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STUDY OF THREE TYPES OF ESSENTIAL OIL OF *VALERIANA OFFICINALIS* L. s.l BY COMBINED GAS CHROMATOGRAPHY–NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

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SUMMARY

OH^- ions have been shown to produce abundant $(M-H)^-$ ions of most oxygenated mono- and sesquiterpenes. Esters are cleaved by an apparent nucleophilic displacement reaction, with the formation of $RCOO^-$ ions.

Three types of essential oil of valerian, having different chemical compositions, were subjected to combined gas chromatography–OH^- negative ion chemical ionization mass spectrometry. Extracted ion current profiles, reconstructed by the data system, of various $(M-H)^-$ ions and $RCOO^-$ ions, have given a clear insight into the presence of the components of interest. The method is particularly suitable for the identification of esters, as shown by the results obtained for acetates and isovalerates.

INTRODUCTION

The analysis of the essential oil of a valerian strain, in which about 70 compounds could be identified, has led to the discovery of three types of essential oil within that strain[1]. Using gas chromatography–electron impact mass spectrometry (GC–EI-MS) and gas chromatography–chemical ionization mass spectrometry (GC–CI-MS), some questions could not be answered satisfactorily for the following reasons: (a) several compounds, such as terpene alcohols and their esters, do not produce an observable molecular ion upon electron impact, and even isobutane CI often fails to give molecular weight information; (b) the molecular weight (M) may be ascribed to different compound classes, *e.g.*, $M = 238$ ($C_{15}H_{26}O_2$) corresponds to both sesquiterpenoids and isovalerates of monoterpene alcohols.

It has been demonstrated in this work that negative ion CI-MS has great potential in improving this situation.

OH^- ions[2], generated in a CI source from a mixture of nitrous oxide and methane, abstract a proton from oxygenated terpenoids with the formation of $(M-1)^-$ ions. With some exceptions, the $(M-1)^-$ ions are observed with a high relative abundance[3]. A nucleophilic attack of the OH^- ions on esters produces $(R\text{–}COO)^-$ ions, presenting a simple and elegant method for the identification of the acid part of an ester, which is hardly possible in positive ion MS.

One of the advantages of GC–MS–computer systems is that the computer can store thousands of spectra, taken by repetitive scanning, on tape or disk. After the data aquisition has been completed a total ion current profile[4] can be reconstructed by the computer which is generally identical with a gas chromatogram when the mass spectrometer is operated in the EI mode.

Identification of a particular compound is greatly assisted by the construction of the extracted ion current profile[4] of an intense ion from the spectrum of the substance of interest. A peak will be observed in this profile at the position where the compound elutes from the gas chromatograph. If the ion is a ubiquitous fragment ion, however, the extracted ion current profile may contain almost as many peaks as the total ion current profile. Unfortunately, the intense fragmentation observed in the EI spectra of terpenoids, combined with the qualitative similarity of many of their EI spectra, renders this method generally unsuccesful for fragment ions, and insensitive for molecular ions. CI spectra are, by their simplicity, better suited for the generation of extracted ion current profiles.

The three types of essential oil of valerian were subjected to OH^- negative ion CI, to explore the potential of a combination of OH^- negative ion CI with extracted ion current profiles when applied to complex mixtures.

EXPERIMENTAL

Plant material and essential oils

The essential oils were obtained by distillation of the roots of a number of individual plants, grown in the experimental garden at Buitenpost. The method according to the Dutch Pharmacopoeia (VIth edition) was used. Three types were distinguished (A, B and C)[1].

Negative ion chemical ionization mass spectrometry

A Finnigan 3300 GC–MS system equipped with a standard CI source was used. Mass spectra were obtained under computer control using the Finnigan 6110 Data System.

Samples were injected on to a 40 m × 0.5 mm SE-30 wall-coated open tubular column with a splitting ratio of approximately 1:20. Data acquisition was started 4.00 min after injection. Mass spectra were taken repetitively with 1-sec cycle time. Total ion current chromatograms and extracted ion current profiles were reconstructed by the computer.

In the negative ion CI mode, the electron collector and the ion repeller were shorted to the source block. To obtain the desired voltages on the source and lens, the appropriate number of 9.5-V batteries were connected in series with the original source and lens supplies. Negative ion detection was accomplished by the conversion dynode method[5]. The system was insensitive owing to the use of an old electron

multiplier. The original flow controller and needle valve in the reactant gas and calibration gas inlets were replaced with Brooks ELF fine needle valves (needle No. 1, equipped with numerical handles; Brooks Instrument, Veenendaal, The Netherlands). Methane and nitrous oxide could thus be introduced in any desired ratio with very smooth and reproducible control. The thermocouple gauge measuring the source pressure was calibrated for methane and nitrous oxide against a McLeod gauge. A 1:1 mixture at a total pressure of 0.6 torr (corrected) was used. The indicated source temperature was kept between 100 and 110° by a CRL 405 digital temperature controller (CRL, Worthing, Great Britain).

RESULTS AND DISCUSSION

The total ion current chromatograms of the three types of essential oil are presented in Fig. 1. A rather good similarity was obtained with the chromatograms obtained previously by capillary GC–EI-MS. Notable differences were as follows.

(a) Mono- and sesquiterpene hydrocarbons are observed with a relatively low sensitivity, probably because the protons in these compounds are more difficult to abstract.

(b) The peaks of some major components have a limited height, which may be ascribed to the large sample size injected to counteract the low sensitivity of the detection system. As a result, the OH^- ions may no longer be present in a large excess, yielding less product ions. Further, the possible formation of $(2M-1)^-$ cluster ions from $(M-1)^-$ ions and non-ionized sample molecules may play a role. They fall outside the mass range of interest during data acquisition and are consequently not included in the total ion current chromatogram, resulting in an apparent loss of sensitivity at the high sample concentrations at which this phenomenon mainly occurs. Normally shaped peaks could be obtained when the sample size was reduced, at the expense, of course, of the quality of the spectra of minor components.

The main differences between the three types have nevertheless been confirmed (percentages were taken from ref. 1).

Type A: elemol scan 1125–1140 (2.4–4.9 %)
valeranone scan 1335–1355 (6.2–8.7 %)
valerenal scan 1415–1430 (13.4–15.9%)
"M=238" (α-kessyl alcohol) and "M=280" (its corresponding acetate) were not observed by EI GC–MS, and were not detectable in the negative ion total ion current profile.

Type B: elemol scan 1125–1140 (9.8–11.7%)
valerenal scan 1415–1430 (10.3–12.0%)
valeranone, "M=238" and "M=280" were absent in the EI and negative ion total ion current profiles.

Type C: elemol scan 1125–1140 (1.9–2.8%)
valeranone scan 1335–1355 (16.2–18.2%)
"M=238" scan 1355–1375 (9.3–10.3%)
valerenal scan 1415–1430 (3.3–3.9%)
"M=280" scan 1740–1755 (3.5–4.3%)

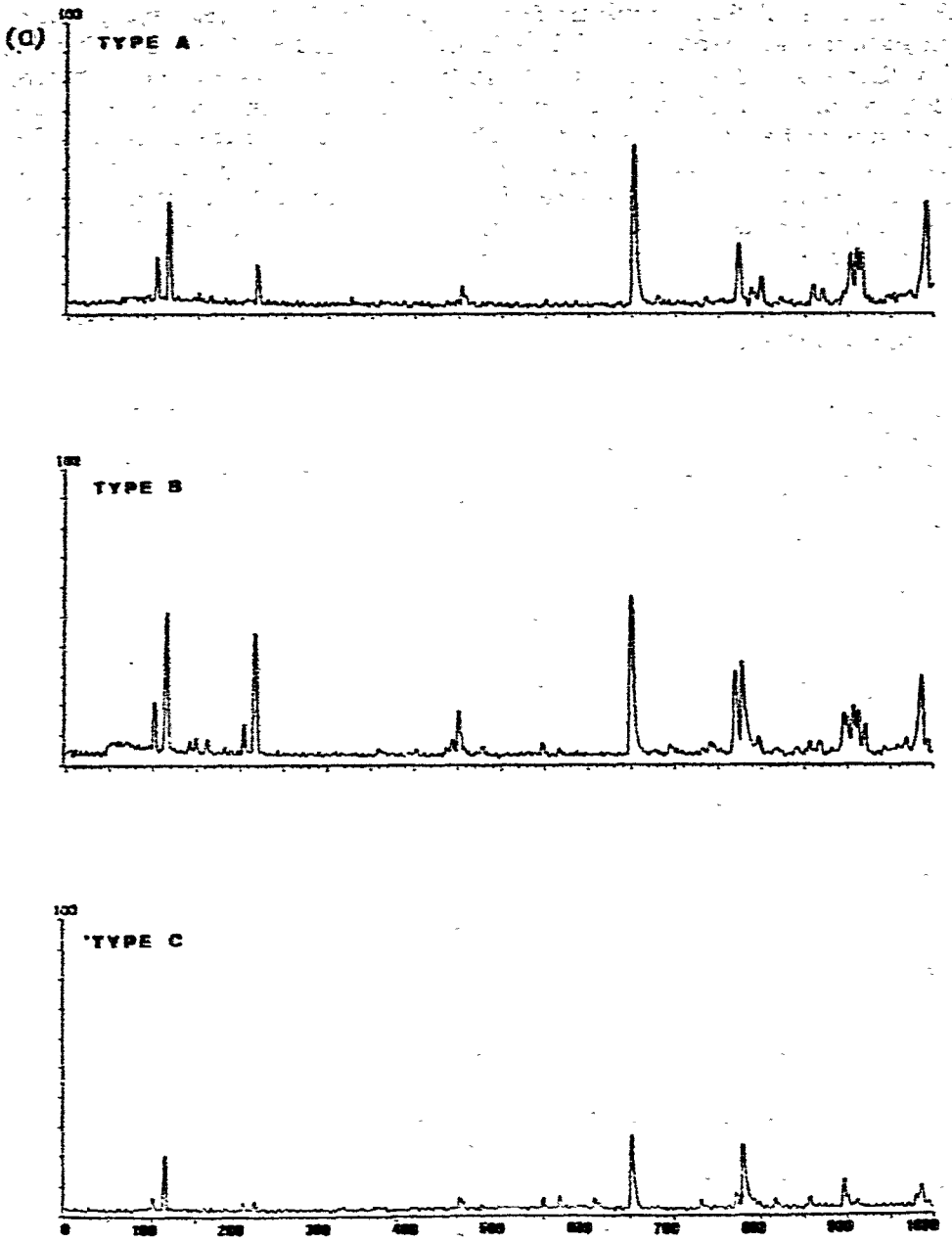


Fig. 1.

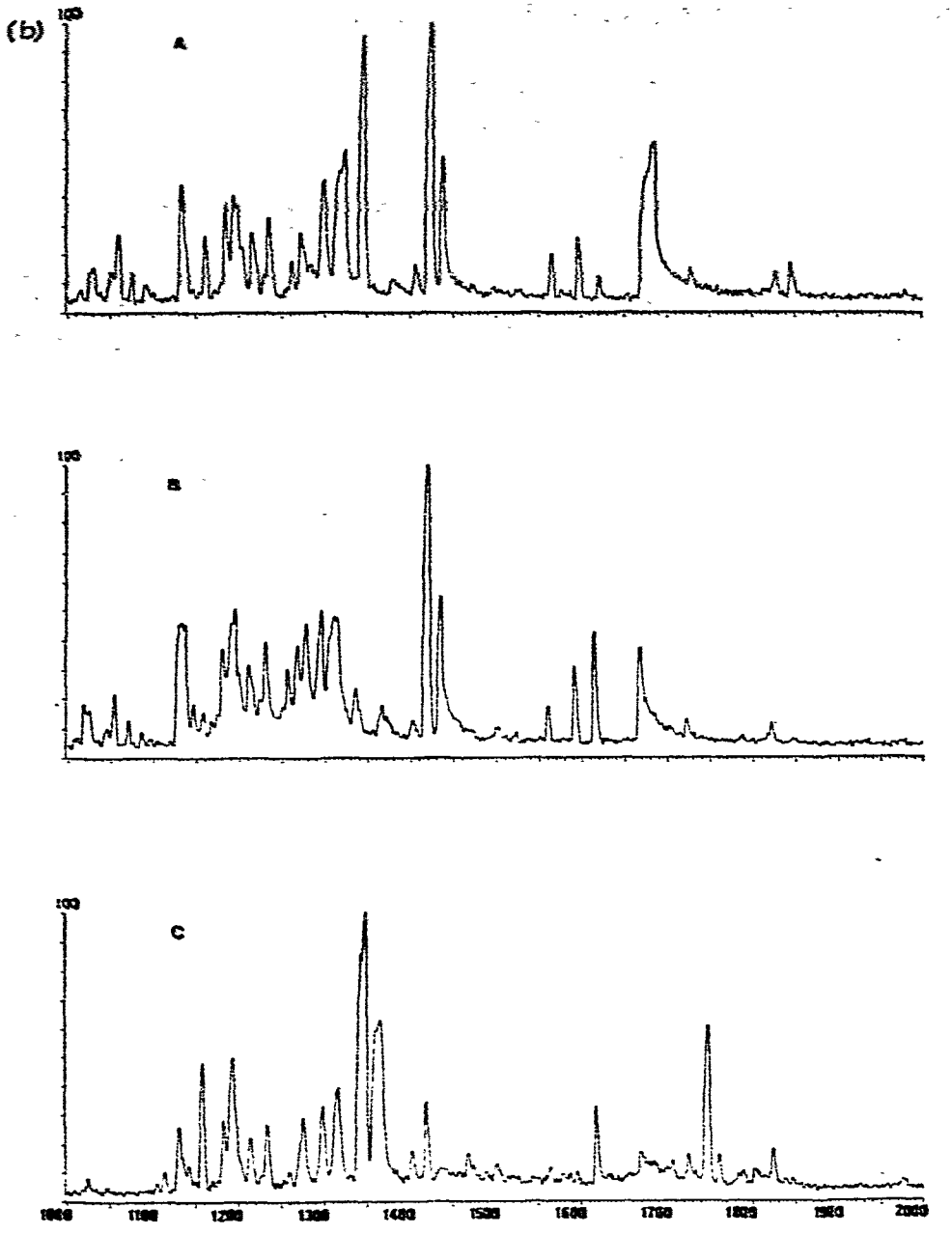
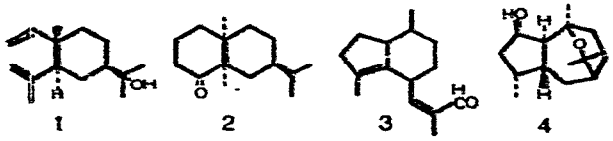


Fig. 1. Total ion current profiles of the three types (A, B and C) of essential oil of *Valeriana officinalis* obtained by OH^- negative ion chemical ionization GC–MS. (a) Scan 1–1000; (b) scan 1000–2000.

Structures of the compounds are shown in Fig. 2.



1 2 3 4

Fig. 2. Structures of (1) elemol, (2) valeranone, (3) valerenal and (4) α-kessyl alcohol.

Extracted ion current profiles were generated from the data files of types A, B and C on $m/e = 279$ $(C_{17}H_{27}O_3)^-$, 239 $(C_{15}H_{27}O_2)^-$, 237 $(C_{15}H_{25}O_2)^-$, 221 $(C_{15}H_{25}O)^-$, 219 $(C_{15}H_{23}O)^-$, 217 $(C_{15}H_{21}O)^-$, 101 [$(C_5H_9O_2)^-$, isovalerate] and 59 [$(C_2H_3O_2)^-$, acetate].

m/e = 279 (Fig. 3)

A peak could be observed in type C between scan 1740 and 1755 ("*M*=280") and in smaller amounts between scan 1700 and 1710 and 1755–1765 (also kesso compounds?) When an amplification (10 ×) was used, small amounts could also be

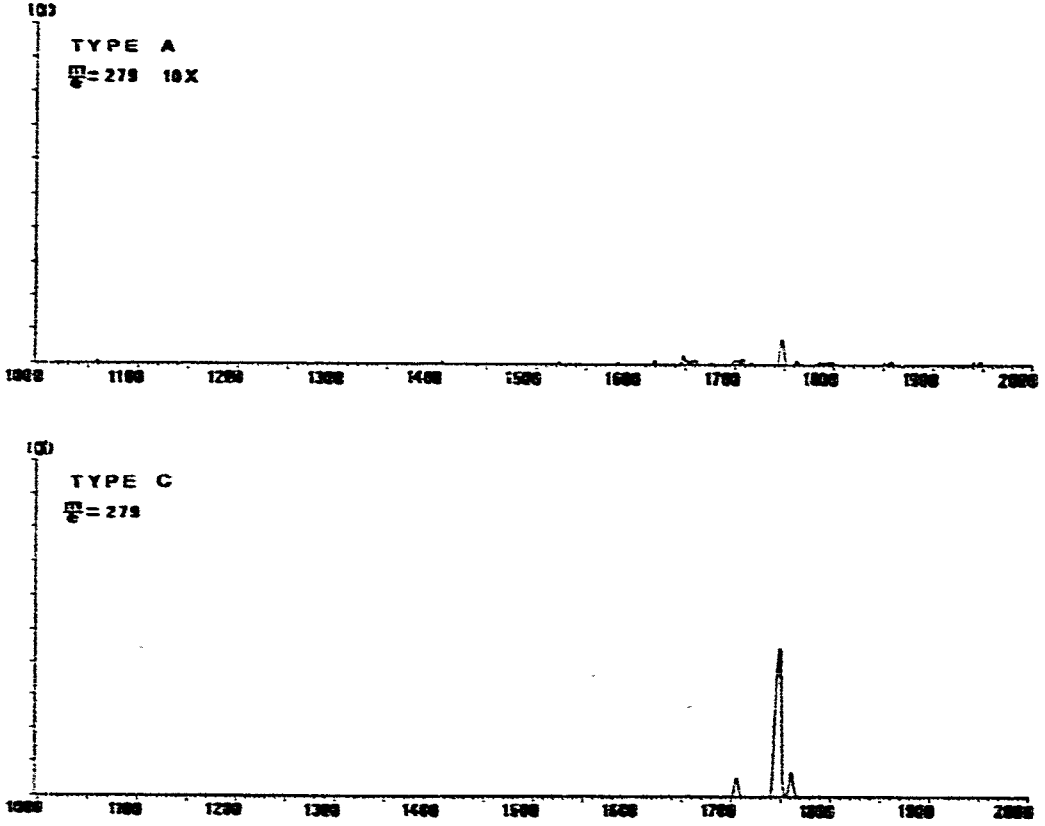


Fig. 3. Extracted ion current profiles ($m/e = 279$) obtained by OH^- negative ion chemical ionization GC–MS of types A and C.

identified in types A (Fig. 2) and B at the same position. This means that "M=280" is present in each type, which could not be established using GC–EI-MS.

m/e = 239

This was present in all three types between scan 1185 and 1195. It corresponds with an isovalerate of a monoterpene alcohol, probably citronellyl isovalerate (M=240), because *m/e*=101 shows a peak between the same scan numbers.

m/e=237

This was present in type C at scan 1355–1375, and represents "M=238" (α-kessyl alcohol). The presence of an isovalerate can be excluded because *m/e*=101 did not show a peak at these scan numbers.

In some other positions both *m/e* 237 and *m/e* 101 show a maximum, which may be ascribed to isovalerates of monoterpene alcohols.

m/e=221, 219 (Fig. 4)

These $(M-1)^-$ ions are present between scan 1100 and 1400, corresponding to oxygenated sesquiterpenes.

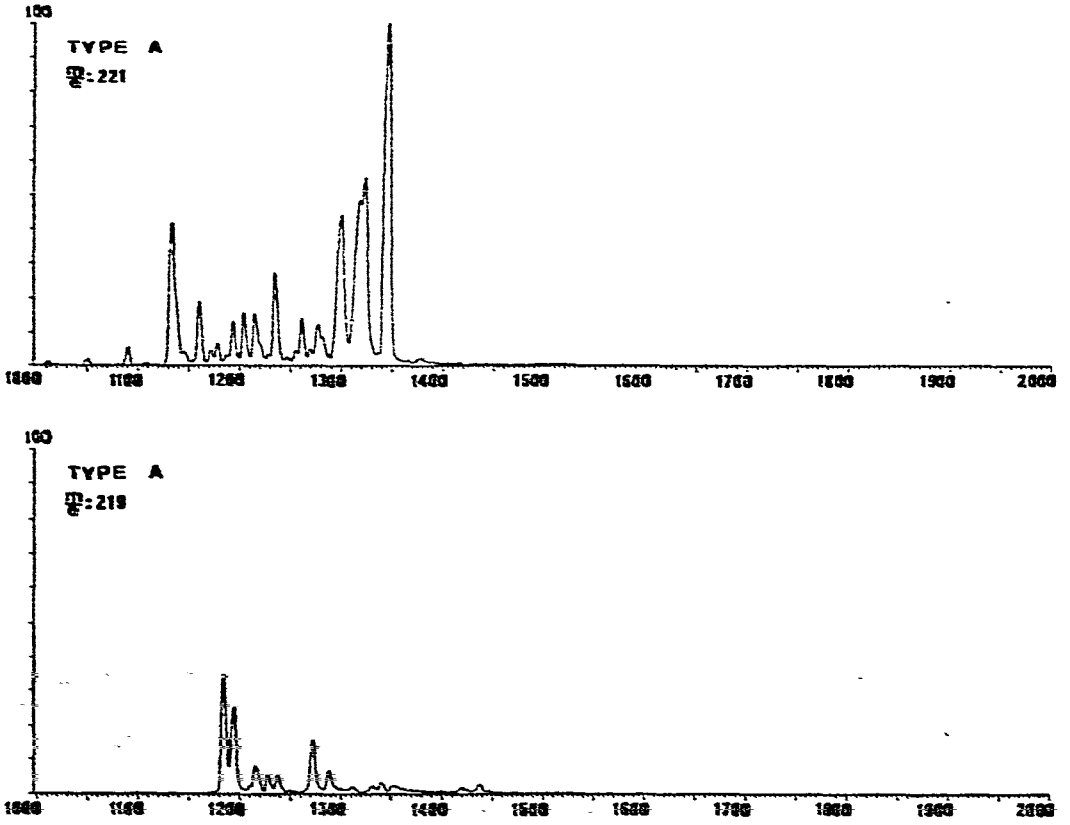


Fig. 4. Extracted ion profiles (*m/e* = 221 and 219) obtained by OH^- negative ion chemical ionization GC–MS of type A.

m/e=217

This was observed in all three types between scan 1415 and 1430 (valerenal) and in some other positions.

m/e=101 (Fig. 5)

This was present in all types between scan 1185 and 1195 (citronellyl isovalerate), 1395–1415 (unknown) and 1495–1510 (*cis*-carveyl isovalerate). At scan 1825 a distinct peak of *m/e*=101 can also be observed (see also *m/e*=59).

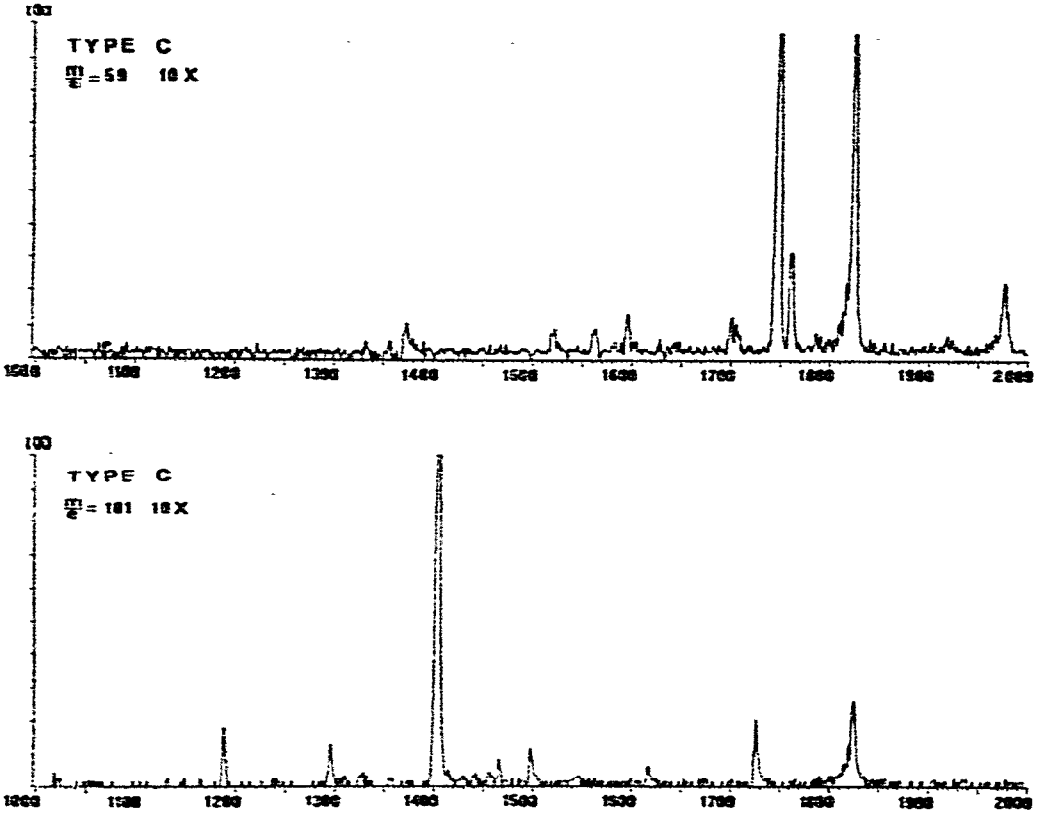


Fig. 5. Extracted ion profiles (*m/e* = 59 and 101) obtained by OH^- negative ion chemical ionization GC–MS of type C.

m/e=59

In the first part of the chromatogram this ion can be observed at scan numbers 648–660, 725–735, 770–777 and 770–790. These peaks correspond to bornyl acetate, *cis*-carveyl acetate, citronellyl acetate and α-terpenyl acetate. In the second part of the chromatogram (Fig. 5) this ion can be found between scan 1500 and 1650, representing sesquiterpene acetates (see *m/e*=261, 263).

Between scan 1820 and 1830 a peak having the same shape is observed in the profile of *m/e*=101. Probably it represents a decomposition product of valepotriates

in which the acetate and isovalerate moieties are still present. The EI spectrum shows some similarities with those of some decomposition products of valepotriates[6].

The presence of isobutyrates, reported to be present in the essential oil of valerian[7] and of propionates, could not be confirmed because the extracted ion current profiles of m/e=87 $(C_3H_7COO)^-$ and m/e=73 $(C_2H_5COO)^-$ did not give any response. Extracted ion current profiles of the ions listed below have been generated from the data file of type C.

m/e=263, 261

Peaks can be observed in the same positions between scans 1500 and 1650, where the acetate ion is also present. Sesquiterpene acetates are thus clearly identified.

m/e=247, 163

These fragments show a response at scan 1470–1480 and scan 1610–1630, corresponding to eugenyl and isoeugenyl isovalerate. A peak could hardly be observed in the extracted ion current profile of m/e 101, which could be predicted from the negative ion mass spectrum of these compounds (247=17%, 163=100%, 101=2%).

m/e=197, 195, 193

This was present in the first part of the chromatogram at the scan numbers where m/e 59 maximized. It represents monoterpene acetates (see above). In one instance the extracted ion current profile of m/e 59 does not show a peak, and represents dihydro-β-ionone (M=194).

m/e=191

This ion is present in the position of β-ionone (scan 1020–1035).

m/e=155, 153, 151

These $(M-1)^-$ ions represent oxygenated monoterpenes (alcohols, aldehydes and ketones) eluting from SE-30 columns after monoterpene hydrocarbons but before sesquiterpene hydrocarbons.

Some unsaturated acyclic alcohols, such as nerol, geraniol and farnesol, produce very weak $(M-H)^-$ ions upon reaction with the OH^- ion[3]. Intense $(M-H-H_2O)^-$ ions are observed instead. These compounds are consequently not observed in the extracted ion current profiles of the M−1 ions of mono- and sesquiterpene alcohols.

CONCLUSION

Although the identities of many components in essential oils can be established by means of EI and positive ion CI, application of OH^- negative ion MS furnishes exact data on the molecular weights of oxygenated terpeneoids, in most instances where positive ion CI fails. With the exception of the esters of substituted phenols, such as (iso)eugenol, information is also obtained concerning the RCOO– part of esters. Extracted ion current profiles of $RCOO^-$ ions thus give a very convenient insight into the presence of esters in essential oils. Our instrumentation for negative

ion MS has not yet been optimized, but it appears that it is a very sensitive method, as demonstrated by the presence of trace amounts of "M=280" in types A and B, which could be distinctly shown. This was not possible using GC–EI-MS.

Finally, it can be easily understood that OH^- negative ion GC–MS can be applied to the quantitative analysis of complex mixtures because very simple spectra are obtained which do not mutually interfere.

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