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Short communication

Rapid detection of high-molecular-mass dienes in beeswax

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Abstract

A rapid procedure for the detection of high-molecular-mass dienes in waxes, using a combination of chromatographic separations and direct inlet mass spectrometric determinations, allowed the determination of the presence of and the approximate relative concentrations on C₃₁–C₃₅ dienes in some beeswaxes.

1. Introduction

Beeswax is a complex mixture of organic compounds belonging to a number of chemical classes. Aliphatic saturated and monounsaturated compounds have been described in several papers [1]. A mixture of alkanes and alkenes can be easily separated from more polar components by means of column absorption chromatography, using silica gel as the stationary phase and hexane as the eluent. Subsequent separation of the alkenes can be carried out by reversible complex-forming column chromatography on silica gel impregnated with silver nitrate. Whereas GC–MS analysis, using high-resolution capillary columns and electron impact ionization, allows the separation of several alkenes present in the mixture and the determination of their molecular masses, evidence for the presence of dienes could not be obtained. Higher molecular mass conjugated dienes and dienes with isolated

double bonds may be expected to exhibit longer retention times because of their lower volatility. Therefore, it was necessary to develop a rapid procedural variation for the resolution of dienes in beeswax.

2. Experimental

The total hydrocarbon (TH) fraction from beeswax was obtained as described in a previous paper [2]. An alkene fraction was obtained by rechromatographing the TH fraction on a silica gel thick layer (Merck, PSC-Fertigplatten Kieselgel 60 F₂₅₄, layer thickness 2 mm) impregnated with silver nitrate using hexane as the eluent [3]. The spot containing the unsaturated hydrocarbons was detected with iodine, scraped off and extracted with hot hexane. The extract was deposited on the solid introduction system of a mass spectrometer, from which it was slowly fully evaporated into the ion source. Mass spectral data were continuously recorded and stored in a computer, from which it was possible to

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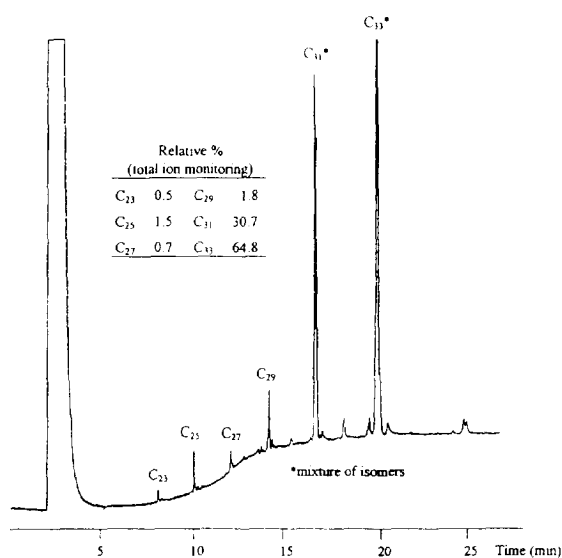


Fig. 1. Typical GC profile (MS detector, positive ions, total ion current, SPB-5 fused-silica capillary column, 30 m × 0.32 mm I.D., 0.25- μ m film thickness, temperature programme, 100–300°C at 10°C min⁻¹) of the unsaturated fraction of hydrocarbons from a European beeswax.

obtain single-ion traces of the evaporated components.

GC-MS and MS measurements were obtained using a Finnigan Model 1020 gas-chromatograph-mass spectrometer, with electron impact ionization in the positive-ion mode (70 eV electron energy).

3. Results and discussion

A typical GC profile of the unsaturated hydrocarbon present in beeswax is shown in Fig. 1. Fig. 2 shows the electron impact (EI) mass spectrum at 70 eV of the peak corresponding to C₃₁H₆₂ alkene (M⁺ = 434 u).

The whole unsaturated mixture was slowly vaporized into the electron impact ion source of the mass spectrometer at 120°C. Under these conditions there was a definite fractionation into two otherwise unresolved mixtures, one made up of the alkenes and a quantitatively much smaller one containing dienes of molecular mass corre-

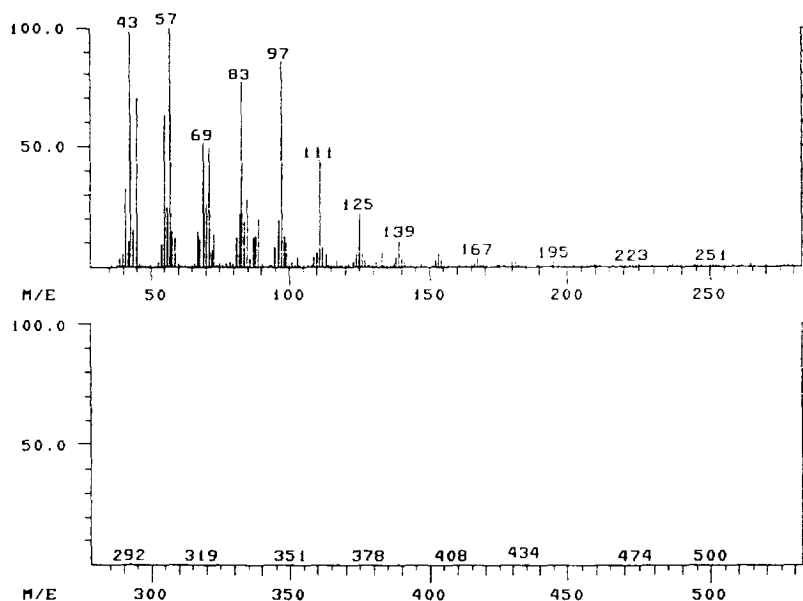


Fig. 2. Typical mass spectrum of an eluate (C₃₁H₆₂) from the capillary GC separation of the components of the alkene fraction of beeswax hydrocarbons.

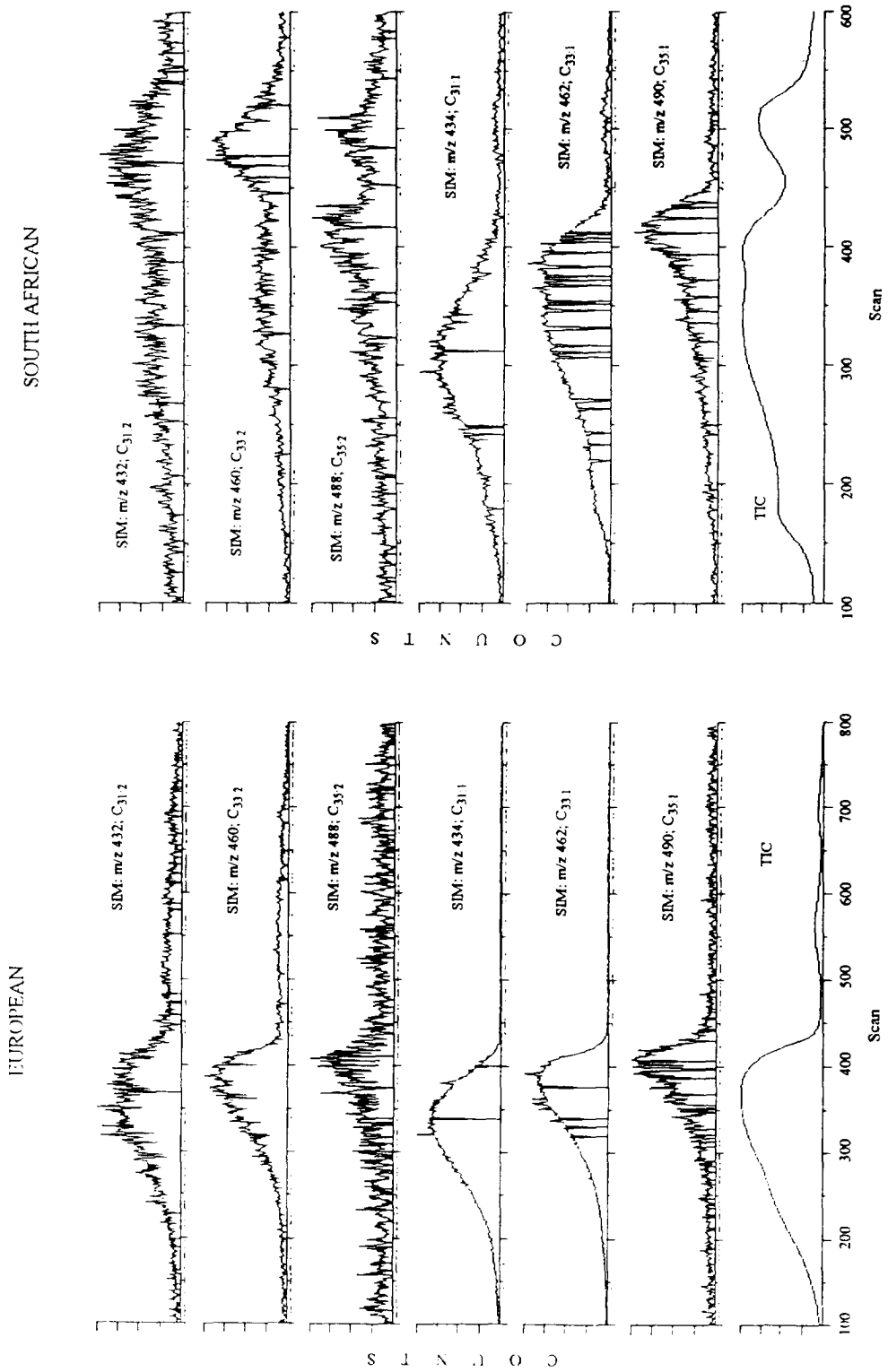


Fig. 3. SIM and total ion current (TIC) of the bulk evaporated unsaturated hydrocarbons in a European and a South African beeswax.

Table 1
Relative abundance of dienes in European and South African beeswax

Carbon No.	European		South African	
	Rel. %	Dienes (%) ^a	Rel. %	Dienes (%) ^a
C ₃₁	25	1.4	19	2.9
C ₃₃	65	3.5	60	9.2
C ₃₅	10	0.7	21	3.2

^a In unsaturated hydrocarbon fraction.

sponding to the GC-detectable alkenes. Single-ion monitoring (SIM) of the molecular ions for individual alkenes and dienes (Fig. 3) plotted against time confirmed the concentration of the dienes in the sublimate at longer times. Integration of the M⁺ ions profiles led to an approximate evaluation of a ratio of 100:6 of the relative molar concentration of alkenes to dienes for a European wax. Also, the ratio of the integrated SIM of the individual parent peaks of the alkenes and dienes allowed the evaluation of their relative abundances (Table 1). The ratios thus obtained for the alkenes matched fairly well with the results of the GC using the MS as a total ion monitor (TIC) (Table 2).

Table 2
Relative abundances determined on GC-separated alkenes (EI-MS) and bulk vaporized alkenes (EI-MS, SIM parent ion) from a South African wax

Carbon No.	GC-MS (%)	SIM (%)
C ₂₃	2	5
C ₂₅	4	9
C ₂₇	3	8
C ₂₉	3	7
C ₃₁	14	16
C ₃₃	57	42
C ₃₅	17	13

Our procedure, in its present form, does not provide structural information on the dienes. A complete study may be effected by double methyl sulfonation of the double bonds, followed by capillary GC-MS [4]. Dienes with internal bonds were found in propolis [5] and in some other material waxes both of animal and plant origin. At least one bond was internal. The type of partial separation obtained in the vaporization process, presenting the C₃₁-C₃₅ alkenes on the far side of the vaporization peak, allows one to infer the structure of unconjugated internal alkenes. The beeswax from the African Cape bee *Apis mellifera capensis* was found to be richer in dienes than that of the European beeswax from *Apis mellifera* var. *ligustica* × *carnica*.

Acknowledgements

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