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Strategy for the Analysis of Cuticular Hydrocarbon Waxes from Insects Using Gas Chromatography/Mass Spectrometry with Electron Impact and Chemical Ionization

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Gas chromatographic/mass spectrometric methods have been developed for the analysis of cuticular hydrocarbon waxes from termites, ants and house flies. A combination of electron impact, chemical ionization with ethylene oxide, methane and ammonia together with methoxy mercuration followed by reductive demercuration, enabled alkane and alkene components of waxes from *Reticulitermes* termites, *Hypoponera eduardi*, *Camponotus Vagus*, and *Cataglyphis cursor* ants and *Calliphora Vomitora* house flies to be characterized.

INTRODUCTION

Cuticular hydrocarbons have been analysed for many insect species.[1–4] These compounds are important for protection against water loss, microorganisms and abrasion, in addition to acting as either pheromones for recognition[5–14] or as sex pheromones[14–22] in social insects. In termites, the importance of cuticular hydrocarbons has been demonstrated in species recognition.[8,23–26] It has been suggested that similar cuticular hydrocarbons allowed integration of termitophiles and myrmecophiles into termite and ant colonies.[26–31]

The role of cuticular hydrocarbons in the recognition of species is to determine what perceptive mechanisms and recognition cues are involved. The chemiotaxonomy describes their behaviour and the method which has been generally used for their analysis is gas chromatography coupled with mass spectrometry (GC/MS) and data system.

The purpose of this present paper is to describe a useful strategy for determination of the cuticular hydrocarbon wax composition for several classes of social insects (Table 1) using GC/MS. The ionization mode generally used was electronic impact (EI). Some papers have mentioned use of methane chemical ionization (CI), but we show here the systematic use of chemical ionization (CI) with methane, ammonia, nitric oxide and ethylene oxide (oxirane) [EO], a new CI reagent gas. This last gas gave good selectivity and all the CI mass spectra were analysed with respect to EI mass spectra, which are also most important for the structural analysis of the branching point of saturated hydrocarbons.

EXPERIMENTAL

Origin of insects[32–34]

Insects came from European species in the laboratory: termite nests from southwestern and southern France;

Table 1. Analysis of cuticular waxes from social insects using GC/MS with EI and CI

Termites (*Reticulitermes*)		
R. santonensis (Feytaud)		R_s
R. (*lugifugus*) *grassei* (Clement)[32–34]		R_g
R. (*lucifugus*) *banyulensis* (Clement)[32–34]		R_b
Ants[35]		
Ponerinae	*Hypoponera eduardi*[30,31]	
Formicae	*Camponotus vagus*[11,12]	
	Cataglyphis cursor[38]	
Myrmicinae	*Monomorium minutum*[36]	
House-fly	*Calliphora vomitora*[37]	

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ant nests from southwestern and southern France; and Dipteres from the Toulouse area of France.

Extraction of insects and GC

We extracted from 60 to 600 samples of cuticular hydrocarbons by immersing each worker, previously killed by freezing, in 3 ml of pentane for 3 min. The samples were dried under pure nitrogen.

The solid part was analysed first by GC using a Delsi 300 gas chromatograph equipped with a flame ionization detector (FID), with a CPSIL-5 (WCOT) capillary column (25 m, 0.22 mm internal diameter, phase thickness 0.13 µm) (Chrompack, The Netherlands) and a Unica-10 integrator. All GC analyses used temperature programming from 140 °C to 320 °C at 1 °C min^{-1}. Retention times were compared to those of alkane standards which were coinjected. Injector and detector temperatures were respectively 260 °C and 325 °C.

Thin layer chromatography (TLC). TLC was used to separate unsaturated cuticular hydrocarbons from extracts;[39,40] 10% silver nitrate silica gel TLC plates were developed in benzene (8%)–hexane (92%) and scraped into vials, followed by addition of 50 µl of pentane.

Methoxy-mercuration–demercuration. This was performed using Plattner's method.[41–43]

GC/MS. Analyses were performed on a Nermag R10-10C quadrupole mass spectrometer coupled with a Girdel 32 gas chromatograph equipped with a CPSIL-5 (WCOT) capillary column (25 m, 0.22 mm internal diameter, phase thickness 0.13 µm. Injector and detector temperatures were 260 and 325 °C) (Chrompack, The Netherlands) and coupled with a SIDAR-8 data system. EI mass spectra were recorded at EI conditions 70 eV with an ion source temperature of 150 °C. Mass spectra under CI conditions were recorded according to the following process:

(i) Primary ionization of methane or ammonia reagent gas, at an indicated source pressure of $\approx$0.05 torr, was accomplished with a primary beam of high-energy (100 eV) electrons emitted from a heated rhenium filament. Ion source temperature was fixed at 150 °C. Ion source conditions (repeller, focus and drawout plate voltages) were optimized.

(ii) For ethylene oxide [E0] CI, the following experimental conditions were selected, after optimization: 100 eV, extractor voltage 6 V, EO pressure 0.03 torr (measured with Nermag vacuum gauge), source temperature 150 °C. The nature of the plasma has been described elsewhere.[45,46] The protonic affinity of ethylene oxide[53,54] is 792.6 kJ mol^{-1}.

Reagents. Methane and ammonia were obtained from Air Liquide (N-45), and ethylene oxide from Matheson Gas Products (99.9% pure). Solvents or chemicals were purchased: methanol (Merck), pentane (RP, normapur), 9-(*Z*)-tricosene, mercury acetate and sodium borohydride (Jansen Chemica).

RESULTS AND DISCUSSION

Termites of the genus *Reticulitermes*

Cuticular waxes of worker termites of some different colonies (see Table 1) were extracted by pentane dipping 1000 of them for 3 min. Chromatographic analysis of the cuticular extracts of the termites were made using a WCOT capillary apolar column, The three chromatograms for a colony of each species R_s, R_g and R_b are presented in Fig. 1. Extraction using a polar solvent such as dichloromethane, which was analysed after the extraction with an apolar solvent, did not give any peak on GC analysis. Quantitative analysis showed that the cuticular hydrocarbons of workers were only 0.001–0.004% of the biomass of the termites. These proportions have been obtained by GC with an internal reference, *n*-heneicosane.

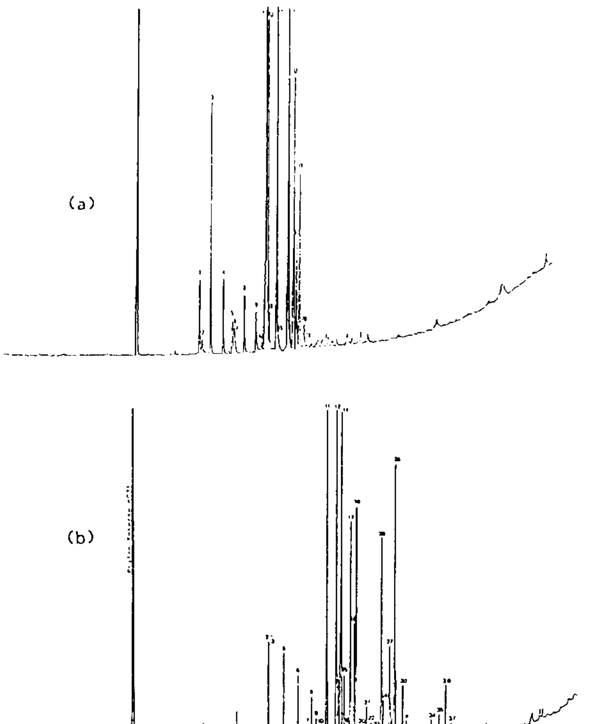
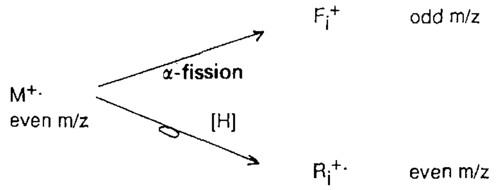


Figure 1. Gas chromatograms of cuticular hydrocarbons of termites in the genus *Reticulitermes* (WCOT apolar column CP-Sil-5, 25 m, 150–320 °C at 5 °C min^{-1}: (a) *R. santonensis*; (b) *R. grassei*; *R. banyulensis*.

GC/MS analyses of pentane extracts were made using different ionization modes: EI, methane CI and ethylene oxide CI, which is a new CI reagent gas.[45,46]

Alkanes. Mass spectra of monomethylalkanes and dimethylalkanes are shown in the Figs 2 and 3, respectively. With methane CI,[55] $[M-H]^+$ ions as base peak were shown in the two classes of branched compounds[56–61] as well as for *n*-alkanes. Alkyl fragment ions were weak for every class of alkanes. In addition, for mono- and dimethylalkanes, methane CI produced important fragment ions characteristic of the branching point. Ethylene oxide CI produced similar information (Fig. 2, Table 2), but the fragment ions characteristic of the branching point were more abundant than those obtained with methane[61] or nitric oxide[44,62,63] CI. Thus ethylene oxide CI will produce easy structural interpretation for this class of compound. However, differentiation between monomethyl- and dimethylalkanes was made using EI. Scammells[64] has demonstrated that EI of branched chain alkanes gives an even-electron fragment ion F_i^+ resulting from an α-fission of the vicinal C—C bond and an odd-electron rearrangement ion $R_i^{+\cdot}$ involving an hydrogen transfer process.

We noticed that the abundances of these ions were different according to the number of branching points remaining in the fragment ion: for monomethylalkanes, only one branching point was possible; the abundance of odd-electron ion $R_1^{+\cdot}$ was greater than that of the even-electron ion F_1^+; for dimethylalkanes,[4,65,66] two classes of fragmentation and rearrangement were possible: (i) when the charge remained on the external part of the hydrocarbon, only one branching point was observed; then the abundance of $R_1^{+\cdot}$ was greater than that of $F_1{}^+$; (ii) when the charge remained on the internal part of the hydrocarbon, two branching points were observed; the abundances were then inversed: the abundance of the even-electron fragment ion $F_2{}^+$ was greater than that of odd-electron rearrangement ion $R_2^{+\cdot}$. So we will call these ions '*diagnostic ions*'.

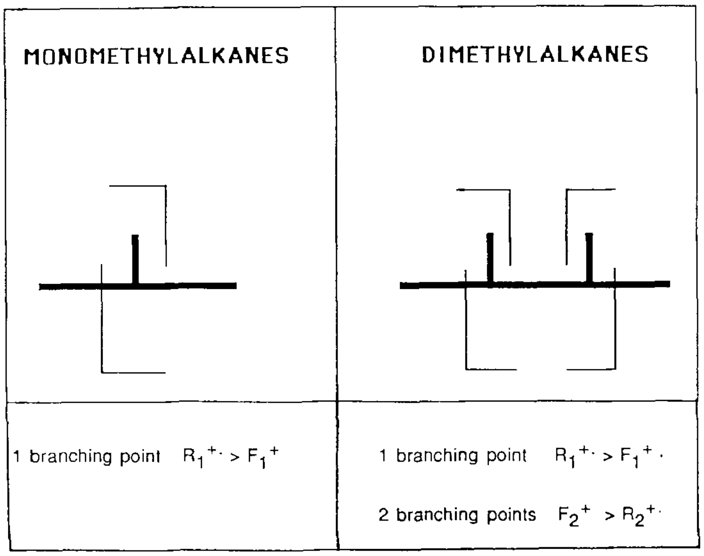


These results are shown in the spectra of 9,13-dimethylpentacosane (Fig. 3). These diagnostic ions were always observed in a reproducible way. It is the only method at present which allows distinction of monomethylalkanes from dimethylalkanes.

Alkenes and Dienes. Identification of unsaturated cuticular hydrocarbon was more complex because of the location of the double bonds, so we used several techniques. We described in a previous paper[45,46] the use of ethylene oxide CI for alkenes. Figure 4(b) shows the mass spectra of 9-(*Z*)-tricosene in these conditions: an abundant protonated molecule was accompanied by $[M+43]^+$ and $[M+57]^+$ adduct ions which were considered valuable diagnostic ions for this class of compounds (Table 3). They probably resulted from electrophilic additions of the $[C_2H_5O]^+$ ion and 57 solvated ions. These adduct ions were not observed in the

Table 2. Mass spectra under ethylene oxide (0.03 torr, 150 °C) of monomethylalkanes. Alkyl fragment ions are weak

	$[M-H]^+$	Other ions
11-Methyltricosane	337 (100)	168 (25), 169 (23), 196 (20), 197 (23)
12-Methyltetracosane	351 (100)	168 (17), 169 (19), 210 (13), 211 (16)
11-Methylpentacosane	365 (100)	168 (40), 169 (36), 224 (20), 225 (30)
13-Methylpentacosane	365 (100)	196 (19), 197 (20)
3-Methylpentacosane	365 (100)	336 (10), 337 (38)



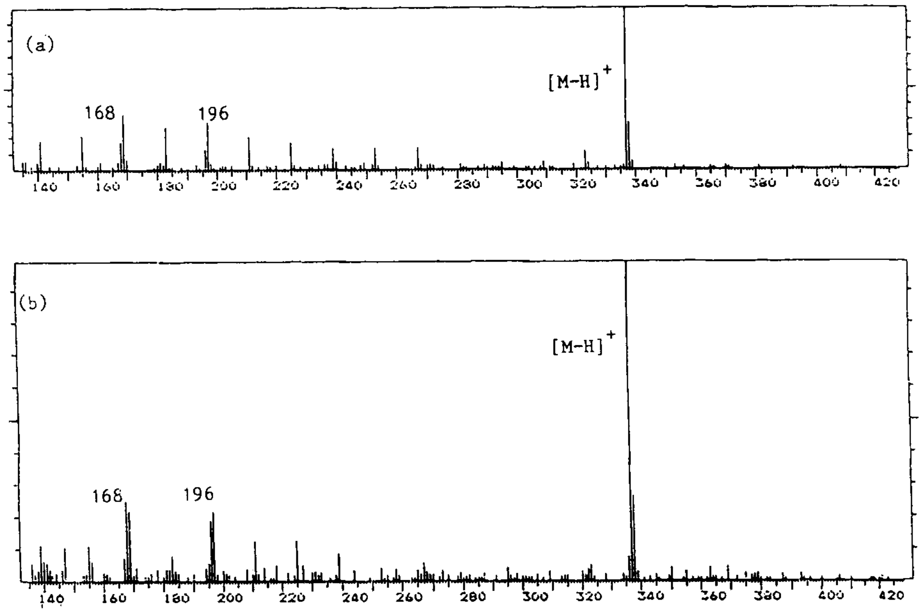


Figure 2. Mass spectra of 11-methyltricosane under (a) methane CI and (b) ethylene oxide CI conditions.

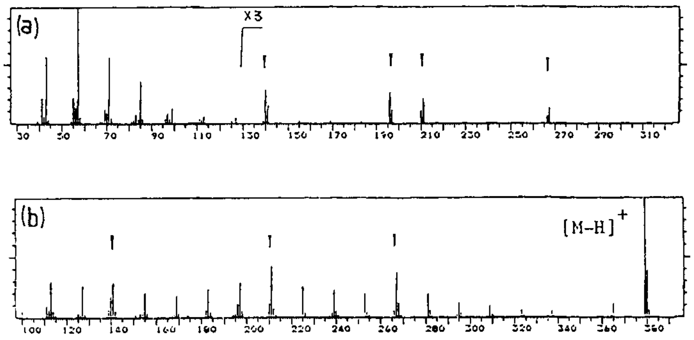


Figure 3. Mass spectra of 9,13-dimethylpentacosane under (a) EI and (b) methane CI conditions.

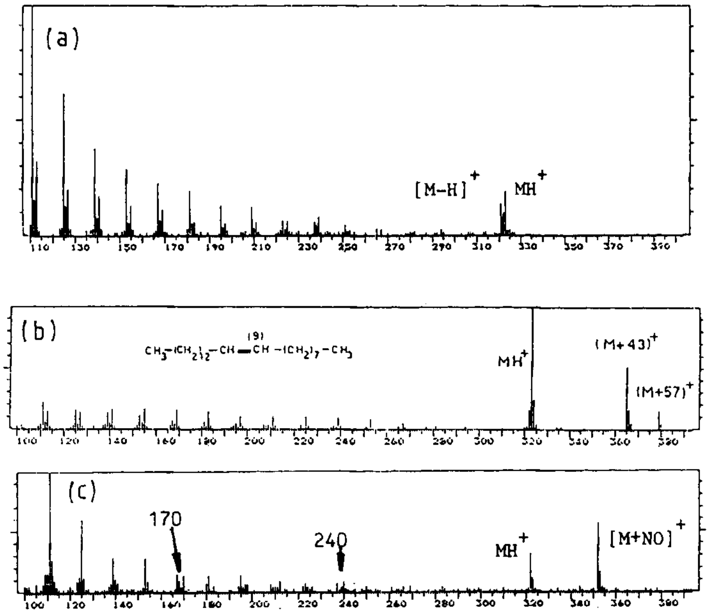


Figure 4. Mass spectra of 9-(*Z*)-tricosene under (a) methane CI, (b) ethylene oxide CI (0.03 torr, 150 °C) and (c) nitric oxide CI (0.045 torr, 180 °C) conditions.

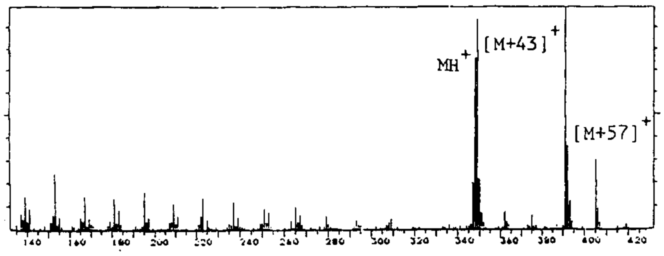


Figure 5. Mass spectra of 7,9-pentacosadiene under ethylene oxide CI (0.03 torr, 150 °C) conditions.

ethylene oxide CI of alkanes. Ethylene oxide CI mass spectra were different from the methane CI spectra.[67] In ethylene oxide CI the protonated molecular ion was the base peak, whereas the alkenyl fragment ions were minimized in these mass spectra compared to those of other gases (methane,[67] isobutane,[68] nitric oxide[68–73] (Fig. 4(c)). Conjugated diene ethylene oxide CI mass spectra (Fig. 5, Table 3) showed weak fragment ions, an abundant protonated molecule and $[M + 43]^+$ and $[M + 57]^+$ adduct ions. The behaviour of 7,9-pentacosadiene was different from those of alkenes in that the $[M + 43]^+$ adduct ion was the base peak.

Ethylene oxide CI thus appeared an efficient method for the detection and analysis of unsaturated long-chain hydrocarbons. It was used in GC/MS using amounts of samples of ~1 ng. A notable feature of ethylene oxide CI is that alkenes give a more intense response than alkanes. The application of ethylene oxide CI for analysis of the cuticular extracts from the termites *Reticulitermes santonensis* has been undertaken and Fig. 6 shows an excellent response for unsaturated hydrocarbons, with saturated hydrocarbons yielding only a very weak response. Such differences were not observed in CI with methane or nitric oxide. As already mentioned,[68] ethylene oxide CI did not provide a method for determining the location of double bonds in olefins when compared to nitric oxide[69–74] or methylvinyl ether.[75–80]

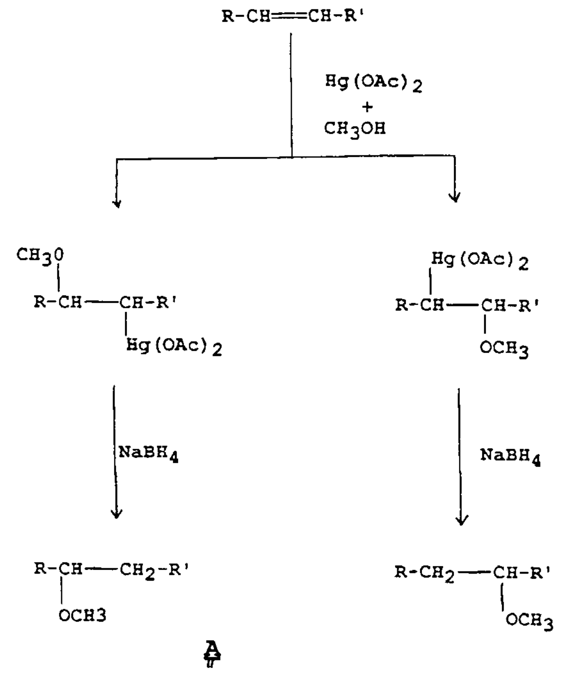


Table 3. **Mass spectra under ethylene oxide of alkenes and dienes; alkenyl fragment ions are weak (0.03 torr, 150 °C)**

	MH^+	$[M + 43]^+$	$[M + 57]^+$	Other ions
1-Eicosene	100	45	10	a
9-Tricosene	100	45	14	a
9-Pentacosene	100	38	12	a
7,9-Pentacosadiene	93	100	30	348 (75)

[a] Alkenyl and alkyl fragment ions.

When we examined extracts using methane CI, very long chain alkanes ($C \geqslant 23$) were found. Using nitric oxide CI, the diagnostic ions found were very small in the 9-(*Z*)-tricosene model (Fig. 4(c)). During GC/MS of natural extracts using nitric oxide CI, these diagnostic ions for higher alkenes became too weak to locate the double bonds, which contrasts with the literature.[68,71,74] So we proceeded to derivatization of unsaturated hydrocarbons using Plattner's method.[41] It consists of methoxy-mercuration followed by a reductive demercuration with sodium borohydride in methanol to give directly a mixture of two isomeric monomethoxy derivatives.[42,43] The mass spectra using EI, methane CI, ammonia CI or ethylene oxide CI modes showed a classical fragmentation involving the ether cleavage of the C—C bond. Figure 7 shows mass

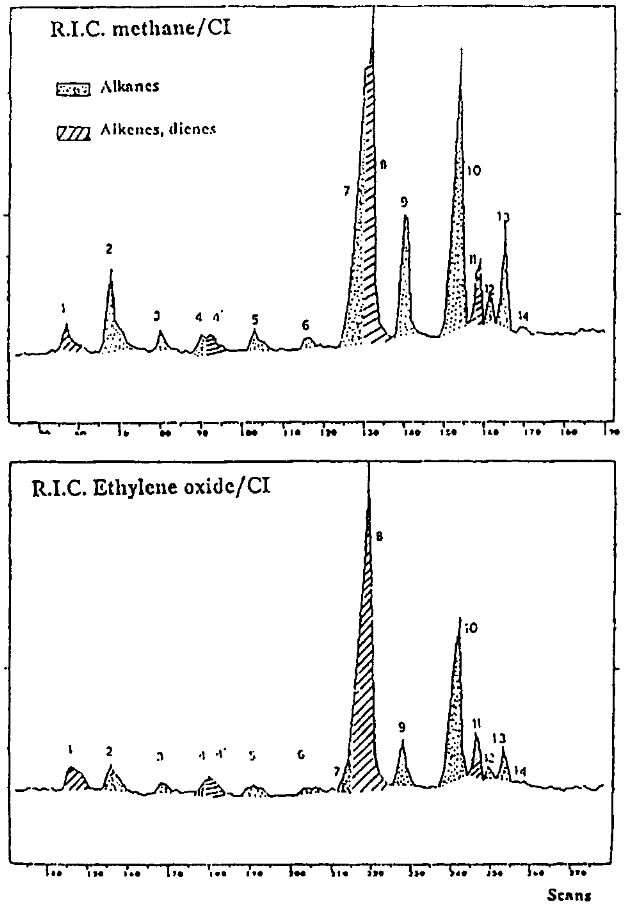


Figure 6. Monotograms (TIC) of cuticular hydrocarbons of termites (*Reticulitermes santonensis*) under (a) methane CI (0.05 torr, 150 °C) and (b) ethylene oxide/CI conditions (0.03 torr, 150 °C).

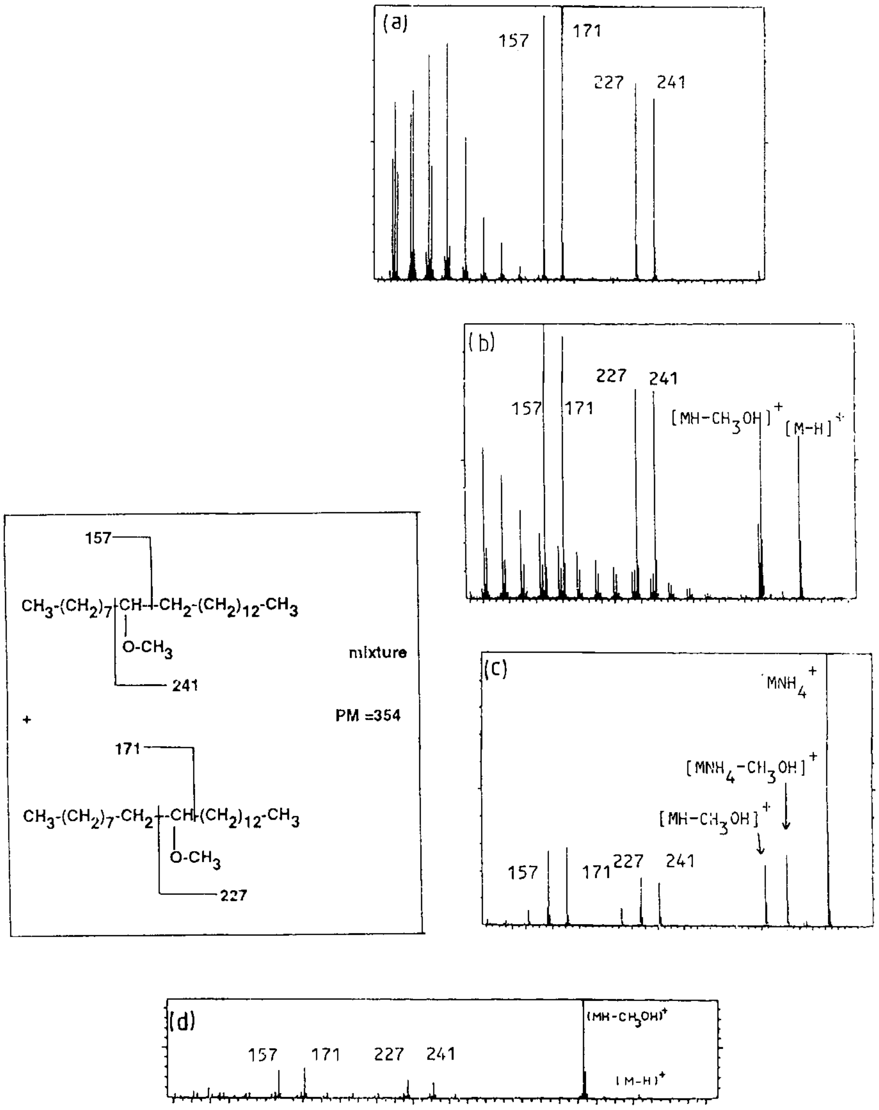


Figure 7. Mass spectra of the mixture of 9-methoxy- and 10-methoxytricosane under (a) EI, (b) methane CI, (c) ammonia CI (0.1 torr, 150 °C) and (d) ethylene oxide CI (0.03 torr, 150 °C) conditions.

spectra of a mixture of 9-methoxy- and 10-methoxy-tricosene which came from derivatization of 9-(*Z*)-tricosene. Location of the double bond resulted from examination of the four diagnostic fragment ions 227/171, 241/157. The double bond was located between C_9 and C_{10}. This technique was applied to the crude cuticular wax extracts. It was also performed on the unsaturated hydrocarbon fraction which was separated from the crude extracts by TLC (silica–$AgNO_3$–benzene). All the locations of double bonds were made in this way.

Results for *Reticulitermes* cuticular waxes. Using these different techniques, we performed the analysis of the three species of *Reticulitermes* of which gas chromatograms are shown in Fig. 1. The identification of all compounds is shown in Table 4. This table presents $[M - H]^+$ ions for alkanes and protonated molecules for alkenes under CI conditions, and diagnostic ions under EI conditions. The principal classes of compounds were found to be linear alkanes, mono- and dimethylalkanes and alkenes. Their nature and their ratio fluctuated according to the genus and the species.

Cuticular waxes of ants

Cuticular waxes of ants of different genera (see Table 1) were extracted by pentane dipping 1000 of them during 3 min. GC analysis of the crude cuticular extracts was made on a WCOT apolar column. GC/MS analysis was performed using the same process previously described for termites.

Ponerinae ants: *Hypoponera eduardi*. The gas-chromatogram is shown in Fig. 8. Mass spectra of the constituents of the crude extract were obtained using GC/MS analysis under EI and methane CI conditions. Table 5 explains the composition resulting from the latter analysis. Only saturated hydrocarbons were found. Beside linear alkanes, the analysis showed a great number of monomethylalkanes and fewer dimethylalkanes.

Formicae ants. Two species of this group have been examined: *Camponotus vagus* and *Cataglyphis cursor*. They were differentiated with regard to unsaturated

Table 4. Identification of cuticular hydrocarbons from *Reticulitermes* termites

Components	ECL	R_s	R_g	R_b	Mol. wt	Diagnostic EI ions[a]	Diagnostic CH_4/CI ions[a] [M − H]$^+$	Others
Alkanes								
n-C$_{22}$	22.00	T	T	T	310		309	
n-C$_{23}$	23.00	3	T	2	324		323	
n-C$_{24}$	24.00	8	T	4	338		337	
n-C$_{25}$	25.00	14	2	7	352		351	
n-C$_{26}$	26.00	21	6	14	366		365	
n-C$_{27}$	27.00	TT	11	18	380		379	
n-C$_{28}$	28.00	–	18	24	394		393	
n-C$_{29}$	29.00	–	25	28	408		407	
n-C$_{30}$	30.00	–	31	32	422		421	
n-C$_{31}$	31.00	–	34	34	436		435	
n-C$_{32}$	32.00	–	–	38	450		449	
n-C$_{33}$	33.00	–	–	40	464		463	
n-C$_{34}$	34.00	–	–	44	478		477	
n-C$_{35}$	35.00	–	–	46	492		491	
Monomethyl alkanes								
11-Me C$_{23}$	23.36	4	–	3	338	168/169, 196/197	337	169, 197, 323
4-Me C$_{23}$	23.64	5	–	–	338	70, 294/295	337	295, 323
11-Me C$_{24}$	24.30	–	–	5	352	168/169, 210/211	351	169, 211, 337
12-Me C$_{24}$	24.35	9	–	–	352	182/183,196/197	351	183, 197, 337
4-Me C$_{24}$	24.65	11	–	–	352	70, 308/309	351	309, 337
13-Me C$_{25}$	25.37	16	–	9	366	196/197	365	197, 351
11-Me C$_{25}$	25.35	16	4	9	366	168/169, 224/225	365	169, 225, 351
5-Me C$_{25}$	25.35	–	5	11	366	84, 308/309	365	309, 351
4-Me C$_{25}$	25.64	18	–	–	366	70, 322/323	365	323, 351
3-Me C$_{25}$	25.75	19	–	–	366	56, 336/337	365	337, 351
13-Me C$_{26}$	26.35	–	–	15	380	196/197, 210/211	379	197, 211, 365
12-Me C$_{26}$	26.35	–	–	15	380	182/183, 224/225	379	183, 225, 365
11-Me C$_{26}$	26.35	–	7	15	380	168/169, 238/239	379	169, 239, 365
6-Me C$_{26}$	26.46	–	8	16	380	98, 308/309	379	309, 365
13-Me C$_{27}$	27.33	–	12	19	394	196/197, 224/225	393	197, 225, 379
11-Me C$_{27}$	27.33	–	12	19	394	168/169, 252/253	393	169, 253, 379
5-Me C$_{27}$	27.52	–	14	21	394	84,336/337	393	337, 379
11-Me C$_{28}$	28.33	–	20	–	408	168/169, 266/267	407	169, 267, 393
6-Me C$_{28}$	28.41	–	21	–	408	98, 336/337	407	337, 393
15-Me C$_{29}$	29.32	–	–	29	422	224/225	421	225, 407
13-Me C$_{29}$	29.32	–	27	29	422	196/197, 252/253	421	197, 253, 407
11-Me C$_{29}$	29.32	–	27	29	422	168/169, 280/281	421	168, 281, 407
5-Me C$_{29}$	29.52	–	28	30	422	84, 364/365	421	365, 407
11-Me C$_{31}$	31.31	–	–	–	450	168/169, 308/309	449	169, 309, 435
Dimethyl alkanes								
9, 13-diMe C$_{25}$	25.69	–	–	12	380	140, 196, 211, 267	379	141, 197, 211, 267, 365
x, x-diMe C$_{27}$	27.63	–	16	–	408			
5, 17-diMe C$_{27}$	27.87	–	17	23	408	84, 168, 267, 351	407	169, 267, 351, 393
5, 17-diMe C$_{29}$	29.82	–	–	31	436	84, 196, 267, 379	435	196, 267, 379, 421
Monoenes								
9-C$_{23}$:1	22,68	1	–	1	322		323[b]	
9-C$_{24}$:1	23.69	6	–	–	336		337[b]	
9-C$_{25}$:1	24.69	12	–	6	350		407[b]	
x-C$_{29}$:1	28.01	–	19	–	406		351[b]	
Dienes								
7, 9-C$_{25}$:2	22.55	17	–	–	348		349[b]	

[a] upon addition of alkyl and alkanyl ions.
[b] MH$^+$.
T = Traces.

hydrocarbons. The gas chromatogram of *Camponotus vagus* extract is shown in Fig. 9. Mass spectra of the different components of the extract were obtained as previously described. The hydrocarbon cuticular wax of this species of ants was composed only of linear, mono- and dimethylalkanes (Table 6). The gas chromatogram of *Cataglyphis cursor* is shown in Fig. 10. This crude extract was very different from the other extracts of ants that we described previously. From the four species examined, the *Cataglyphis* extract was the only one which contained some alkenes. So we applied all the strategy adjusted for the termites: using TLC, unsat-

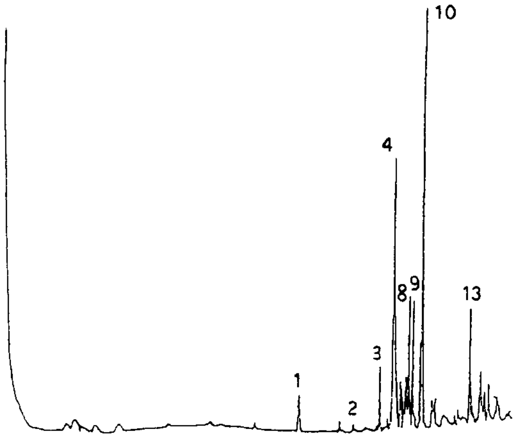


Figure 8. Gas chromatogram of cuticular hydrocarbons of ants (*Hypoponera eduardi*) (WCOT apolar column CP-Sil-5, 25 m, 150–320 °C at 5 °C min^{-1}).

urated hydrocarbons were separated; methoxymercuration was made on the latter fraction. Table 7 shows the identifications of all components; protonated molecule and diagnostic ions under EI and CI are reported.

Calliphora vomitora house-fly

Cuticular waxes of *Calliphora* house-flies were extracted by immersing 300 of them in pentane for 3 min. GC analysis of the crude extract was made on a WCOT apolar column (Fig. 11). GC/MS analysis was performed for the crude wax and the unsaturated hydrocarbon fraction derivatized as previously described. The cuticular hydrocarbons of *Calliphora* extract were composed of linear mono- and dimethylalkanes and of some alkenes (Table 8).

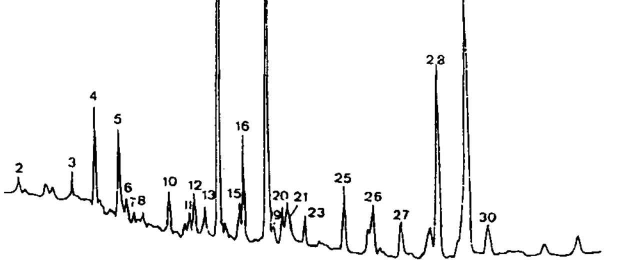


Figure 9. Gas chromatogram of cuticular hydrocarbons of ants (*Camponotus vagus*) (WCOT apolar column CP-Sil-5, 25 m, 150–320 °C at 5 °C min^{-1}).

CONCLUSION

Discrimination between genera, species, nestmate or alien individuals can be based on the components of cuticular waxes. As well as providing protection against outside aggressions (desiccation, abrasion, shocks, toxins of predators, insecticides, etc.), cuticular waxes play a predominant part in the communication between social insects.

GC/MS has been developed here for the analysis of complex cuticular mixtures and required the use of selective means of detection such as CI with ethylene oxide. Consequently, alkenes showed a better response

Table 5. Identification of cuticular hydrocarbons from *Hypoponera eduardi* ants

Peaks	Components	Mol. wt	EI diagnostic ions	CH_4/CI diagnostic ions $[M-H]^+$	Others
1	11-Me-pentacosane	366	168/169, 224/225	365	169, 225, 351
	15-Me-pentacosane	366	196/197	365	197, 351
2	7,10-diMe tetracosane	366	112, 169, 224, 281	365	113, 169, 225, 281, 351
3	*n*-Heptacosane	380		379	
4	9-Me-Heptacosane	394	140/141; 280/281	393	141, 281, 379
	11-Me-Heptacosane	394	168/169, 252/253	393	169, 253, 379
	13-Me-Heptacosane	394	196/197, 224/225	393	197; 225, 379
5	7-Me-Heptacosane	394	112/113, 308/309	393	113, 309, 379
6	5-Me-Heptacosane	394	84, 336/337	393	337, 379
7	9, 14-diMe-Heptacosane	408	140, 211, 224, 295	393	141, 211, 225, 295, 393
8	3-Me-Heptacosane	394	56, 364/365	393	365, 379
9	5, 17-diMe Heptacosane	408	84, 168, 267, 351	407	169, 267, 351, 393
10	3,9-diMe-Heptacosane	408	155, 280, 379	407	155, 281, 379, 393
	5,9-diMe-Heptacosane	408	155, 280, 351	407	155, 281, 351, 393
	3,7-diMe-Heptacosane	408	127, 308, 379	407	127, 309, 379, 393
	5,7-diMe-Heptacosane	408	127, 308, 351	407	127, 309, 351, 393
11	15-Me octacosane	408	210, 224	407	211, 225, 393
12	13-Me octacosane	408	182, 252	407	183, 253, 393
13	11-Me-nonacosane	422	168/169, 280/281	421	169, 281, 407
	13-Me-nonacosane	422	196/197, 252/253	421	197, 253, 407
	15-Me-nonacosane	422	224/225	421	225, 407
14	13,15-diMe-nonacosane	436	196, 224, 239, 267	435	197, 225, 239, 267, 421
15	10,15-diMe-nonacosane	436	154, 224, 239, 309	435	155, 225, 239, 309, 421
16	3,15-diMe-nonacosane	436	224, 239, 379	435	225, 239, 379, 421
	3,17-diMe-nonacosane	436	196, 267, 379	435	197, 267, 379, 421

Table 6. Identification of cuticular hydrocarbons from *Camponotus vagus* ants

Peaks	Components	Mol. wt	Diagnostic IE/MS ions	Diagnostic IC CH_4 ions	
				$[M-H]^+$	Others
1	n-C_{25}	352		351	
2	n-C_{27} +	380		379	
	2-Me C_{26}	380	43, 364/365	379	365
3	4-Me C_{28}	408	70, 71, 364/365	407	365, 393
4	n-C_{29}	408		407	
5	11-Me C_{29} +	422	168/169, 280/281	421	169, 281, 407
	13-Me C_{29} +	422	196/197, 252/253	421	197, 253, 407
	15-Me C_{29}	422	224/225	421	225, 407
6	7-Me C_{29}	422	112/113, 336/337	421	113, 337, 407
7	11,15-diMe C_{29} +	436	168, 224, 239, 295	435	169, 225, 239, 295, 421
	13,17-diMe C_{29}	436	196, 267	435	197, 267, 421
8	5,15-diMe C_{29} +	436	84, 224, 239, 379	435	225, 239, 379, 421
	5,17-diMe C_{29} +	436	84, 196, 267, 379	435	197, 267, 379, 421
	5,19-diMe C_{29}	436	84, 168, 295, 379	435	169, 295, 379, 421
9	3-Me C_{29} +	422	56/57, 392/393	421	393, 407
	6-Me C_{29}	422	84/85, 350/351	421	351, 407
10	n-C_{30}	422		421	
11	11-Me C_{30} +	436	168/169, 294/295	435	169, 295, 421
	13-Me C_{30}	436	196/197, 266/267	435	197, 267, 421
12	x-Me C_{30}	436		435	
13	y-Me C_{30}	436		435	
14	4-Me C_{30}	436	70/71, 392/393	435	393, 421
15	11,15-diMe C_{30} +	450	168, 238, 239, 309	449	169, 239, 309, 435
	13,17-diMe C_{30}	450	196, 210, 267, 281	449	197, 211, 267, 281, 435
16	n-C_{31}	436		435	
17	3,25-diMe C_{31}	464	56, 112, 379, 435	463	113, 379, 435, 449
	4,26-diMe C_{31}	464	70, 98, 393, 421	463	393, 421, 449
18	11-Me C_{31} +	450	168/169, 308/309	449	169, 309, 435
	13-Me C_{31} +	450	196/197, 280/281	449	197, 281, 435
	15-Me C_{31} +	450	224/225, 252/253	449	225, 253, 435
19	7-Me C_{31} +	450	112/113, 364/365	449	113, 365, 435
	9-Me C_{31}	450	140/141, 336/337	449	141, 337, 435
20	5,11-diMe C_{31}	464	84, 183, 308, 407	463	183, 309, 407, 449
21	5,13-diMe C_{31} +	464	84, 211, 280, 407	463	211, 281, 407, 449
	5,15-diMe C_{31}	464	84, 239, 252, 407	463	239, 253, 407, 449
22	5,17-diMe C_{31} +	464	84, 224, 267, 407	463	225, 267, 407, 449
	5,19-diMe C_{31}	464	84, 196, 295, 407	463	197, 295, 407, 449
23	5,21-diMe C_{31}	464	84, 168, 323, 407	463	169, 323, 407, 449
	5,23-diMe C_{31}	464	84, 140, 351, 407	463	141, 351, 407, 449
24	5,25-diMe C_{31} +	464	84, 112, 379, 407	463	113, 379, 407, 449
	5,27-diMe C_{31}	464	84, 407	463	407, 449
25	11-Me C_{32} +	464	168/169, 322/323	463	169, 323, 449
	13-Me C_{32}	464	196/197, 294/295	463	197, 295, 449
26	7-Me C_{32} +	464	112/113, 378/379	463	113, 379, 449
	5,20-diMe C_{32} +	478	84, 196, 309, 421	477	197, 309, 421, 463
	5,23-diMe C_{32}	478	84, 154, 351, 421	477	155, 351, 421, 463
27	11,20-diMe C_{32}	478	168, 196, 309, 337	477	169, 197, 309, 337, 463
28	11-Me C_{33} +	478	168/169, 336/337	477	169, 337, 463
	13-Me C_{33} +	478	196, 197, 308/309	477	197, 309, 463
	15-Me C_{33}	478	224/225, 280/281	477	225, 281, 463
29	2,19-diMe C_{33}	492	224, 295, 477	491	225, 295, 477
	3,21-diMe C_{33}	492	56, 323, 196, 463	491	197, 323, 463, 477
	4,23-diMe C_{33}	492	70, 168, 351, 449	491	169, 351, 449, 477
30	5,11-diMe C_{33} +	492	84, 183, 336, 435	491	183, 336, 435, 477
	5,13-diMe C_{33}	492	84, 211, 308, 435	491	211, 309, 435, 477
31	5,15-diMe C_{33} +	492	84, 239, 280, 435	491	239, 281, 435, 477
	5,17-diMe C_{33}	492	84, 252, 267, 435	491	253, 267, 435, 477
32	5,19-diMe C_{33} +	492	84, 224, 295, 435	491	225, 295, 435, 477
	5,21-diMe C_{33}	494	84, 196, 323, 435	491	197, 323, 435, 477

Table 7. Identification of cuticular hydrocarbons from *Cataglyphis cursor* ants

Peak no.	Carbon no.	Identification	Mol. wt	Diagnostic EI ions	Diagnostic CH_4Cl ions	
					$[M-H]^+$	Others
Alkanes						
2	25	n-C_{25}	352		351	
7	26	n-C_{26}	366		365	
13	27	n-C_{27}	380		379	
17	28	n-C_{28}	394		393	
25	29	n-C_{29}	408		407	
31	30	n-C_{30}	422		421	
38	32	n-C_{32}	450		449	
Monomethylalkanes						
3	26	11-Me C_{25}	366	168, 169, 224, 225	365	169, 225, 351
	26	13-Me C_{25}	366	196, 197	365	197, 351
4	26	5-Me C_{25}	366	84, 308, 309	365	309, 351
5	26	3-Me C_{25}	366	56, 336, 337	365	337, 351
9	27	10-Me C_{26}	380	154, 155, 252, 253	379	155, 253, 365
	27	11-Me C_{26}	380	168, 169, 238, 239	379	169, 239, 365
	27	13-Me C_{26}	380	196, 197, 210, 211	379	197, 211, 365
10	27	4-Me C_{26}	380	70, 336, 337	379	337, 365
14	28	11-Me C_{27}	394	168, 169, 252, 253	393	169, 253, 379
	28	13-Me C_{27}	394	196, 197, 224, 225	393	197, 225, 379
15	28	5-Me C_{27}	394	84, 336, 337	393	337, 379
16	28	3-Me C_{27}	394	56, 364, 365	393	365, 379
19	29	x-Me C_{28}	408		407	393
26	30	11-Me C_{29}	422	168, 169, 280, 281	421	169, 281, 407
	30	13-Me C_{29}	422	196, 197, 252, 253	421	197, 253, 407
	30	15-Me C_{29}	422	224, 225	421	225, 407
27	30	5-Me C_{29}	422	84, 364, 365	421	365, 407
28	30	4-Me C_{29}	422	70, 378, 379	421	379, 407
32	31	13-Me C_{30}	436	196, 197, 266, 267	435	197, 267, 421
	31	15-Me C_{30}	436	224, 225, 238, 239	435	225, 259, 421
36	32	13-Me C_{31}	450	196, 197, 280, 281	449	197, 281, 435
	32	15-Me C_{31}	450	224, 225, 252, 253	449	225, 253, 435
39	33	x-Me C_{32}	464		463	435
Dimethylalkanes						
6	27	5,9-diMe C_{25}	380	84, 155, 252, 323	379	155, 253, 323, 365
8	27	3,18-diMe C_{25}	380	56, 126, 281, 351	379	127, 281, 351, 365
12	28	4,8-diMe C_{26}	394	70, 141, 280, 351	393	141, 281, 351, 379
	28	4.12-diMe C_{26}	394	70, 197, 224, 351	393	197, 225, 351, 379
18	29	3,11-diMe C_{27}	408	56, 183, 252, 379	407	183, 255, 379, 393
20	30	3,8-diMe C_{28} or	422	56, 141, 308, 393	421	141, 309, 393, 407
		3,13-diMe C_{28}	422	56, 211, 238, 393	421	211, 238, 393, 407
23	30	6,10-diMe C_{28}	422	98, 169, 280, 351	421	169, 281, 351, 407
	30	6,18-diMe C_{28}	422	98, 168, 281, 351	421	169, 281, 351, 407
24	30	4,12-diMe C_{28}	422	70, 197, 252, 379	421	197, 253, 379, 407
	30	4,14-diMe C_{28}	422	70, 224, 225, 379	421	225, 379, 407
29	31	5,13-diMe C_{29}	436	84, 211, 252, 379	435	211, 253, 379, 421
30	31	3,11-diMe C_{29}	436	56, 183, 280, 407	435	183, 281, 407, 421
Alkenes						
1	25	$C_{25}:1$	350		351[a]	
11	27	$C_{27}:1$	378		379[a]	
34	32	$C_{32}:1$	448		449[a]	
37	33	$C_{33}:1$	462		463[a]	
40	34	$C_{34}:1$	476		477[a]	

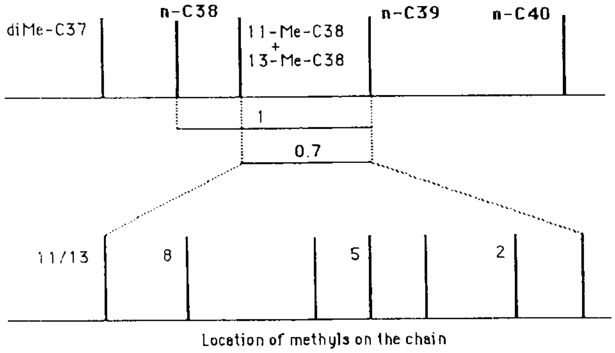
[a] MH^+.

Table 8. Identification of cuticular hydrocarbons of *Calliphora vomitora* house-flies

Peak no	ECL	Carbon no.	Identification	Mol. wt	Diagnostic EI ions	Diagnostic CH_4/CI ions	
						$[M-H]^+$	Others
Alkanes							
1	20.0	20	n-C_{20}	282	267	281	
3	21.0	21	n-C_{21}	296	281	295	
7	22.0	22	n-C_{22}	310	295	309	
11	23.0	23	n-C_{23}	324	309	323	
16	24.0	24	n-C_{24}	338	323	337	
20	25.0	25	n-C_{25}	352	337	351	
26	26.0	26	n-C_{26}	366	351	365	
30	27.0	27	n-C_{27}	380	365	379	
38	28.0	28	n-C_{28}	394	379	393	
40	29.0	29	n-C_{29}	408	393	407	
46	30.0	30	n-C_{30}	422	407	421	
48	31.0	31	n-C_{31}	436	421	435	
Monomethylalkanes							
2	20.7	21	3-Me C_{20}	296	57, 267, 268	295	267, 281
4	21.4	22	11-Me C_{21}	310	168, 169	309	169, 295
5	21.5	22	7-Me C_{21}	310	224, 225, 112, 113	309	113, 225, 295
6	21.7	22	3-Me C_{21}	310	57, 280, 281	309	281, 295
8	22.4	23	9-Me + 11-Me C_{22}	324	140, 141, 210, 211 + 168, 169, 182, 183	323	141, 211 + 169, 183, 309
9	22.5	23	7-Me C_{22}	324	112, 113, 238, 239	323	113, 239, 309
10	22.6	23	4-Me C_{22}	324	71, 280, 281	323	281, 309
12	23.3	24	9-Me + 11-Me C_{23}	338	140, 141, 220, 221 + 168, 169, 196, 197	337	141, 221 + 169, 197, 323
13	23.5	24	7-Me C_{23}	338	112, 113, 252, 253	337	113, 253, 323
14	23.6	24	5-Me C_{23}	338	85, 280, 281	337	281, 323
15	23.7	24	3-Me C_{23}	338	57, 308, 309	337	309, 323
17	24.3	25	11-Me + 12Me + C_{24}	352	168, 169, 210, 211 + 182, 183, 196, 197	351	169, 211 + 183, 197, 337
18	24.6	25	4-Me C_{24}	352	71, 308, 309	351	309, 337
21	25.3	26	11-Me + 13-Me C_{25}	366	168, 169, 224, 225 + 196, 197	365	169, 225 + 197, 351
22	25.4	26	7-Me + 9-Me C_{25}	366	112, 113, 280, 281 + 140, 141, 252, 253	365	113, 281 + 141, 253, 351
23	25.5	26	5-Me C_{25}	366	85, 308, 309	365	309, 351
24	25.7	26	3-Me C_{25}	366	57, 336, 337	365	337, 351
27	26.3	27	11-Me + 13-Me C_{26}	380	168, 169, 238, 239 + 196, 197	379	169, 239 + 197, 365
28	26.7	27	3-Me C_{26}	380	196, 197, 350, 351	379	197, 351, 365
31	27.3	28	13-Me C_{27}	394	196, 197, 222, 223	393	197, 223, 379
32	27.4	28	11-Me C_{27}	394	168, 169, 252, 253	393	169, 253, 379
33	27.5	28	9-Me C_{27}	394	140, 141, 280, 281	393	141, 281, 379
34	27.7	28	3-Me C_{27}	394	57, 364, 365	393	365, 379
37	28.3	29	11-Me + 13-Me C_{28}	408	168, 169, 266, 267 + 196, 197, 238, 239	407	169, 267 + 197, 239, 393
38	28.6	29	4-Me C_{28}	408	85, 364, 365	407	365, 393
41	29.3	30	13-Me C_{29}	422	196, 197, 252, 253	421	197, 253, 407
42	29.4	30	11-Me C_{29}	422	168, 169, 280, 281	421	169, 281, 407
43	29.5	30	9-Me C_{29}	422	140, 141, 308, 309	421	141, 309, 407
44	29.6	30	7-Me C_{29}	422	112, 113, 336, 337	421	113, 337, 407
45	29.7	30	3-Me C_{29}	422	57, 392, 393	421	393, 407
47	30.6	31	4-Me C_{30}	436	71, 392, 393	435	393, 421
49	31.3	32	15-Me C_{31} + 13-Me C_{31} + 11-Me C_{31}	450	222, 223, 252, 253 + 196, 197, 280 281 + 168, 169, 308, 309	449	223, 253 + 196, 281 + 169, 309, 435
Dimcalkenes							
25	25.8	27	DiMe-4,15 C_{25}	380	71, 168, 239, 337	379	169, 239, 337, 365
35	27.8	29	DiMe-4,15 C_{27}	408	71, 196, 239, 367	407	197, 239, 367, 393
19	24.9	25	Pentacosene	350		351[a]	
29	26.8	27	Heptacosene	380		381[a]	
39	28.7	29	Nonacosene	408		409[a]	

[a] Footnote to Table 8.

Table 9. Elution of branched alkanes with apolar WCOT column CP-Sil-5 (25 m)



than alkanes. But location of double bonds of unsaturated hydrocarbons required the use of TLC separations followed by derivatization methods and GC/MS under CI conditions. The difficulty of these analyses for branched-chain saturated hydrocarbons has required the use of the chromatographic logic (Table 9).

These techniques have been used to study a number of social insects. Termites, ants and house-fly waxes were all composed of linear alkanes, mono- and dimethylalkanes (tricosane to tritriacontane). Alkenes (9-tricosene to 9-nonacosene) and dienes (pentacosadiene to dotriacontadiene) have only been observed in termite waxes and in *Cataglyphis* ant waxes.

From the GC/MS analysis, we have contributed to definition of a new chemotaxonomy which is complementary to the biotaxonomy of entomologists. It enables explanation of different behaviours, which can be briefly described:

(i) European *Reticulitermes* waxes have been differentiated from those of USA species. The European species *Reticulitermes santonensis*, *R. grassei* and *R. banyulensis* have been discriminated, amongst themselves. Their analysis might explain aggressions or non-aggressions noted between different colonies of *R. banyulensis*.

(ii) Examination of the analysis of waxes has been made. Now it will be possible to undertake a biological explanation of the presence of the ponerine *Hypoponera eduardi*, which parasitizes *Reticulitermes* nests.

(iii) Other explanations of the behaviour have been made with regard to the contituents of waxes of two *Formicae* species (*Camponotus vagus* and *Cataglyphis cursor*) and a *Myrmicinae* (*Monomorium*).

(iv) These techniques have been performed for the analysis of cuticular waxes of the female *Calliphora vomitora* house-flies (Diptera). During the ontogenesis of this house-fly, qualitative and quantitative modifications of epicuticular hydrocarbons have been observed.

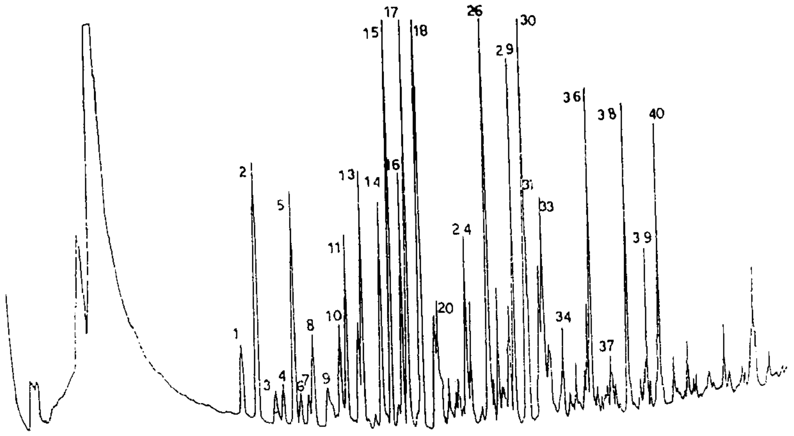


Figure 10. Gas chromatogram of cuticular hydrocarbons of ants (*Cataglyphis cursor*) (WCOT apolar column CP-Sil-5, 25 m, 150–320 °C at 5 °C min^{-1}).

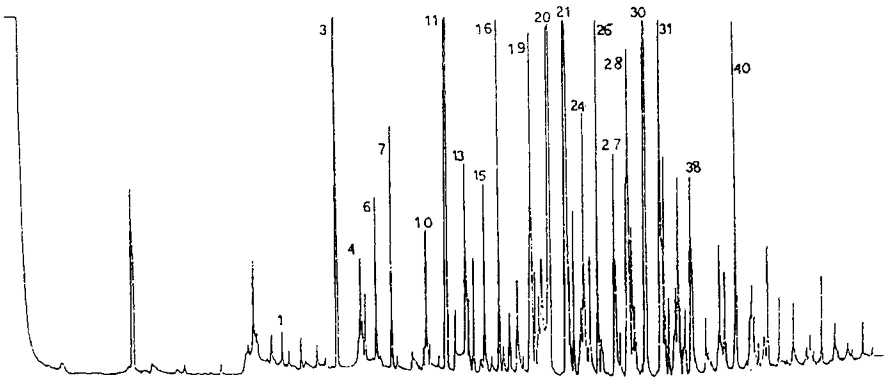


Figure 11. Gas chromatogram of cuticular hydrocarbons of house-flies (*Calliphora vomitora*) (WCOT apolar column CP-Sil-5, 25 m, 150–320 °C at 5 °C min^{-1}).

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