

Structure determination of sesquiterpenes in Chinese vetiver oil by gas chromatography–tandem mass spectrometry

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ABSTRACT

In Chinese vetiver oil, 118 compounds were characterized according to their chromatographic (GC) and mass spectral (MS) data. Twenty of these compounds were completely identified (name, structure). The GC–MS technique applied involved the use of efficient capillary columns and the acquisition of mass spectra in three different ionization modes (electron impact and positive- and negative-ion chemical ionization). The specificity and sensitivity of the method were greatly enhanced by developing GC–MS–MS experiments under collisionally activated dissociation conditions, the results of which permitted the confirmation of the presence of minor constituents and the determination of the structures of previously undescribed compounds.

INTRODUCTION

The analysis of complex mixtures such as essential oils requires high-resolution chromatographic separation. However, even the use of very efficient columns cannot prevent the overlapping of some peaks. In order to overcome this difficulty, we used a relatively new technique that is particularly well suited to the analysis of complex mixtures, namely tandem mass spectrometry [1] coupled with capillary gas chromatography (GC–MS–MS).

Correct identification of sesquiterpenes is important as a means of checking the authenticity of essential oils, as the profile of sesquiterpenes provides a “fingerprint” for each oil and its particular origin.

In previous work we studied the alcoholic fraction of Bourbon vetiver oil from Reunion [2] and the acetylated fraction of the oil from Java [3]. The aim of this work was to identify the constituents of Chinese vetiver oil by using GC–MS with three different ionization methods: electron impact (EI), positive-ion chemical ionization

(PCI) and negative-ion chemical ionization (NCI). However, in such complex mixtures as vetiver oil, a single chromatographic peak may contain several compounds, therefore making the recorded spectrum difficult to interpret. Tandem mass spectrometry (MS-MS) coupled with GC enables us to analyse each component of such peaks separately. Using GC-MS-MS we have confirmed the presence of minor compounds such as α -cedrene, acoradiene and khusimene and determined two new esters of molecular weights (MW) 324 and 436, which had not been described previously.

EXPERIMENTAL

Chinese vetiver oil was obtained from Sanofi (Grasse, France) and from L'O-réal (Chevilly-Larue, France) and reference compounds α -cedrene from Fluka (Buchs, Switzerland) and cadinenes from A. Michet of Clermont-Ferrand II University (Clermont-Ferrand, France).

Preliminary GC studies with flame ionization detection (FID) were performed on a Varian (Les Ulis, France) Model 3400 gas chromatograph.

GC-MS experiments were performed on a Nermag R10-10C combined gas chromatograph-mass spectrometer controlled by a Digital PDP11-23 Plus system (Delsi-Nermag, Agenteuil, France). Two fused-silica capillary columns were used, one (50 m \times 0.23 mm I.D.) coated with SE-54 liquid phase (0.2 μ m) (Chrompack-France, Les Ulis, France) and the other (60 m \times 0.247 mm I.D.) coated with DB-WAX (0.15 μ m) (J&W Scientific, Folsom, CA, USA). The oven temperature was programmed from 100 to 160°C at 2°C/min, and then to 290°C at 5°C/min for the first column and to 250°C at 5°C/min for the second.

GC-MS analysis of the oxygenated fraction of Chinese vetiver oil was also carried out. This oil was retained on a column filled with silica (40 cm \times 2.5 cm I.D.) with diethyl ether as eluent [4].

Different ionization methods were employed: EI, PCI using NH₃ [5] and N²H₃ [6] (Air Liquide, Le Plessis-Robinson, France) as reagent gas at a pressure of 0.1 Torr (1 Torr = 133.3 Pa) in the ion source and NCI with OH⁻ as reagent from a nitrous oxide-methane (1:4) mixture (Air Liquide) [7,8].

Subsequent MS-MS studies were carried out using a Finnigan MAT (Hemel Hempstead, UK) TSQ70 triple quadrupole mass spectrometer under collisionally activated dissociation (CAD) conditions. Argon was used as collision gas at a pressure of $1.2 \cdot 10^{-3}$ Torr and the collision energy was 10 eV. Three different scan modes were used: daughter scan, parent scan and neutral loss scan.

The peak matching technique [9] was used to establish the exact mass of the esters and was carried out on a VG70E double-focusing mass spectrometer (VG Instruments, Le Chesnay, France) possessing electric and magnetic sectors.

RESULTS AND DISCUSSION

Gas chromatographic separation

The gas chromatogram of Chinese vetiver oil (Fig. 1) exhibits about 150 peaks, and 118 compounds were detected from these peaks and characterized according to their retention times and mass spectra obtained in the EI, PCI and NCI ionization modes. We identified specifically twenty of these compounds (name and structure)

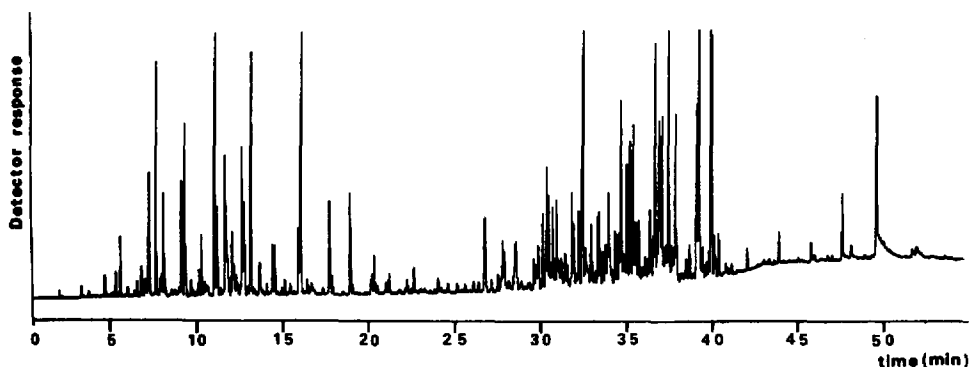


Fig. 1. GC-FID of Chinese vetiver oil. Column, DBWAX (60 m \times 0.247 mm I.D.); pressure, 2.2 atm.

(Fig. 2) either with literature data [2,3,10–13] or with reference compounds when available [14].

Although we used a high-resolution capillary column, some peaks unfortunately overlapped (Fig. 3), making mass spectrum interpretation more difficult. GC-EI-MS-MS enabled us to analyse each component of such peaks separately [1].

Tandem mass spectrometry

Chinese vetiver oil does not have exactly the same composition as Bourbon vetiver oil. It was therefore necessary to confirm the presence of α -cedrene, acoradiene and khusimene, which are minor and isomeric compounds; their EI mass spectra are mixed together in the GC-MS analysis and consequently correct identification by comparison with EI spectra of the standards cannot be ensured.

α -Cedrene GC-EI-MS-MS. The EI mass spectrum of α -cedrene standard (Fig. 4a) does not fit the EI mass spectrum of α -cedrene in the mixture (Fig. 4b), which shows extra peaks.

To confirm the presence of this compound in the vetiver oil, GC-EI-MS-MS experiments were carried out. The CAD mass spectra daughter ions of m/z 204 and neutral loss of 28 u for standard α -cedrene (Fig. 4b and c) are very close to the CAD spectra obtained at the relevant point of α -cedrene in the mixture (Fig. 4e and f) and confirm that this compound is undoubtedly α -cedrene.

Acoradiene and khusimene GC-EI-MS-MS. The EI mass spectra of acoradiene (Fig. 5a) and khusimene (Fig. 5b) show base peaks at m/z 136 and 134, respectively, produced by a retro-Diels-Alder reaction. Consequently, the GC-EI-MS-MS experiments concerned the neutral loss of 68 u (Fig. 5c) and the parent ions of m/z 136 (Fig. 5e) for acoradiene and the neutral loss of 70 u (Fig. 5d) and the parent ions of m/z 134 (Fig. 5f) for khusimene. These results enabled us to assign the structures of acoradiene and khusimene for these two compounds.

GC-EI-MS-MS of two new products. The GC-EI-MS of Chinese vetiver oil (Fig. 6) shows the presence of unknown compounds of molecular weights 324 and 436, just at the end of the chromatogram. Two groups of peaks are observed in the chromatogram; we only analysed peaks A and B (Fig. 6) as the others are isomers of these compounds. Using GC-MS and GC-MS-MS we tried to establish the structure of these new, natural products observed in Chinese vetiver oil.

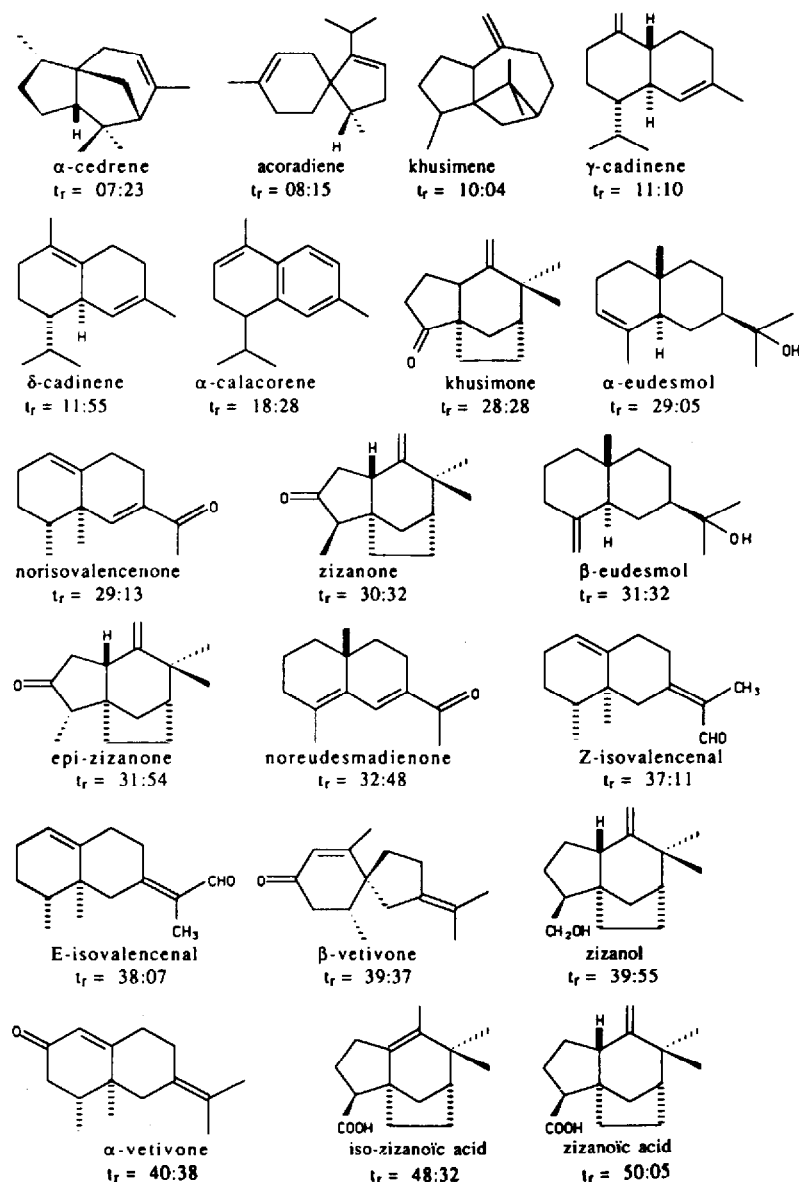


Fig. 2. Twenty sesquiterpenes identified in Chinese vetiver oil with names, formulae and retention times (min:s).

Figs. 7a and b show the EI mass spectra at 70 eV of the unknown compounds of molecular weights 324 (A) and 436 (B). We observed for A and B characteristic ions of sesquiterpenes such as at $m/z = 131, 133, 145, 159, 173$ and 187 . For compound A, the base peak is $m/z = 91$ and an important fragment ion is observed at $m/z = 233$. For compound B, the base peak is $m/z 202$.

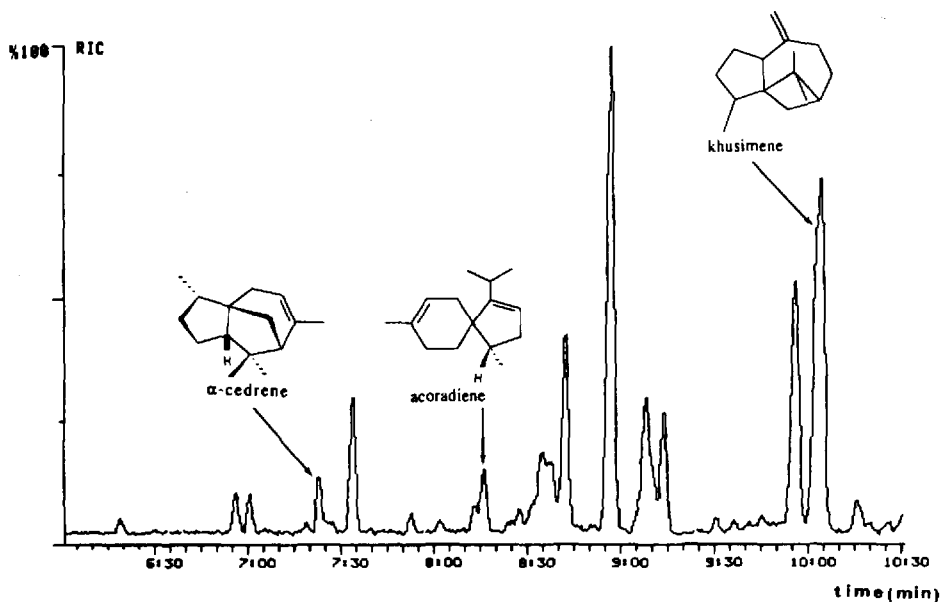


Fig. 3. GC-EI-MS expanded chromatogram of the region concerning α -cedrene, acoradiene and khusimene in Chinese vetiver oil.

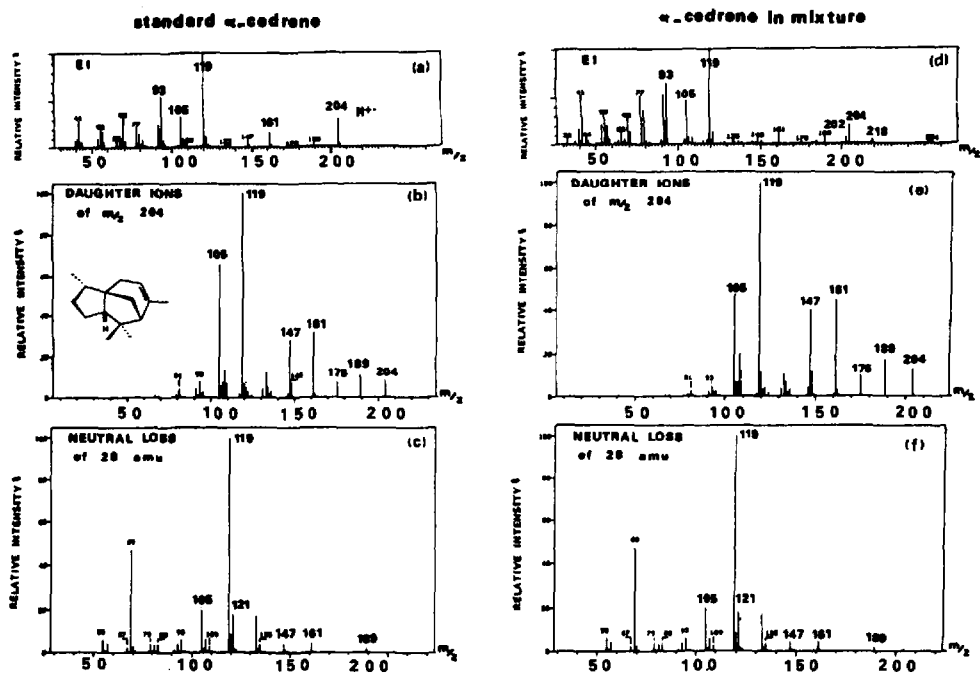


Fig. 4. (a) EI mass spectrum of standard α -cedrene at 70 eV; (b), (c) CAD mass spectra of standard α -cedrene at 10-eV collision energy; (d) EI mass spectrum of α -cedrene in the mixture at 70 eV; (e), (f) CAD mass spectra of α -cedrene in the mixture at 10-eV collision energy.

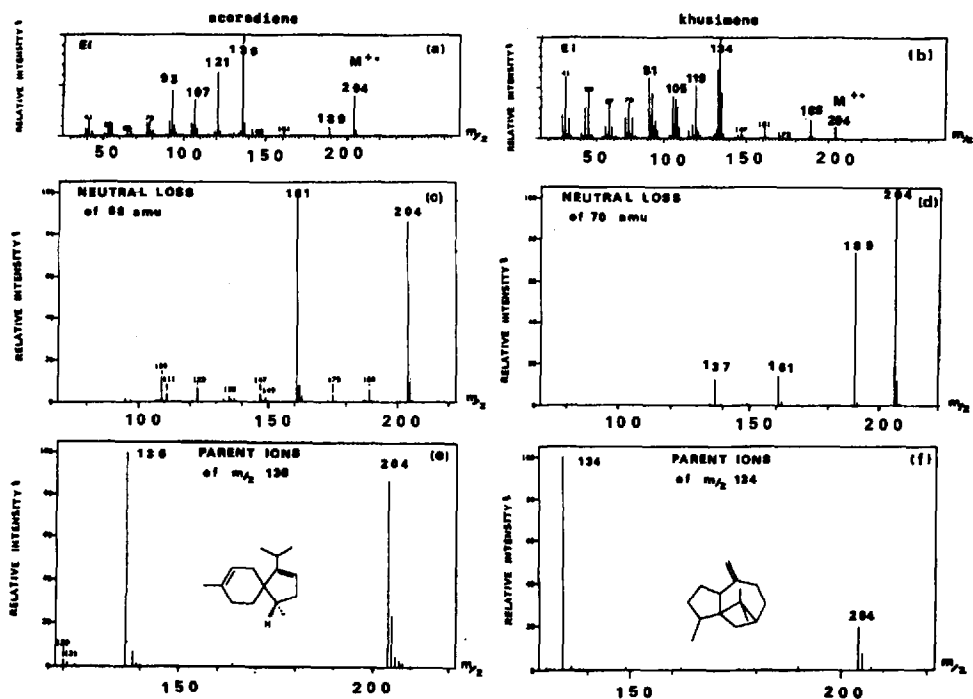


Fig. 5. (a) EI mass spectrum of acoradiene at 70 eV; (b) EI mass spectrum of khusimene at 70 eV; (c), (e) CAD mass spectra for acoradiene at 10-eV collision energy; (d), (f) CAD mass spectra for khusimene at 10-eV collision energy.

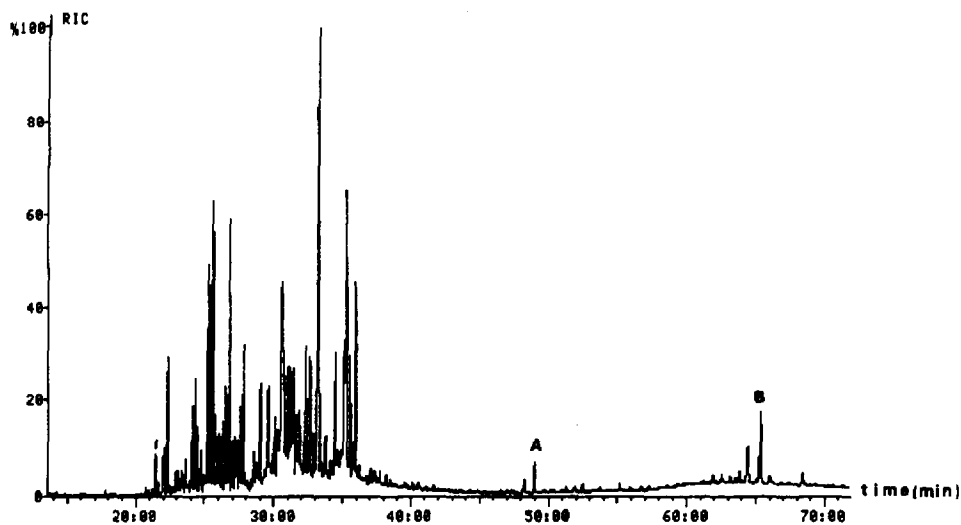


Fig. 6. GC-EI-MS chromatogram of Chinese vetiver oil. Column, SE-54 (50 m \times 0.23 mm I.D.); pressure, 1.8 atm.

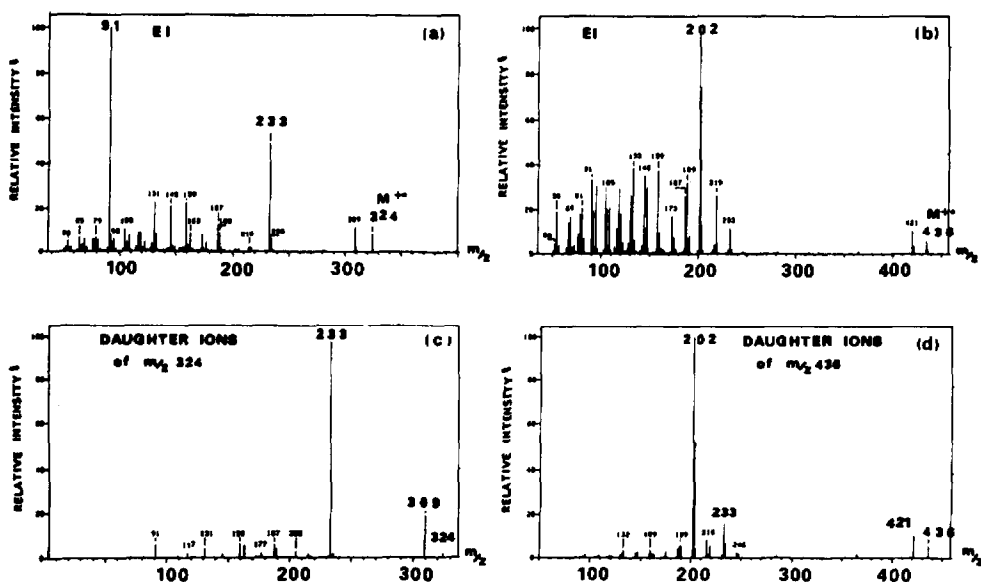


Fig. 7. (a) EI mass spectrum of compound A at 70 eV; (b) EI mass spectrum of compound B at 70 eV; (c) CAD mass spectrum daughter ions of m/z 324 for compound A at 10-eV collision energy; (d) CAD mass spectrum daughter ions of m/z 436 for compound B at 10-eV collision energy.

The spectra of compounds A and B were also obtained by GC-PCI ($N^2H_4^+$)-MS and this technique confirmed that no labile hydrogen exists in these two molecules.

To complete these investigations we used CAD experiments with a collision energy of 10 eV to examine the daughter ions of m/z 324 (Fig. 7c), the parent ions of m/z 233 and 91 and the neutral loss of 15 u from compound A. We also determined the daughter ions of m/z 436 (Fig. 7d) and the parents of m/z = 202 and 233 for compound B. In conclusion, the ions at m/z 233 from A and m/z 202 from B are only generated by the molecular ions of m/z 324 and 436, respectively.

Exact masses and formulae were obtained for compounds A and B by the peak matching technique. The results are given in Table I.

All the MS and MS-MS experiments confirmed the presence of esters of formula $C_{22}H_{28}O_2$ (MW = 324) and $C_{30}H_{44}O_2$ (MW = 436).

Two possible suggestions for compound A are $RCOOC_6H_4CH_3-p$ or $RCOOCH_2C_6H_5$. If RCOOH is an acid of Chinese vetiver oil, it could be isozizanoic acid (Fig. 2), whose fragmentation under EI ionization is the same as that observed in the spectrum of compound A (Fig. 7a). The benzyl ester formula is the more likely of the two with regard to the base peak at m/z 91 observed in the EI mass spectrum (Fig. 7a).

For the ester B we propose the formula $RCOOR'$, in which RCOOH is an acid (MW = 234) and R'OH is an alcohol (MW = 218) both of which are present in vetiver oil.

It is difficult to determine the structures of the esters precisely because they are present in very small proportions in Chinese vetiver oil (0.27% of the total). There-

TABLE I
EXACT MASSES AND FORMULAE OF COMPOUNDS A AND B

Data were obtained on a VG70E double-focusing mass spectrometer.

Compound	Nominal mass	Exact mass	Formula
A	324	324.2087	C ₂₂ H ₂₈ O ₂
	309	309.1853	C ₂₁ H ₂₅ O ₂
	233	233.1540	C ₁₅ H ₂₁ O ₂
B	436	436.3340	C ₃₀ H ₄₄ O ₂
	421	421.3104	C ₂₉ H ₄₁ O ₂
	203	203.1799	C ₁₅ H ₂₃
	202	202.1720	C ₁₅ H ₂₂

fore, GC-infrared measurements are not possible. Other vetiver oils from Bourbon Reunion, Java and Haiti were also analysed with the same techniques but the esters A and B were found only in Chinese vetiver oil.

In conclusion, although GC-MS is a very useful tool for the analysis of complex mixtures such as essential oils, a more sophisticated technique such as GC-MS-MS is required to confirm the presence and to determine the structures of minor compounds found in such oils. This paper has illustrated the successful application of tandem mass spectrometry and confirmed the specificity, sensitivity and speed of this technique.

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