Journal of Chromatography, 294 (1984) 269–279
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 16,732

ANALYSIS OF PEPPER AND CAPSICUM OLEORESINS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY AND FIELD DESORPTION MASS SPECTROMETRY

DAVID E. GAMES* and NICHOLAS J. ALCOCK
Department of Chemistry, University College, P.O. Box 78, Cardiff CF1 1XL (U.K.)
and
JAN VAN DER GREEF, LAMBERTUS M. NYSSEN, HENK MAARSE and MICHAEL C. TEN NOEVER DE BRAUW
CIVO Institutes TNO, P.O. Box 360, 3700 AJ Zeist (The Netherlands)
(Received March 1st, 1984)

SUMMARY

Using a combination of high-performance liquid chromatography–mass spectrometry with a moving-belt interface, field desorption mass spectrometry and high-resolution accurate mass electron impact mass spectrometry, the major components of the oleoresins of black pepper and capsicum have been identified. Nordihydro-, dihydro- and homo-capsaicin together with capsaicin and a series of fatty acids were found in the capsicum oleoresin. A series of N-isobutyltrienamides and dienamides, together with piperettin and piperine isomers and piperolein A and B, piperanine and piperylin were identified in the black pepper oleoresin.

INTRODUCTION

Analysis of the pungent principles of pepper[1–12] and capsicum oleoresins[13–19] have been the subject of many studies. A variety of sample clean-up procedures and chromatographic techniques have been used in these investigations and mass spectrometry (MS) has played an important role in the characterization of oleoresin components. Because of the thermal lability and low volatility of many of the compounds present, mass spectral studies have used either direct insertion probe analysis of purified components[1,2] or gas chromatography (GC)–MS of suitable derivatives[13] for identification.

The advent of newer mass spectral ionization techniques and sophisticated sample introduction systems for mass spectral analysis offer new possibilities for the analysis of these compounds. Field desorption (FD) is a soft ionization technique which can be used to directly examine complex mixtures without prior separation[20]. This is because the ions formed are predominantly molecular ions and protonated molecular or cationized molecules according to compound type and relative molecular mass profiles can be obtained providing an indication of the overall composition

0021-9673/84/$03.00

of the sample. Recently high-performance liquid chromatography (HPLC) has been directly combined with mass spectrometry and combined HPLC–MS can be used for the analysis of mixtures not amenable to GC–MS because of their low volatility and/or thermal instability[21–24].

We report here on our studies of the oleoresins of black pepper and capsicum using both HPLC–MS and FD-MS. The HPLC–MS studies were performed using a moving-belt interface which enabled both electron impact (EI) and chemical ionization (CI) mass spectral data to be obtained.

EXPERIMENTAL

HPLC–MS measurements were performed at University College Cardiff, and the EI, FD and off-line liquid chromatographic experiments were conducted at CIVO-TNO.

A Finnigan 4000 quadrupole mass spectrometer equipped with a moving-belt interface and interfaced to an Incos 2300 data system was used for the on-line HPLC–MS measurements.

Typical conditions were: EI, source temperature 250°C (indicated), source pressure $8 \cdot 10^{-7}$ Torr, vaporizer 180°C, clean-up heater 215°C; CI (ammonia), source temperature 150°C, source pressure $2.4 \cdot 10^{-5}$ Torr. A Partisil 5-μm 300 × 4.6 mm column was used for the liquid chromatographic separation of the black pepper oleoresin with ethanol–hexane–acetic acid (4:95:1) as the mobile phase. For the capsicum oleoresin analysis a Spherisorb ODS 5-μm 250 × 4.6 mm column with methanol–water–acetic acid (70:28:2) as the eluent was used for HPLC–EI-MS and a Hypersil ODS 3-μm 150 × 4.6 mm column with ethanol–water–acetic acid (50:49:1) was used for HPLC–CI-MS. In both cases the mobile phase flow-rate was 1 ml min^{-1}.

High-resolution ($2 \cdot 10^{+4}$) electron impact measurements were performed by peak matching on a Varian MAT 711 mass spectrometer using a direct-probe inlet system with perfluorokerosene as the reference substance.

FD measurements were made on a Varian MAT 731 mass spectrometer equipped with a combined EI–field ionization (FI)–FD source and interfaced to a SS 100 data system. The emitter temperature was programmed with an emission control unit and the mass spectrometer was cyclicly scanned at 6 sec/decade. All the spectra from one analysis were summed resulting in a total profile of the sample. A methanol solution of the oleoresins was applied to the indene activated emitter using the syringe technique.

Off-line HPLC was conducted in the following manner. A solution (10 μl) of the black pepper oleoresin (*Piper nigrum*) in chloroform (150 mg l^{-1}) was injected onto a Polygossil 60-5 column (250 × 4.6 mm) and eluted with chloroform–hexane (70:30) which was partially saturated with water. The procedure used was to saturate half of the mobile phase system with water and to then mix this solution with the other half of the solution which had not come into contact with water. The flow-rate was 1.5 ml min^{-1} and the UV detector (343 nm) was used. During these studies light was excluded as much as possible. A 10-μl volume of a capsicum oleoresin solution (1–10 g/100 ml) in tetrahydrofuran–methanol (1:1) was injected onto a Hypersil ODS column (125 × 4.6 mm) and eluted with a methanol–0.1 *N* silver nitrate (60:40) solution. The flow-rate was 2 ml min^{-1} and a fluorescence detector was used.

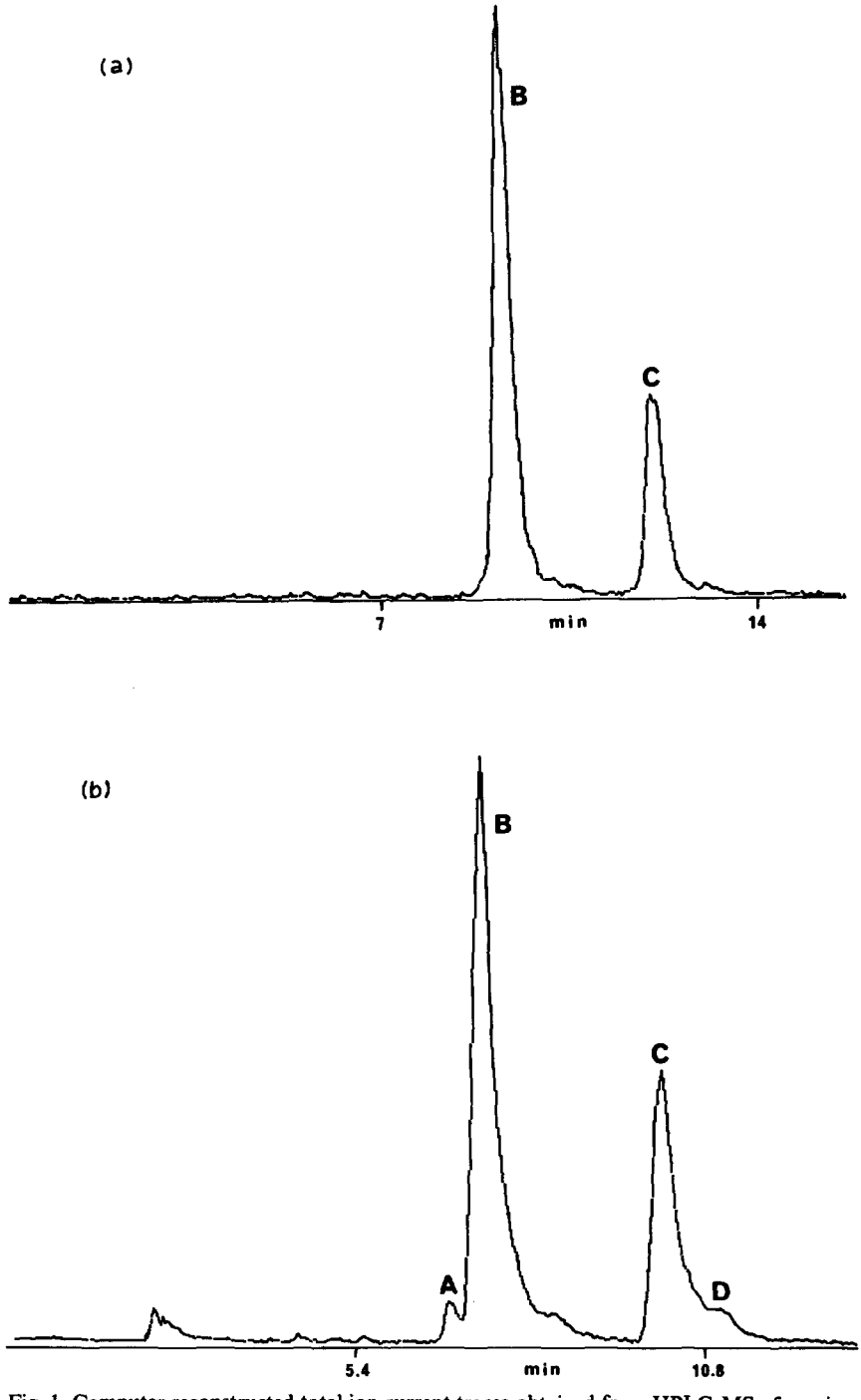


Fig. 1. Computer reconstructed total ion current traces obtained from HPLC–MS of capsicum oleoresin. (a) EI conditions; (b) ammonia CI conditions.

RESULTS AND DISCUSSION

Capsicum oleoresin

Fig. 1a and b show the computer-reconstructed total ion current traces obtained using HPLC–EI-MS and HPLC–(ammonia) CI-MS from the capsicum oleoresin. Different liquid chromatographic conditions were used for the CI study to see if minor components known to be present in the mixture from liquid chromatographic studies (Fig. 2) could be resolved. The mass spectra obtained from the HPLC–MS studies are summarized in Table I. Only two peaks were observed in the HPLC–EI-MS trace and search for minor components by mass chromatographic techniques failed to show their presence. The major component (peak B) showed a molecular ion at *m/z* 305 whereas the minor component (peak C) gave *m/z* 307 for its molecular ion. Both spectra contained *m/z* 137 as their base peaks and a series of minor peaks at *m/z* 122, 152 and 195. The HPLC–(ammonia) CI-MS data for these components confirmed their relative molecular mass measurements and in addition, in contrast to their EI spectra differences were observed in the lower mass region of their spectra. Ions at *m/z* 187, 170 being present for the major component and ions at *m/z* 189, 172 for the minor component. These ions must arise from the acyl portion of the molecule and are probably thermally induced. The *m/z* 170, 172 ions arising through rearrangement and cleavage of the nitrogen benzylic carbon bond with the corresponding *m/z* 187 and 189 ions being ammonia adduct ions. These data suggest capsaicin (1) and dihydrocapsaicin (1a) as the structures of these compounds. Accurate mass measurements under EI conditions of the samples obtained gave elemental compositions $C_{18}H_{27}NO_3$, $C_{18}H_{29}NO_3$ and $C_8H_9O_2$ to *m/z* 305, 307 and 137 confirming these assignments.

HPLC–CI-MS showed the presence of two further components. Peak A showed a $(M + 1)^+$ ion at *m/z* 294 and fragment ions at *m/z* 175, 158, 152 and 137 suggesting it to be nordihydrocapsaicin (1c). Peak D gave a $(M + 1)^+$ ion at *m/z* 320 and fragment ions at *m/z* 201 and 184 suggesting it to be homocapsaicin (1d).

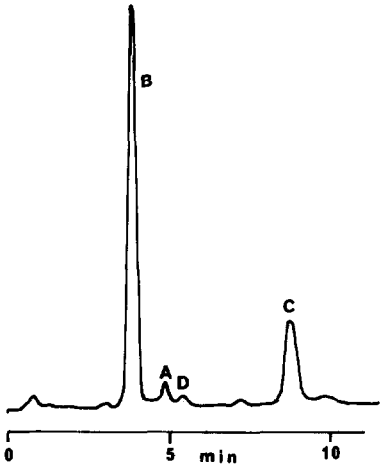


Fig. 2. Liquid chromatogram of capsicum oleoresin.

TABLE I

ELECTRON IMPACT AND AMMONIA CHEMICAL IONIZATION MASS SPECTRA OBTAINED FROM HPLC–MS OF CAPSICUM OLEORESIN

Peak	*Ionization*	*m/z (% rel. int.)*
A: nordihydrocapsaicin (1c)	CI	294 (14), 175 (87), 158 (65), 152 (49), 137 (100)
B: capsaicin (1a)	EI	305 (6), 195 (2), 152 (10), 137 (100), 122 (6)
	CI	306 (36), 187 (20), 170 (51), 154 (22), 152 (2), 137 (100)
C: dihydrocapsaicin (1b)	EI	307 (11), 195 (5), 152 (10), 137 (100), 122 (6)
	CI	308 (31), 189 (54), 172 (47), 154 (20), 152 (33), 137 (100)
D: homocapsaicin (1d)	CI	320 (4), 201 (31), 184 (100)

The presence of these compounds known to be present in capsicum oleoresin was confirmed by high resolution accurate mass measurements and comparison of liquid chromatographic retention times with authentic samples. These liquid chromatographic studies (Fig. 2) were performed under different conditions to the HPLC–MS studies (see Experimental) and nordihydrocapsaicin and homocapsaicin have different elution orders.

1a R=$(CH_2)_4CH{=}CHCH(CH_3)_2$

1b R=$(CH_2)_6\ CH(CH_3)_2$

1c R=$(CH_2)_5\ CH(CH_3)_2$

1d R=$(CH_2)_5CH{=}CHCH(CH_3)_2$

The FD mass spectrum of a methanol solution of the capsicum oleoresin is shown in Fig. 3. The spectrum is dominated by peaks assigned to fatty acids which were not observed under the liquid chromatographic conditions used. Palmitic acid (mol.wt. 256), hexadecanoic acid (mol.wt. 254), stearic acid (mol.wt. 284), oleic acid (mol.wt. 282), linoleic acid (mol.wt. 280) and linolenic acid (mol.wt. 278) are found. The concentration ratio of these fatty acids from the FD spectrum, 28.3:6.4:8.7:35.9:100:11.4 agrees well with the literature values[25] of 30.7:3.1:6.3:32.6:100:8.6. Further examination of the spectrum shows the presence of capsaicin (*m/z* 305) and dihydrocapsaicin (*m/z* 307). The peak at *m/z* 137 is attributed to fragmentation of the molecular ions of capsaicin and related compounds at higher emitter temperatures. Peaks in the *m/z* 830–890 region are probably due to triglycerides and those in the *m/z* 570–630 region may be fragment ions or diglycerides. We have as yet been unable to identify the nature of the peak at *m/z* 338.

Black pepper oleoresin

The computer-reconstructed total ion current trace obtained by HPLC–EI-MS of the black pepper oleoresin is shown in Fig. 4. Thirteen peaks can be identified and the EI and CI mass spectral data obtained are summarized in Table II. Using com-

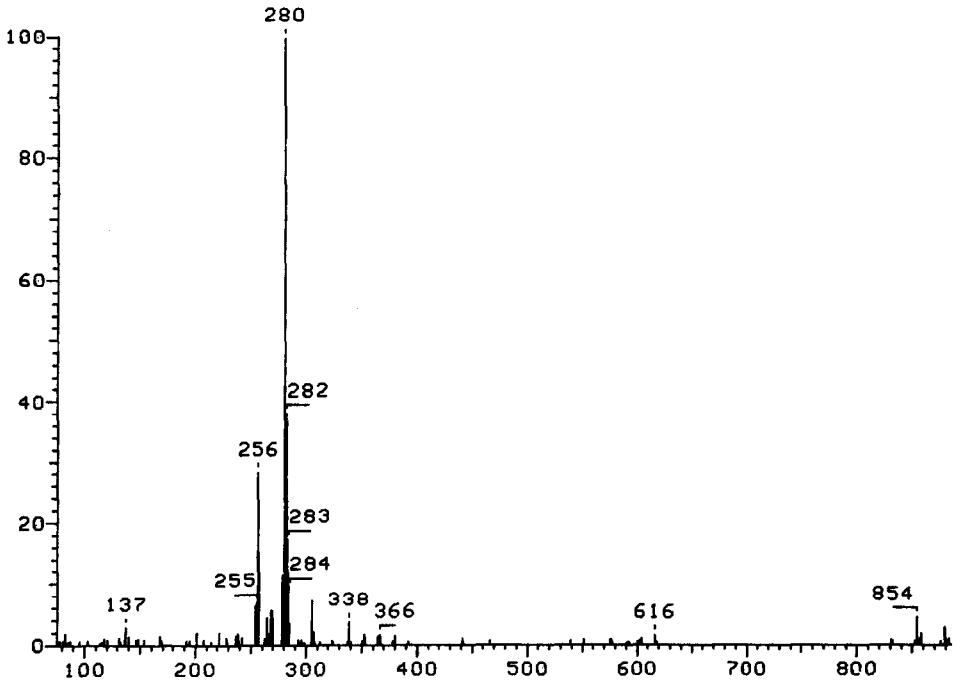


Fig. 3. Field desorption mass spectrum of capsicum oleoresin.

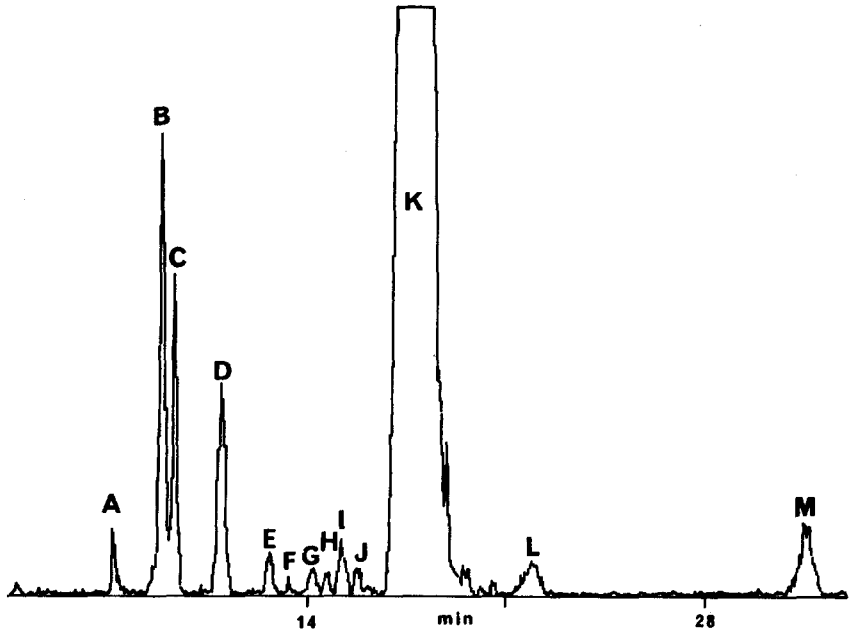


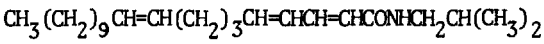
Fig. 4. Computer-reconstructed total ion current trace obtained from HPLC–EI-MS of black pepper oleoresin.

TABLE II

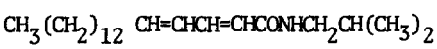
ELECTRON IMPACT AND CHEMICAL IONIZATION DATA OBTAINED BY HPLC–MS OF BLACK PEPPER OLEORESIN AND ELEMENTAL COMPOSITIONS OBTAINED BY ACCURATE MASS MEASUREMENTS OF CRUDE SAMPLE

Peak	*Identification*	*Elemental composition*	*Mol.wt.*	*Ionization*	*m/z (% rel. int.)*
A	Sitosterol (2)	$C_{29}H_{50}O$	414	EI	416 (6), 396 (6), 255 (8), 213 (10), 166 (22), 138 (76), 127 (94), 69 (100)
B	N-Isobutyleicosa-trienamide (3)	$C_{24}H_{43}NO$	361	EI	361 (12), 335 (7), 263 (38), 180 (20), 152 (72), 115 (33), 96 (47), 81 (100)
	N-Isobutyloctadecadienamide (4)		335	CI	362 (69), 336 (100), 236 (19)
C	N-Isobutyloctadecatrienamide (5)	$C_{22}H_{39}NO$	333	EI	333 (18), 261 (12), 180 (21), 152 (61), 115 (38), 95 (37), 81 (100), 67 (58)
				CI	334 (100), 177 (5), 110 (5)
D	Piperolein B (6)[26]	$C_{21}H_{29}NO_3$	343	EI	343 (28), 208 (26), 182 (18), 140 (71), 135 (38), 131 (43), 127 (100), 112 (33), 103 (41), 84 (50)
				CI	344 (100), 252 (5), 150 (5), 140 (8), 128 (10)
E	Mixture containing piperolein A (7)[26]		315	EI	354 (13), 315 (12), 169 (25), 161 (8), 135 (100), 127 (80), 112 (18)
F	Piperettin isomer (8)	$C_{19}H_{21}NO_3$	311	EI	311 (35), 198 (52), 169 (55), 141 (100), 139 (37), 115 (50), 112 (44), 78 (43)
G	Unknown			EI	248 (13), 161 (56), 152 (29), 135 (98), 131 (100), 103 (52), 81 (54), 77 (35)
H	Dihydropiperettin isomer (9)	$C_{19}H_{23}NO_3$	313	EI	313 (10), 161 (66), 131 (100), 103 (34)
				CI	314 (100), 152 (18)
I	Piperanine isomer (10)	$C_{17}H_{21}NO_3$	287	EI	287 (7), 202 (2), 174 (5), 135 (100)
				CI	288 (100)
J	Piperettin isomer (8)	$C_{19}H_{21}NO_3$	311	EI	311 (38), 227 (20), 198 (58), 169 (47), 141 (100), 115 (70), 112 (40)
				CI	312 (100)
K	Piperettin isomer (8)	$C_{19}H_{21}NO_3$	311	EI	311 (55), 227 (30), 198 (48), 169 (79), 141 (100), 115 (40)
				CI	312 (100)
	Two piperine isomers (11)	$C_{17}H_{19}NO_3$	285	EI	285 (45), 201 (88), 173 (37), 143 (30), 115 (100), 84 (36)
				CI	286 (100)
L	Piperine isomer (11)	$C_{17}H_{19}NO_3$	285	EI	285 (42), 201 (100), 173 (35), 143 (27), 115 (93), 84 (40)
				CI	286 (100)
M	Piperylin isomer (12)	$C_{16}H_{17}NO_3$	271	EI	271 (40), 201 (100), 173 (32), 143 (23), 115 (74)
				CI	272 (100)

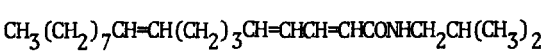
puter enhancement routines peak 11 could be resolved into at least four components. Using a combination of high-resolution accurate mass measurements made on the total black pepper oleoresin and the HPLC–MS mass spectral data we have been able to identify most of the components in the mixture.



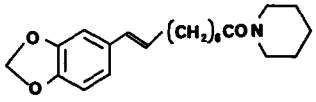
3



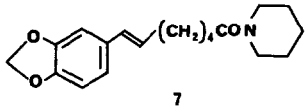
4



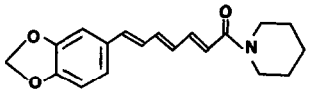
5



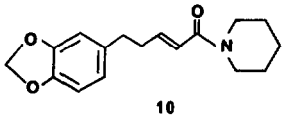
6



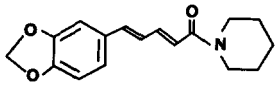
7



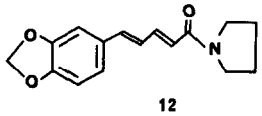
8



10



11



12

Compounds 2–8 and 10–12 have been reported previously[1,26]. The CI data enabled peak B to be confirmed as a multicomponent peak and on the basis of the

EI and CI data is a mixture of N-isobutyleicosatrienamide (3) and N-isobutyloctadecadienamide (4). Identification of peak E was hampered by it consisting of two components of mol.wt. 354 and 315. The latter is probably piperoleine A[26] because two significant ions were found at *m/z* 135 assigned to a methylenedioxytropylium (or methylenedioxybenzyl) ion and *m/z* 127 (which is formed by fission of the band *β* to the carbonyl group and hydrogen migration (McLafferty rearrangement)[2].

The FD mass spectrum of the black pepper oleoresin is shown in Fig. 5. The two major peaks at *m/z* 285 and 287 can be ascribed to piperine and piperanine isomers. In addition to some fatty acids (*m/z* 256, 280 and 282) an interesting series of probable homologues are found at *m/z* 333, 347, 361 and 375. Two of them *m/z* 333 and 361 were also detected by HPLC–MS and using high resolution mass measurement this series can be identified as N-isobutyl-R-trienamides with R = octadeca, nonadeca, eicosane and heneicosane. This series of compounds have been reported previously[1]. By use of high-resolution EI measurements the presence of N-isobutyldienamides with mol.wt. 335 ($C_{22}H_{41}NO$) and 377 ($C_{25}H_{47}NO$) was indicated. The former having been located in the HPLC–MS studies.

A further study of the oleoresin was made using off-line HPLC–FD-MS. The oleoresin of black pepper was injected onto a Polyglossil 60-5 column and the fractions containing the peaks indicated in Fig. 6 were collected and studied by FD. Based on the relative molecular mass information obtained by FD, the LC retention times and high-resolution EI mass spectra, peaks A, D and E were identified as piperettin isomers. Peaks F and I were assigned to piperine (2-*trans*,4-*trans*) and piperylin respectively. No identification could be made for peaks, B, G and H.

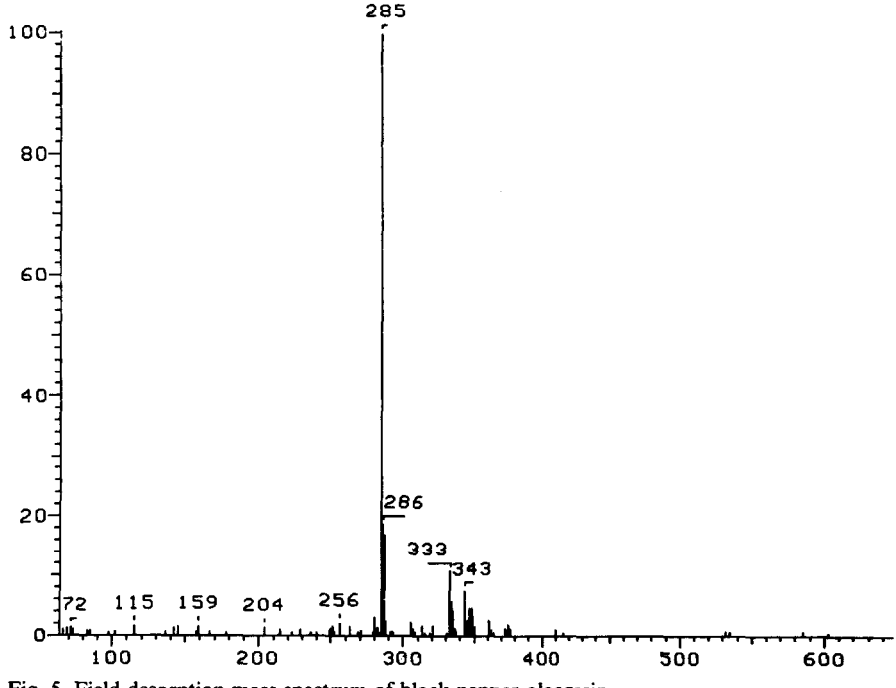


Fig. 5. Field desorption mass spectrum of black pepper oleoresin.

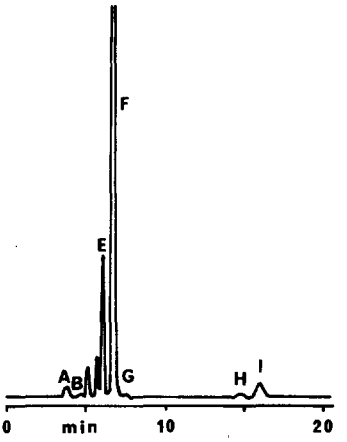


Fig. 6. Liquid chromatogram of black pepper oleoresin.

CONCLUSIONS

HPLC–MS using a moving-belt interface is well suited for the identification of compounds present in oleoresins especially when high-resolution accurate mass data are also available. A clear advantage of the moving-belt interface is the ability to obtain EI spectra which can be directly compared with existing EI libraries. In addition in cases where it is necessary to confirm the molecular weight of compounds the system can be used in the chemical ionization mode of operation.

FD-MS provides a useful complementary technique to HPLC–MS since it enables a total profile of the sample to be obtained and hence indicates if components have been degraded or lost during the HPLC–MS investigation. The presence of various isomers cannot be detected by this method, however, the advantages of using a combination of techniques are well illustrated by these identification of fatty acids present in the oleoresins by FD-MS, which were not found in the HPLC–MS study.

Combination of on-line HPLC–MS, FD-MS, high-resolution EI-MS and off-line HPLC–FD-MS has enabled the major pungent principles of the oleoresins of capsicum and black pepper to be identified.

ACKNOWLEDGEMENTS

N.J.A. thanks the Analytical Trust Fund of the Royal Society of Chemistry for a studentship. We thank the SERC and Royal Society for financial assistance for the purchase of mass spectral and liquid chromatography equipment.

REFERENCES

1 J. Borup-Grochtmann, *Thesis*, University of Munich, Munich, April 1978.
2 J. W. Loder, A. Moorhouse and G. B. Russell, *Aust. J. Chem.*, 22 (1969) 1531.
3 M. Rathnawathie and K. A. Buckle, *J. Chromatogr.*, 264 (1983) 316.
4 S. Narasimham and D. Rajalakshmi, *J. Food Sci. Technol.*, 18 (1981) 11.
5 K. G. Rashuveer and S. M. Anathakrishna, *J. Food Sci. Technol.*, 17 (1980) 268.
6 L. J. van Gemert, L. M. Nijssen, A. T. H. J. de Bie and H. Maarse, in A. A. Williams and R. K. Atkin (Editors), *Sensory Quality in Food and Beverages*, Ellis Horwood, Chichester, 1980, p. 266.

7 H. Maarse and L. M. Nijssen, *Nahrung*, 24 (1980) 29.
8 H. Verzele, P. Mussche and S. A. Qureshi, *J. Chromatogr.*, 172 (1979) 493.
9 R. de Cleijn and M. Verzele, *Chromatographia*, 5 (1972) 346.
10 W. G. Galetto, D. E. Walger and S. M. Levy, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 951.
11 M. A. Sumathikutty, K. Rajaraman, B. Sankarikutty, C. S. Narayanan and A. G. Mathew, *Lebensm.-Wiss.-Technol.*, 14 (1981) 225.
12 V. S. Govindarajan, *CRC Crit. Rev. Food Sci. Nutr.*, 9 (1977) 115.
13 Y. Massada, K. Hashimoto, T. Inoue and M. Suzuki, *J. Food Sci.*, 35 (1978) 858.
14 E. L. Johnson, R. E. Majors, L. Werum and P. Reihe, in G. Charalambous (Editor), *Analysis of Food and Beverages*, Academic Press, London, 1979, p. 17.
15 J. Juvenitsch, E. Bingler, H. Becker and W. Kubelka, *Planta Med.*, 36 (1979) 54.
16 J. Juvenitsch, M. David, F. Heresch and W. Kubelka, *Planta Med.*, 36 (1979) 61.
17 J. A. Maga, *CRC Crit. Rev. Food Sci. Nutr.*, 6 (1975) 177.
18 A. Saria, F. Lembeck and G. Skofitsch, *J. Chromatogr.*, 208 (1981) 41.
19 P. H. Todd Jr., M. G. Bensinger and T. Biftu, *J. Food Sci.*, 42 (1977) 660.
20 G. W. Wood, *Tetrahedron*, 38 (1982) 1125.
21 P. J. Arpino, in T. M. Vickery (Editor), *Liquid Chromatography Detectors*, Marcel Dekker, New York, 1983, p. 243.
22 D. E. Games, *Advan. Chromatogr.*, 21 (1983) 1.
23 R. C. Willoughby and R. F. Browner, in J. F. Lawrence (Editor), *Trace Analysis*, Vol. 2, Academic Press, New York, 1982, p. 69.
24 Z. F. Curry, *J. Liquid Chromatogr.*, 5 (Suppl. 2) (1982) 257.
25 U. J. Salzer, *Fette, Seifen, Anstrichm.*, 77 (1975) 446.
26 R. Grewe, W. Freist, H. Neumann and S. Kersten, *Chem. Ber.*, 103 (1970) 3752.