MS methane chemical ionization mass spectrum of a mixed triglyceride from bayberry wax formed with one palmitate and two myristate groups esterified to glycerol. The identity of the major ions is discussed in the text.

The mass spectrum of the mixed MP diglyceride is shown in Fig. 9. Characteristic diglyceride ions are observed at $(M - 1)^+$ (*m/z* 539) and at $(M - 17)^+$ (*m/z* 523). The intense fragment ions that occur at masses characteristic of the loss of palmitic acid (at *m/z* 285) and the loss of myristic acid (at *m/z* 313), confirm the presence of these acid groups. The mixed MMP triglyceride spectra shown in Fig. 10 also shows the characteristic loss of each acid group. Sufficient standards were not available during this study to determine if the mass spectra could be used to determine at which position each acid group is attached to the glycerol molecule.

CONCLUSIONS

Supercritical fluid chromatography coupled with mass spectrometry provides a rapid and powerful method for the separation and identification of individual wax components. Coupled with a flame ionization detector, supercritical fluid chromatography also allows individual wax components to be quantitated. In order to ensure the accuracy of quantitative measurements, appropriate calibration standards are needed to determine the FID relative response factors and the extent of any injector discrimination.

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