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SUPERCRITICAL FLUID CHROMATOGRAPHY OF SESQUITERPENE HYDROCARBONS ON SILICA PACKED COLUMNS WITH ON-LINE FOURIER TRANSFORM INFRARED DETECTION

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SUMMARY

The on-line coupling of carbon dioxide supercritical fluid chromatography with Fourier transform infrared (FT-IR) spectrometry allows the separation and identification of sesquiterpene hydrocarbon mixtures on bare silica as stationary phase. Separations of these non-polar but extremely thermosensitive organic compounds required low-temperature rather than low-density experimental conditions. These chromatographic conditions preserved structural information about these sesquiterpene hydrocarbons, such as the differentiation of methyl groups ($1375\text{--}1385\text{ cm}^{-1}$) from methylene groups and the geminal or single nature of methyl groups (twin bands near $1360\text{--}1390\text{ cm}^{-1}$ or a single band near 1380 cm^{-1}). The method was applied to the identification of the sesquiterpene hydrocarbon fraction of several essential oils; important structural features provided by their FT-IR spectra are reported.

INTRODUCTION

Sesquiterpene hydrocarbons occur in detectable amounts in most essential oils. They are generally found as complex mixtures of closely related isomers which are mainly analysed by fused-silica capillary gas chromatography (GC) at high temperatures ($150\text{--}200^\circ\text{C}$) and identified by gas chromatography–mass spectrometry (GC–MS)¹. However, unequivocal identification by MS alone is sometimes difficult; compounds within the same terpenoid structure class have very similar ion fragmentation patterns and differ only in ion intensities¹; hence GC with Fourier transform infrared (FT-IR) spectrometric detection offers a complementary technique to GC–MS analysis. Kalasinsky and co-workers^{2,3} studied the analysis of monoterpenoids by GC–FT-IR on packed stainless-steels columns and concluded that it allows the positive identification of solutes. However, sesquiterpene hydrocarbons are thermosensitive organic compounds⁴ and destructive chemical reactions may occur at elevated temperatures.

The increasing interest in supercritical fluid chromatography (SFC) can be at-

tributed to the more rapid solute mass transfer than in the liquid phase and to the development in SFC of GC- and LC-type detectors⁵.

A number of papers have recently appeared on the development of a combined SFC-FT-IR system with a carbon dioxide mobile phase. The two types of interfacing approaches result from the use of solvent elimination techniques and a transmission high pressure-resistant flow cell. Several workers have developed a combined SFC-FT-IR system with a transmission light-pipe flow cell; first, Shafer and Griffiths⁶ reported in 1983 the result of FTIR detection in carbon dioxide SFC, Olesik *et al.*⁷ in 1984 and Johnson *et al.*⁸ in 1985 studied the bands due to Fermi resonance in liquid and supercritical carbon dioxide and particularly their dependence on carbon dioxide pressure⁹.

Two important groups of bands are obscured by the carbon dioxide IR spectrum in the 2200–2500 and 3500–3800 cm^{-1} regions, which means that the functional groups of compounds such as alcohols [$\nu(\text{OH})$], amines [$\nu(\text{NH})$], lactams [$\nu(\text{NH})$], amides [$\nu(\text{NH})$], disubstituted alkynes [$\nu(\text{C}\equiv\text{C})$] and nitriles [$\nu(\text{C}\equiv\text{N})$], cannot be identified by their main stretching frequencies¹⁰.

The first band is the asymmetric stretch near 2349 cm^{-1} and the second is due to combination and overtone bands. The fundamental bending vibration mode at 667 cm^{-1} is approximately 300 cm^{-1} wide and consequently rules out spectral information below 820 cm^{-1} . Unfortunately, the spectral range from 800 down to 600 cm^{-1} is important and particularly useful for the identification of *cis*-disubstituted alkenes and substitution patterns of aromatic compounds [the out-of-plane bending $\gamma(\text{CH})$ modes absorbing near 900–650 cm^{-1} indicate the substitution pattern on the benzene ring]. In addition, organic chlorinated compounds have $\nu(\text{CCl})$ stretching frequencies near 750–700 cm^{-1} , brominated compounds show two bands in the 600–500 cm^{-1} region and alkyl iodides absorb in the general region of 500 cm^{-1} ; consequently, the carbon-halogen stretching vibration cannot be detected by carbon dioxide SFC-FTIR.

Mercury-cadmium telluride (MCT) detectors differ in spectral bandwidth and broad-band detectors are generally less sensitive than narrow-band detectors. However, the IR transparency of supercritical carbon dioxide avoids the need for a compromise between sensitivity and useful spectral range. In all instances a narrow-band MCT detector with high sensitivity is chosen, but no information can be obtained at wavenumbers below 820–800 cm^{-1} . Moreover, two additional pairs of bands (Fermi resonance bands) appear in the IR spectrum of carbon dioxide in the supercritical state. The intensity and the width of the Fermi resonance bands increase on going from the supercritical to the liquid state¹⁰.

Hence an increase in carbon dioxide density induces a reduction in the IR transparency of the mobile phase in the 2060–2072, 1350–1410 and 1275–1285 cm^{-1} regions. Consequently, some bending frequencies, such as the $\delta(\text{CH})$ bending mode of a methyl group or geminal methyl groups, the $\delta(\text{CH})$ bending mode of a gem-disubstituted or a monosubstituted olefinic double bond, the $\delta(\text{CH})$ bending mode of α -methyl or α -methylene groups in a ketone and finally the $\delta(\text{OH})$ bending vibration of an alcohol, which occur in Fermi resonance band region (1350–1410 cm^{-1}), cannot be observed by SFC-FT-IR when the carbon dioxide density is greater than 0.72 g ml^{-1} (10-mm path length cell).

Nevertheless, this direct flow cell approach in SFC-FT-IR is an interesting

technique for the separation and on-line identification of non-volatile compounds such as polycyclic aromatic hydrocarbons¹¹, free fatty acids¹² and phenolic compounds¹³.

Other fluids have been used as the mobile phase in flow cell SFC-FTIR; Freon 23 complements the opaque regions of carbon dioxide by exhibiting transparency from 4000 to 2500 cm^{-1} (ref. 14), and xenon is transparent from the vacuum UV up to the NMR region^{15,16}.

An interesting and detailed review on the interfacing between SFC and FT-IR spectrometry was published by Jinno¹⁷.

The aim of this work was to determine the retentions of sesquiterpene hydrocarbons by carbon dioxide SFC, especially at low temperatures which minimize the thermal degradation of these solutes, and to establish the capability of carbon dioxide as a supercritical mobile phase to provide solute IR spectra of sufficient quality (spectral data range, signal-to-noise ratio, useful structural information).

EXPERIMENTAL

Instrumentation

SFC-FT-IR experiments were performed on a Nicolet Model 5SX-B FT-IR spectrometer system equipped with a KBr/Ge beam splitter, a liquid nitrogen-cooled MCT-A photodetector ($D = 4.3 \cdot 10^{10} \text{ cm Hz}^{1/2} \text{ W}^{-1}$) with a narrow bandwidth 500–750 cm^{-1} . This spectrometer was used to collect time-resolved 8 cm^{-1} resolution spectra with a mirror velocity of 2.221 cms^{-1} and a data collection rate of 1.2 spectra per second. All experiments were made employing the standard GC software provided by the manufacturer. A 4X beam condenser (Barnes Analytical, Stramford, U.S.A.) was used to reduce the 4-mm diameter focused beam to an approximate spot diameter of 1 mm.

A Varian Model 5500 liquid chromatograph was modified for SFC¹⁰. To improve the pump efficiency, the carbon dioxide, kept in a container with an eductor tube, was passed through an ethylene glycol-water cooling mixture which was circulated through a jacket surrounding the pump head. Sample injection was made via a Rheodyne Model 7010 injector with a 10- μl loop. A series of thermocouples (N 225; Thermocoax, Suresnes, France) were used to check the carbon dioxide temperature within the pumping system, the heat exchanger and the outlet of the flow cell. The pressure was controlled by a TESCOM manually adjustable back-pressure regulator (Model 26-3220-24004; GEC Composants, Asnières, France) situated after the FT-IR detector.

The IR flow cell had dimensions of 1 mm I.D. \times 10 mm pathlength (8- μl volume) and was designed to maintain a 3500 p.s.i. pressure gradient across the windows by using 2 mm thick ZnSe discs of 13 mm diameter.

A 250 \times 4.6 mm I.D. Nucleosil 100 or Spherisorb silica (5- μm particle diameter) packed column was used for the study of sesquiterpene hydrocarbon SFC retention. A constant-temperature water-bath provided temperature control for the column. The capacity factors were calculated by SFC-FT-IR from the retention time of the analyte (t_R) and the retention time of the void volume peak (t_0) using *n*-pentane as an unretained compound.

Carbon dioxide (N 45, 99.995% purity) (Alpha Gaz, Bois d'Arcy, France) was employed.

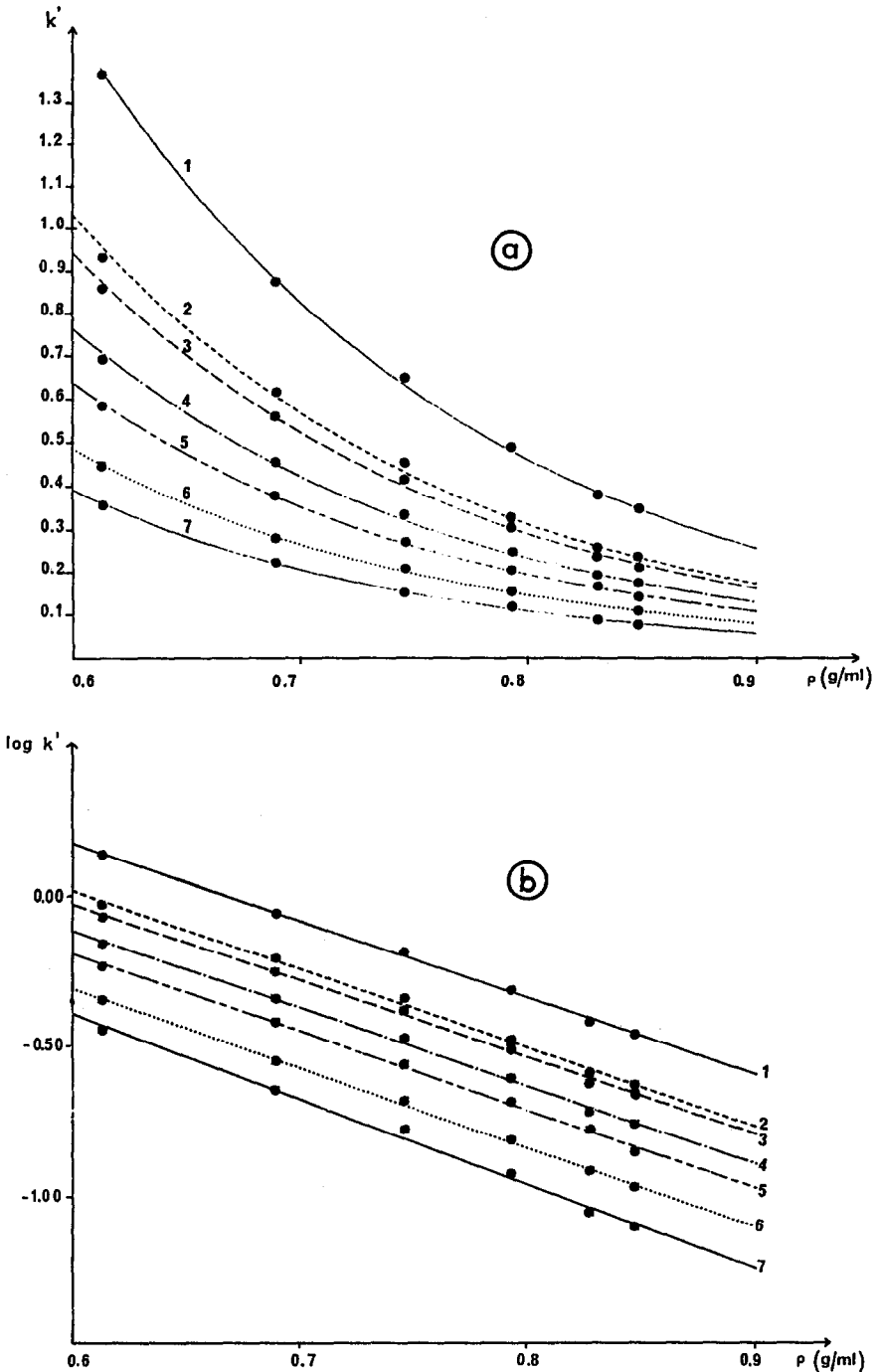


Fig. 1. (a) Influence of supercritical carbon dioxide density on the capacity factors (k') of sesquiterpene hydrocarbons on bare silica. (b) Plots of $\log k'$ versus carbon dioxide density. Column, 250×4.6 mm I.D.; stationary phase, Nucleosil 100 ($5 \mu\text{m}$); temperature, 40°C ; flow-rate, 2 ml min^{-1} ; FT-IR detection. Solutes: 1 = humulene; 2 = *trans*-calamenene; 3 = valencene; 4 = ledene; 5 = aromadendrene; 6 = longifolene; 7 = longicyclene.

Sesquiterpene hydrocarbon solutes

γ -Gurjunene, longipinene, thujopsene, longicyclene, aromadendrene and ledene were purchased from Fluka (Buchs, Switzerland), humulene and valencene from Sarsynthex (Merignac, France) and longifolene from Aldrich (Milwaukee, WI, U.S.A.). *trans*-Calamenene, *cis*-calamenene and β -farnesene were from our own collection.

Semi-preparative liquid chromatography

The prefractionation of an essential oil was carried out by semi-preparative liquid chromatography¹⁸. Reversed-phase chromatography on octadecyl-bonded silica was used and the separation of the essential oil was achieved at ambient temperature, giving four fractions (oxygenated monoterpenes, oxygenated sesquiterpenes, monoterpene hydrocarbons and sesquiterpene hydrocarbons). Finally, the sesquiterpene hydrocarbon fraction was analysed by carbon dioxide SFC-FT-IR.

RESULTS AND DISCUSSION

Chromatographic study

A preliminary study with a synthetic mixture containing sesquiterpene hydrocarbons was carried out to determine their retention behaviour on bare silica with supercritical carbon dioxide. Capacity factors of several sesquiterpene hydrocarbons are reported on Nucleosil 100 silica *versus* carbon dioxide density (Fig. 1a). The experimental variations confirmed the general rule according to which an increase in carbon dioxide density results in enhanced solute solubility and consequently a decrease in retention; on this stationary phase, the capacity factors of these apolar solutes are generally smaller than 2 within the carbon dioxide density range 0.6–0.9 g ml⁻¹. The dependence of the capacity factors on supercritical carbon dioxide density was determined by numerical analysis and confirmed that a logarithmic equation best fits the experimental retention data (Fig. 1b). Hence for each solute at a given temperature:

$$\log k' = a + b\rho \quad (1)$$

where k' = capacity factor and ρ = carbon dioxide density (g ml⁻¹). The numerical constants a and b were determined by linear regression analysis with correlation coefficients greater than 0.998 (Table I).

TABLE I

COEFFICIENTS IN EQN. 1 OBTAINED FROM LINEAR REGRESSION ANALYSIS OF EXPERIMENTAL RETENTION DATA ON SILICA

Solute	a	b	$\sigma(a)^a$	$\sigma(b)^a$	r^b
Humulene	1.69	-2.54	0.03	0.04	0.999
<i>trans</i> -Calamenene	1.57	-2.59	0.05	0.07	0.998
Valencene	1.51	-2.56	0.04	0.05	0.999
Ledene	1.43	-2.58	0.04	0.06	0.999
Aromadendrene	1.36	-2.58	0.05	0.06	0.998
Longifolene	1.26	-2.62	0.04	0.06	0.999
Longicyclene	1.28	-2.81	0.05	0.06	0.999

^a Average deviation of experimental data points from the regression curve.

^b Correlation coefficient.

FT-IR detection

IR window absorbance reconstruction. The power of FT-IR detection results from the ability to reconstruct SFC-FT-IR chromatograms from single-scan interferometric data by various methods. During an SFC-FT-IR separation several hundred interferograms can be collected. However, such a collection of single-scan interferograms gives no indication of which should be transformed in order to obtain the effluent IR spectra. A method is needed to select which interferograms should be transformed and which contain only background information. Two methods are

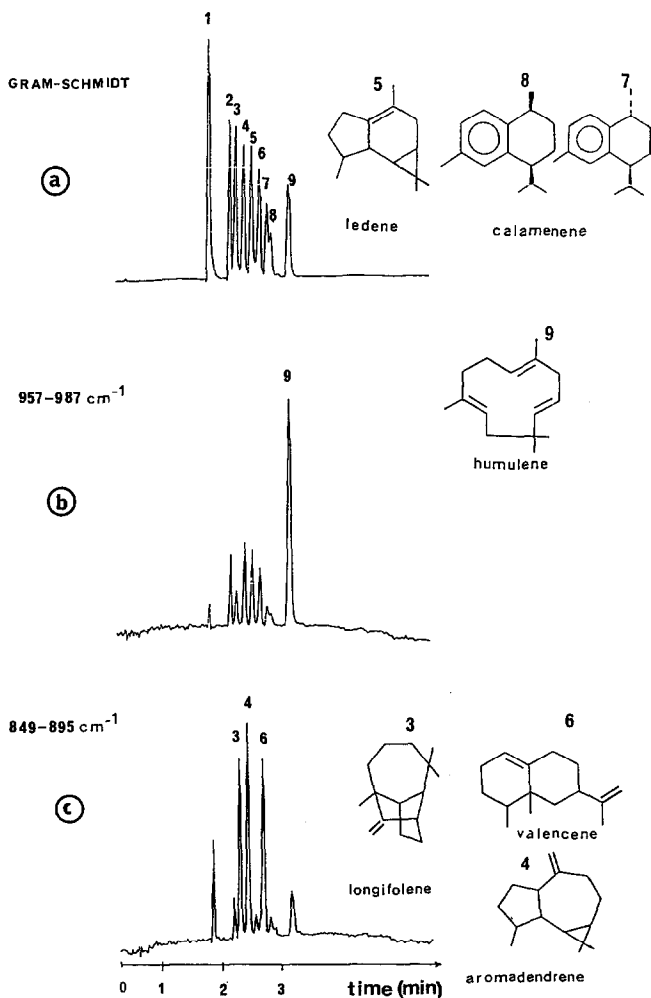


Fig. 2. Gram-Schmidt and spectral window chromatograms of a mixture of eight sesquiterpene hydrocarbons. Two columns in series (250×4.6 mm I.D.); stationary phases, Nucleosil 100 and Spherisorb ($5 \mu\text{m}$); column temperature, 40°C ; average pressure, 130 atm; flow-rate, 4 ml min^{-1} . Solutes: 1 = hexane; 2 = longicyclene; 3 = longifolene; 4 = aromadendrene; 5 = ledene; 6 = valencene; 7 = *trans*-calamenene; 8 = *cis*-calamenene; 9 = humulene. (a) Gram-Schmidt total IR chromatogram; (b) IR chromatogram, $957\text{--}989 \text{ cm}^{-1}$; (c) IR chromatogram, $849\text{--}895 \text{ cm}^{-1}$.

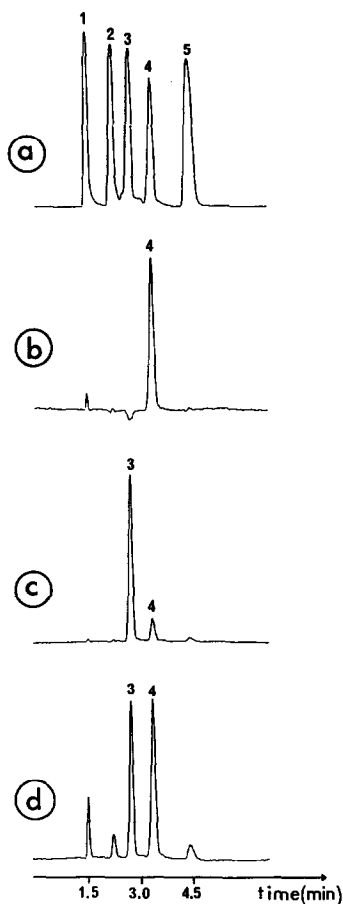


Fig. 3. Gram-Schmidt and spectral window chromatograms of a mixture of four sesquiterpene hydrocarbons. Stationary phase, Nucleosil 100 ($5\ \mu\text{m}$); column temperature, 40°C ; average pressure, 107 atm; flow-rate, $2\ \text{ml min}^{-1}$. Solutes: 1 = hexane; 2 = longicyclene; 3 = aromadendrene; 4 = β -farnesene; 5 = humulene. (a) Gram-Schmidt total IR chromatogram; (b) IR chromatogram, $1589\text{--}1604\ \text{cm}^{-1}$; (c) IR chromatogram, $1635\ \text{cm}^{-1}$; (d) IR chromatogram, $3086\text{--}3101\ \text{cm}^{-1}$.

often used from constructing chromatograms from interferometric data: the first is the Gram-Schmidt orthogonalization procedure¹⁹, which works directly from the interferograms, and the second involves fast Fourier transformation of a 1024-point section of each interferogram to obtain a low-resolution (16 wavenumbers) IR absorbance spectrum which can be integrated to determine a chromatogram detector response²⁰.

In order to compare these two methods, a standard mixture of eight sesquiterpene hydrocarbons, dissolved in hexane, was separated on a silica column in less than 4 min; $20\ \mu\text{g}$ of each component were injected. Our work involves the use of a 4.6 mm I.D. packed column which has fairly high sample capacities (of the order of $100\ \mu\text{g}$ per component). The Gram-Schmidt reconstruction chromatogram is identical in form with a chromatogram obtained by traditional chromatographic detectors and

shows good resolution, except for compounds 7 and 8 (Fig. 2a); *cis*- and *trans*-calamenene differ only in the position of the methyl and isopropyl substituents on the cyclohexane ring. However, these two stereoisomers can be identified by their supercritical carbon dioxide FT-IR spectra, particularly in the 1400–1000 cm^{-1} region.

A different possibility for calculating IR chromatograms is the construction of IR group-specific or IR window chromatograms. A number of subspectral integration windows can be chosen to monitor specific functional groups simultaneously.

IR absorptions due to bending vibrations of olefinic CH groups are more intense than those caused by C=C stretching vibrations, so they can be used to determine the nature of substituent groups. Fig. 2b and c show chromatograms obtained with an IR window specific to the out-of-plane $\delta(\text{CH})$ bending modes of olefinic groups. In the 975–987 cm^{-1} region, the trace is closely related to a *trans* substitution for a disubstituted alkene (humulene) and in the 849–895 cm^{-1} region to a *gem*-disubstituted olefinic group (longifolene, aromadendrene and valencene).

Fig. 3 shows the results of an SFC-FT-IR run on a mixture of longicyclene, aromadendrene, β -farnesene and humulene dissolved in hexane; the trace at the top is the Gram-Schmidt total IR chromatogram. The IR window chromatogram at 1589–1604 cm^{-1} specific to the $\nu(\text{C}=\text{C})$ stretching vibration for conjugated alkenes, exhibits only one peak for β -farnesene. The 1635 and 3086–3101 cm^{-1} window chromatograms are specific to $\nu(\text{C}=\text{C})$ and $\nu(\text{CH})$ stretching vibrations, respectively, for *gem*-disubstituted olefinic groups (β -farnesene, aromadendrene).

SFC-FT-IR spectra of sesquiterpene hydrocarbons. Whereas the mass spectra of sesquiterpene hydrocarbons are often similar, their infrared spectra are sufficiently different for positive identification, particularly for *cis-trans* and aromatic positional isomers. Sesquiterpene hydrocarbons can be identified in an SFC-FT-IR separation by their main stretching frequencies (Table II).

The 1375–1385 cm^{-1} band in sesquiterpene hydrocarbons reveals the presence of a methyl group and allows its differentiation from a methylene group. Moreover,

TABLE II

INFRARED ABSORPTIONS OF FUNCTIONAL GROUPS PRESENT IN SESQUITERPENE HYDROCARBONS

<i>Vibrational mode</i>	<i>Stretching $\nu(\text{CH})$</i>	<i>Double bond stretching $\nu(\text{C}=\text{C})$</i>	<i>In-plane bending $\delta(\text{CH})$</i>	<i>Out-of-plane bending $\gamma(\text{CH})$</i>
<i>Alkene</i>				
Monosubstituted (vinyl)	3040–3010 and 3095–3075	1660–1640	1420–1410 and 1300–1290	995–985 and 915–900
Disubstituted, <i>gem</i>	3095–3075	1655–1650	1420–1410	895–885
Disubstituted, <i>trans</i>	3040–3010	1675–1670	1310–1290	980–965
Disubstituted, <i>cis</i>	3040–3010	1660–1655	–	715–665
Trisubstituted	3040–3010	1670	–	840–790
<i>Alkane</i>				
CH_2	2925–2850	–	1485–1445	–
CH_3	2960–2870	–	1470–1430 and 1380–1370	–
<i>gem</i> -Dimethyl	–	–	1385–1380 and 1370–1365	–

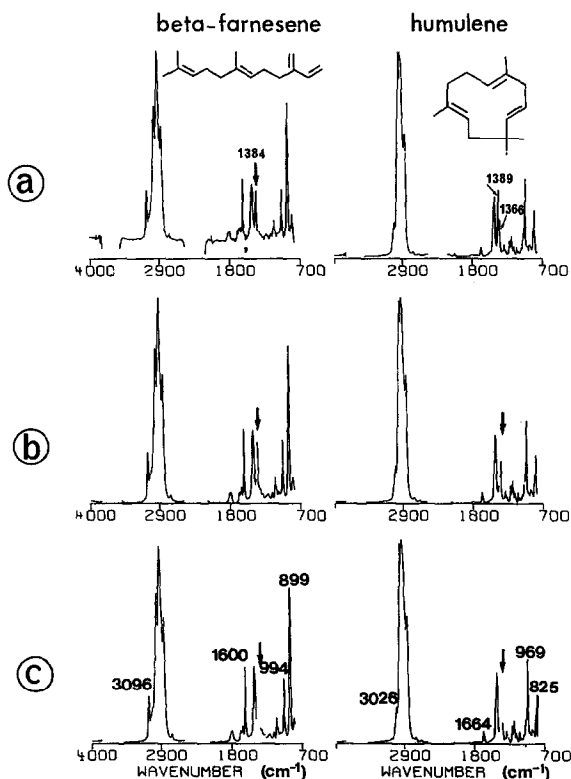


Fig. 4. Influence of carbon dioxide density on carbon dioxide FT-IR spectra of β -farnesene and humulene. Optical resolution, 8 cm^{-1} . Density: (a) 0.45 ; (b) 0.74 ; (c) 0.92 g ml^{-1} .

the main information obtained in the Fermi band region is relative to geminal methyl groups (twin bands near $1360\text{--}1390\text{ cm}^{-1}$). Finally, the in-plane $\delta(\text{CH})$ bending mode of a *gem*-disubstituted or monosubstituted olefinic group absorbs in the $1410\text{--}1420\text{ cm}^{-1}$ region.

With increasing carbon dioxide density, the Fermi doublet in the carbon dioxide spectrum absorbs all the IR radiation so none reaches the MCT detector. As soon as the carbon dioxide density is greater than about 0.74 g ml^{-1} with a 10-mm path-length flow cell¹¹, the presence of a single methyl group (single band at 1384 cm^{-1} in the β -farnesene spectrum, Fig. 4) or a *gem*-dimethyl group (twin bands at 1366 and 1389 cm^{-1} in the humulene spectrum, Fig. 4) is not revealed by their IR spectrum. In spite of these restrictions, considerable structural information is given by a carbon dioxide FT-IR spectrum, as follows. For β -farnesene, several IR bands indicate the presence of a vinyl group ($3096, 994, 899\text{ cm}^{-1}$), a methylene geminal group (899 cm^{-1}) and a conjugated olefinic system (1600 cm^{-1}). For humulene, several IR bands prove the presence of a *trans*-disubstituted alkene ($3026, 1664, 969\text{ cm}^{-1}$) and of trisubstituted double bonds (825 cm^{-1}).

In a carbon dioxide FT-IR spectrum of a sesquiterpene hydrocarbon, the substituted nature of a $\text{C}=\text{C}$ double bond is always detected whatever the carbon dioxide density; indeed, the main stretching [$\nu(\text{C}=\text{C})$], olefinic $\nu(\text{CH})$ and bending (out-

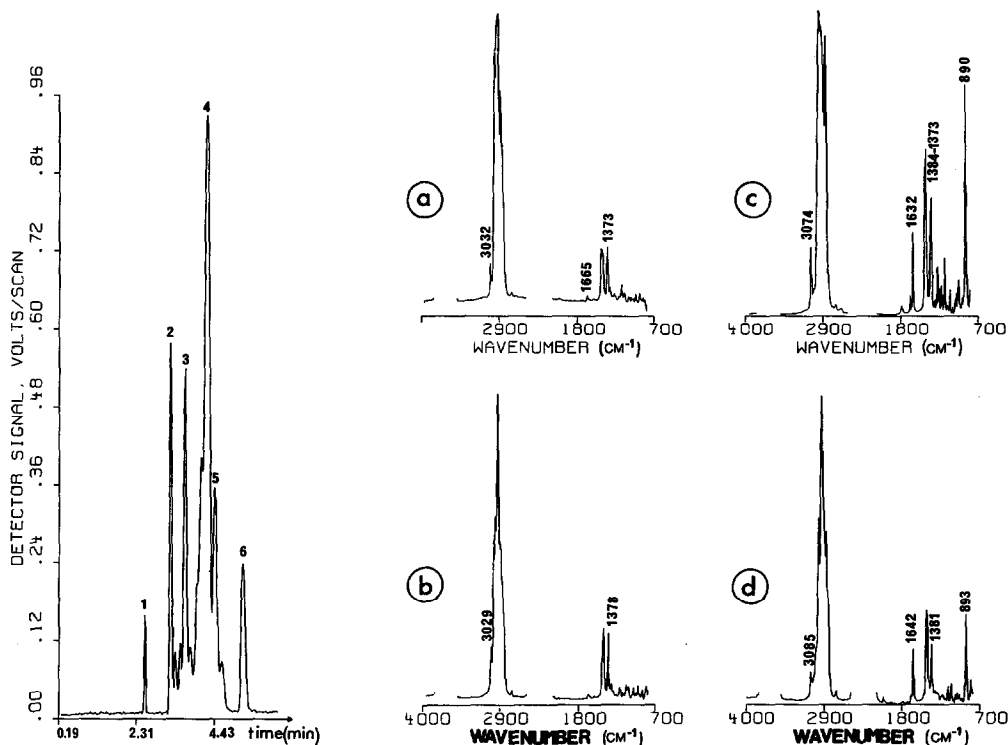


Fig. 5. SFC-FT-IR separation of sesquiterpene hydrocarbons from Copaiba balsam oil. Two columns in series (250×4.6 mm I.D.); stationary phases, Nucleosil 100 and Spherisorb ($5 \mu\text{m}$); average pressure, 140 atm; flow-rate, 4 ml min^{-1} ; column temperature, 40°C . Solutes: 1 = hexane (solvent); 2 = α -copaene; 3 = *trans*- α -bergamotene; 4 = β -caryophyllene; 5 = β -bisabolene; 6 = humulene.

Fig. 6. Carbon dioxide FT-IR spectra of sesquiterpene hydrocarbons. Cell pressure, 92 atm; cell temperature, 33°C , carbon dioxide density, 0.71 g ml^{-1} ; optical resolution, 8 cm^{-1} . Solutes: (a) α -copaene; (b) *trans*- α -bergamotene; (c) β -caryophyllene; (d) β -bisabolene.

of-plane $\delta(\text{CH})$ vibrational modes absorb in the carbon dioxide transparency domain.

Analysis of sesquiterpene hydrocarbons in essential oils

The sesquiterpene hydrocarbon fraction was isolated at room temperature from essential oils by semi-preparative liquid chromatography on octadecyl-bonded silica as described previously¹⁸. Prefractionation by liquid chromatography and SFC-FT-IR analysis are well adapted to thermosensitive compounds such as terpenoids and experiments can be made at ambient temperature.

The characterization of the products are carried out by interpretation of their carbon dioxide FT-IR spectra and comparison with those of authentic samples or those published in the literature^{21,22}.

Copaiba balsam oil. The SFC-FT-IR analysis of the sesquiterpene hydrocarbon fraction of this essential oil²³ was carried out using silica as the stationary phase in less than 5 min with the following chromatographic conditions: inlet pressure, 186 atm; outlet pressure, 92 atm; flow-rate, 4 ml min^{-1} ; and column temperature, 40°C .

The Gram-Schmidt reconstruction chromatogram corresponding to the separation is shown in Fig. 5. Several sesquiterpene hydrocarbons (α -copaene, α -transbergamotene, β -caryophyllene, β -bisabolene, humulene) were identified by comparison of their spectra with those published by Wenninger *et al.*^{21,22}

However a change in physical parameters (pressure, temperature) modifies the carbon dioxide mobile phase polarity and consequently alters the IR absorption bands. Stretching vibrations are more disturbed than bending vibrations by variations in carbon dioxide. Frequency shifts are relatively moderate for apolar functional groups such as CH or C=C, but noteworthy with regard to the precision of spectral data measurements; they become more significant for polar functional groups, such as carbonyl or sulphone, where they can reach up to 10 wavenumbers²⁴.

Fig. 6 shows FT-IR spectra of compounds relative to peaks 2 and 3; the absorption bands centered respectively at 3032, 1665 cm^{-1} for the former and at 3029, 1661 cm^{-1} for the latter indicate the presence of a trisubstituted olefinic group. However, the MCT detector cut-off, near 815 cm^{-1} , prevents us from detecting the out-of-plane $\gamma(\text{CH})$ bending vibration of this unsaturated functional group in the 780–800 cm^{-1} region. The FT-IR spectrum of the fourth peak matches that of β -caryophyllene; the absorptions centred at 3074, 1632 and especially 890 cm^{-1} relate to a *gem*-disubsti-

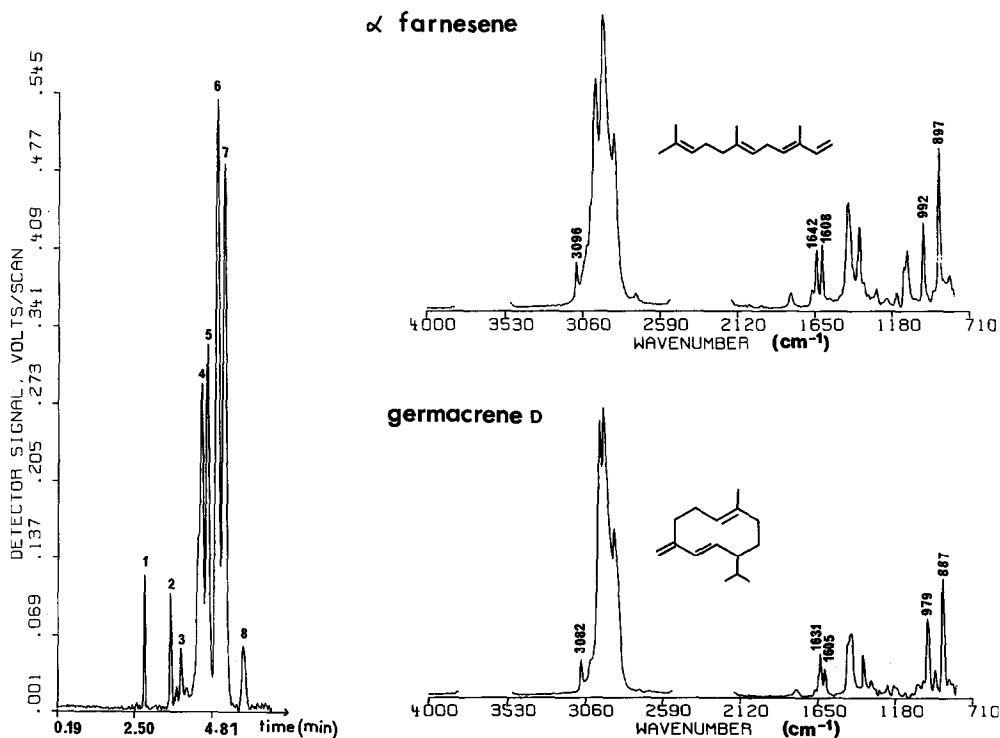


Fig. 7. SFC-FT-IR separation of sesquiterpene hydrocarbons from ylang-ylang oil. Conditions as in Fig. 5. Solutes: 1 = hexane; 2 = α -copaene; 3,4 = unknown; 5 = β -caryophyllene; 6 = α -farnesene; 7 = germacrene D; 8 = unknown.

Fig. 8. Carbon dioxide FT-IR spectra of α -farnesene and germacrene D. Cell pressure, 92 atm; cell temperature, 33°C; carbon dioxide, 0.71 g ml^{-1} ; optical resolution, 8 cm^{-1} .

tuted olefinic group, and the twin bands at 1373 and 1384 cm^{-1} to a *gem*-dimethyl group. Finally, the FT-IR spectrum of the fifth peak suggests the presence of a *gem*-disubstituted olefinic group (3085, 1642, 893 cm^{-1}) and of a trisubstituted olefinic group (3050, 3012, 1674, 830 cm^{-1}), and agrees with that of β -bisabolene.

Ylang-ylang oil. The sesquiterpene hydrocarbon fraction of ylang-ylang 3th²⁵ was separated by SFC-FT-IR on a silica stationary phase (Fig. 7) and further identified by comparison of the FT-IR spectra with those published in the literature. The presence of β -caryophyllene, α -copaene, germacrene D and α -farnesene was confirmed.

The relevant features of the FT-IR spectrum of the sixth elution peak (Fig. 8a) are absorptions at 3096, 992 and 897 cm^{-1} , indicative of a vinyl group, and two absorption bands at 1642 and 1608 cm^{-1} which indicate the presence of a conjugated diene. Also bands at 1667 and 834 cm^{-1} may be assigned to a trisubstituted double bond. These features agree with those of α -farnesene²⁶⁻²⁸.

The FT-IR spectrum of the seventh elution peak (Fig. 8b) suggests the presence of a *gem*-disubstituted olefinic group (3082, 887 cm^{-1}), a *trans*-double bond (979 cm^{-1}), the presence of a conjugated diene (1631, 1605 cm^{-1}) and a *gem*-dimethyl group (twin bands around 1373 cm^{-1}). The results are in good agreement with those obtained with germacrene D. This sesquiterpene hydrocarbon has been observed to photoisomerize to α - and β -bourbonene or to transform thermally in a GC column or on contact with silica gel into several sesquiterpene hydrocarbons²⁹, so the different steps of essential oil analysis (prefractionation by LC, separation by carbon dioxide SFC) always achieved at room temperature allow the identification of this extremely thermally labile compound.

CONCLUSION

At nearly ambient temperatures, sesquiterpene hydrocarbons are conveniently resolved by carbon dioxide SFC-FT-IR on bare silica as stationary phase. The IR transparency domain of supercritical carbon dioxide, *i.e.*, 3500-2500 and 2200-800 cm^{-1} , and the chromatographic parameters applied to resolve a mixture of sesquiterpene hydrocarbons allow the successful identification of all IR vibration modes due to functional groups present in sesquiterpene hydrocarbons. Structural information relative to the nature of the C=C double bond will always be provided by the out-of-plane CH bending vibrations. It is important to emphasize the low temperature required for this separation rather than the low-density mobile phase.

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