From wasps to ion chemistry

The outer surface of the insect cuticle (epicuticle) is covered by a layer of low volatility hydrocarbons. This layer is impermeable and thus limits water loss from the insect body. Furthermore, according to several authors, it is also a barrier to pathogens. In some species the mixture of hydrocarbons is used in intraspecific recognition, for example, recognition of sexual partners. In social insects it has been demonstrated that the hydrocarbon mixture is used to recognize individuals belonging to the same colony. In a society of wasps of the genus *Polistes*, the workers and foundresses are therefore capable of discriminating among individuals with differing hydrocarbon signatures, and act aggressively in response to these individuals to prevent exploitation of their food reserves.

To identify saturated and unsaturated hydrocarbons present on the cuticle of these insects, we used positive chemical ionization (PICI) with acetonitrile as the reagent gas in a GC/MS system. This gas, in the presence of hydrocarbons, favours the formaton of the cation $(M + C_2H_2N)^+$. The abundance of this ion $(M + 40)^+$ increases in proportion to the chain length of the hydrocarbon (in contrast, in EI, the opposite occurs). In the presence of unsaturated hydrocarbons, in addition to the ion $(M + 40)^+$, the ion $(M + 54)^+$ is also apparent. Furthermore, PICI spectra with acetonotrile of these hydrocarbons allow for the determination of the position of the double bond: a pair of ions appear with distinct intensities and with masses corresponding to the resultant fragments after cleavage of the chain in the position of the double bond.

The basic mechanism of the formation of these products and diagnostic ions is still unclear, but we have demonstrated that PICI with acetonotrile allows for the identification both of the molecular weight of hydrocarbons and the position of the double bond in mono-unsaturated alkenes.

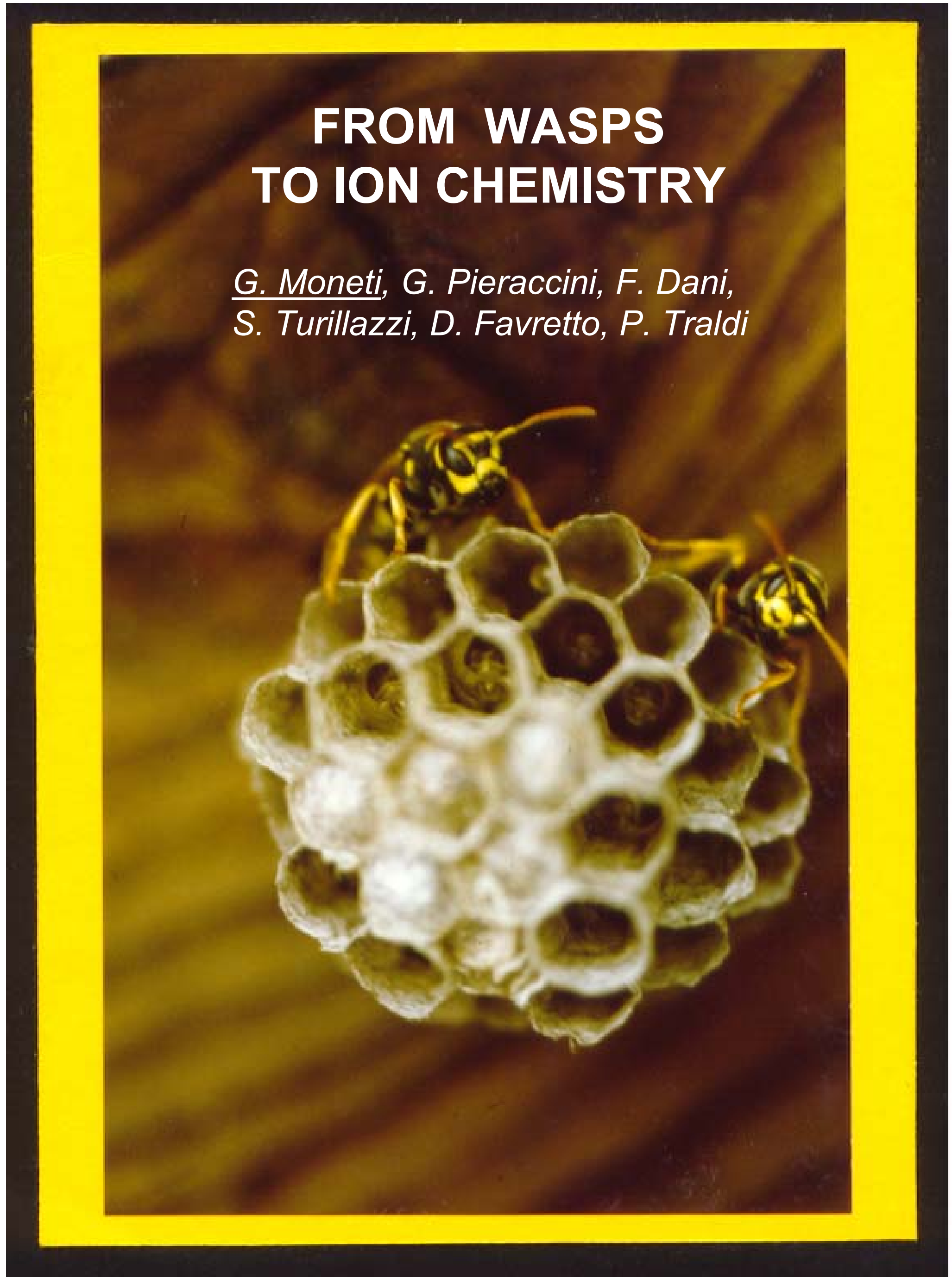
Bibl.: - RCM, 11, 857-862 (1997)
- J. Mass Spectrom. 32 (1997)

•The insect cuticle is covered by a layer of lipids. This layer protect the insect from infection and to reduce water loss.

•In social insects (wasps, bees, ants, and termites) cuticular compounds also allow individuals to recognize each other. Thus, individuals are able to discriminate colony members on the basis of the cuticular signature.

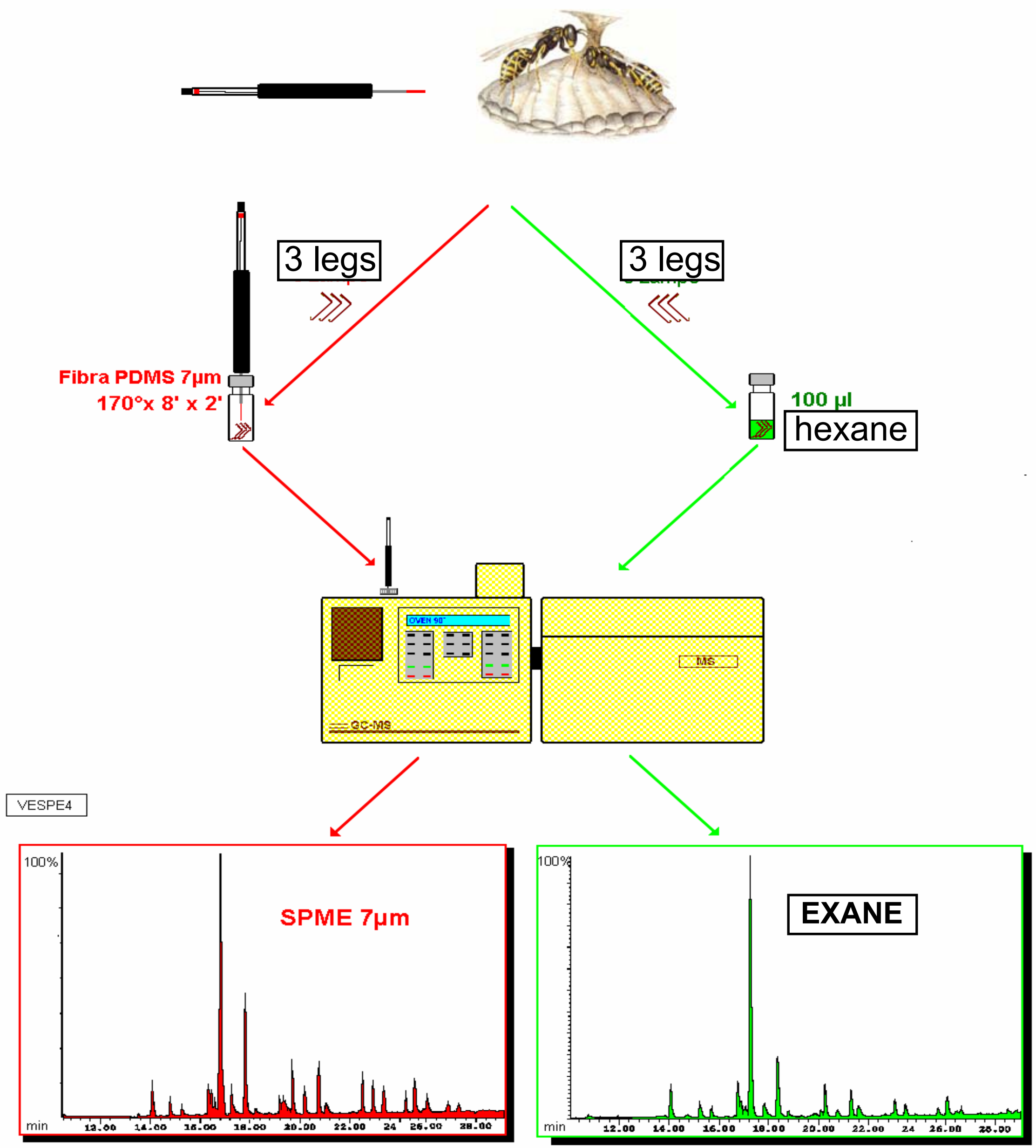
•In social insects the major compounds found on the cuticle are hydrocarbons. These are usually long-chained (C20 to C37) and may be saturated or unsaturated.

•In social wasps (*Polistes* sp.) cuticular hydrocarbons have been found to differ between colonies within species and allow colony members to recognize nestmates. Furthermore, the nest has been shown to be an important source of these hydrocarbons as well as glands present in the wasps.



COMPARISON OF EXTRACTION BY HEXANE AND SPME FROM LEGS OF *Vespa crabro*

- We compared the classical solvent extraction method (with hexane) for hydrocarbons with solid phase micro-extraction (SPME) in the headspace.
- Results from SPME analyses compare well with those of the classical solvent method
- SPME was a simpler and more efficient method for analysing cuticular hydrocarbons.
- An added advantage of the SPME fibre is that extractions can be made directly off <u>live wasps</u> allowing for the analysis of changes in individuals over time.



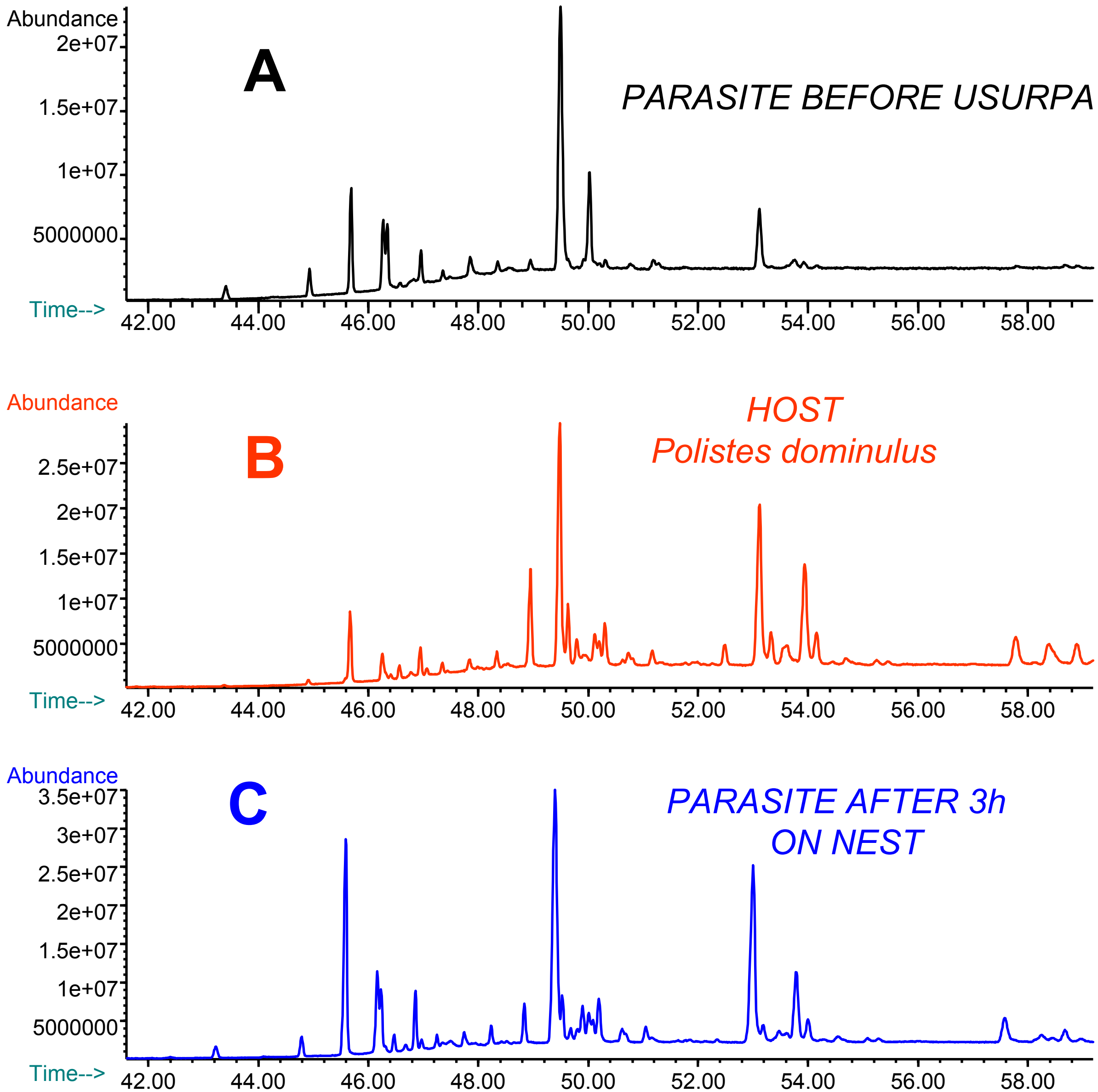
CUTICULAR HYDROCARBON PROFILE OF A WASP SOCIAL PARASITE BEFORE AND AFTER USURPATION OF A NEST OF *Polistes dominulus*

•*Polistes sulcifer* is a species of wasp that has lost the ability to establish nests and workers as other social wasps do. Instead this species is a social parasite. It takes over the nests of other *Polistes* species and forces the host workers to raise its eggs. Using SPME on live individuals, we have shown that this species is able to integrate itself into nests of the host by sequentially changing the cuticular hydrocarbon profile to match that of the host.

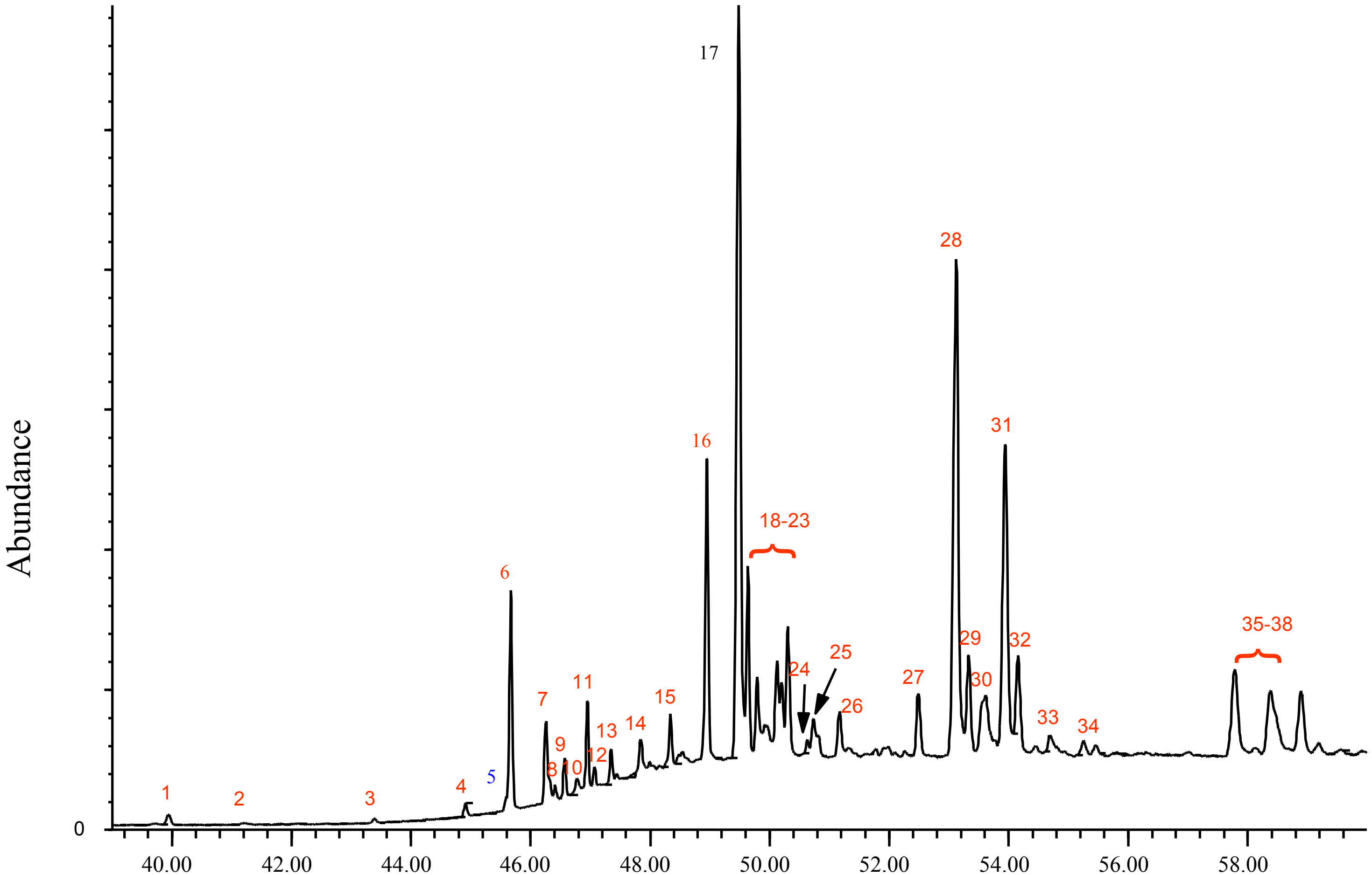
•A - The hydrocarbon profile of the parasite before usurpation of a nest.

•B - The hydrocarbon profile of the host species, *Polistes dominulus*

•C - The cuticular signature of the parasite 40 days after it has taken over the nest of the host



Polistes dominulus: cuticular hydrocarbons



1. C_{25}
2. 13-/ 11-/ 9-, meC_{25}
3. C_{26}
4. 2-meC_{26}
5. $C_{27:1}$
6. C_{27}
7. 13-/ 11- meC_{27}
8. 7-meC_{27}
9. 5-meC_{27}
10. 9, *y* dimetil-C_{27}
11. 3-meC_{27}
12. 5, 15 dimetil-C_{27}
13. C_{28}
14. 14-/ 13-/ 12- meC_{28}
15. 2-meC_{28}
16. C_{29}
17. 15-/,13- meC_{29}
18. 7-meC_{29}
19. 5-meC_{29}
20. 11, *y* dimetil-C_{29}
21. 7, *y* dimetil-C_{29}
22. 3-meC_{29}
23. 5, *y* dimetil-C_{29}
24. C_{30}
25. *x*, y dimetil-C_{29}
26. Unknown
27. C_{31}
28. 15-/, 13-, meC_{31}
29. 7-meC31
30. 13, 17 dimetil-C_{31}
31. 7, 15 dimetil-C_{31}
32. 5, 15 dimetil-C_{31}
33. *x*, *y* dimetil-C_{33}
34. Unknown
35. 17-/, 15-/, 13-, meC_{33}
36. 13, *y* dimetil-C_{33}
37. 7, *y* dimetil-C_{33}
38. 5, *y* dimetil-C_{33}

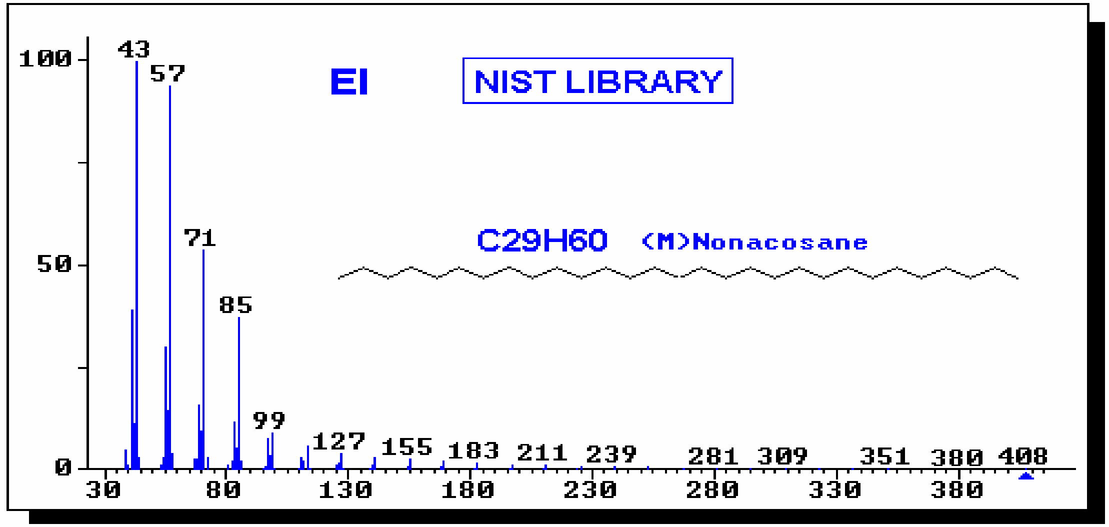
- Chromatogram after SMPE of a live individual of *Polistes dominulus*
- The cuticular signature consists mainly of saturated hydrocarbons ranging from chain length C25 to C33.
- Many of these are mono- or di-methylated, unsaturated hydrocarbons are present in very small quantities

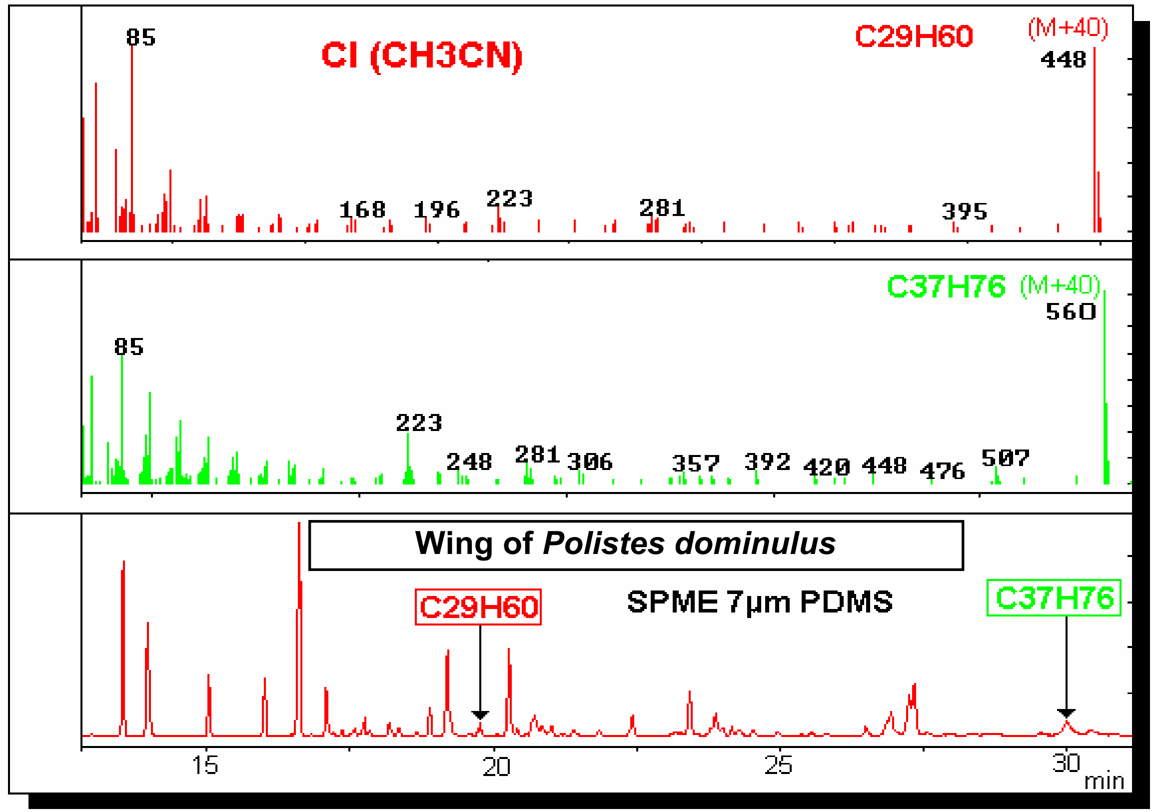
•A - In the EI NIST library the molecular ions of hydrocarbons decrease in proportion with the length of the carbon chain. This means that identification of hydrocarbons with more than 28 carbon atoms is very difficult by MS.

•B - Instead, in CI with CH3CN the intensity of (M+40) ion increases with an increase in chain length

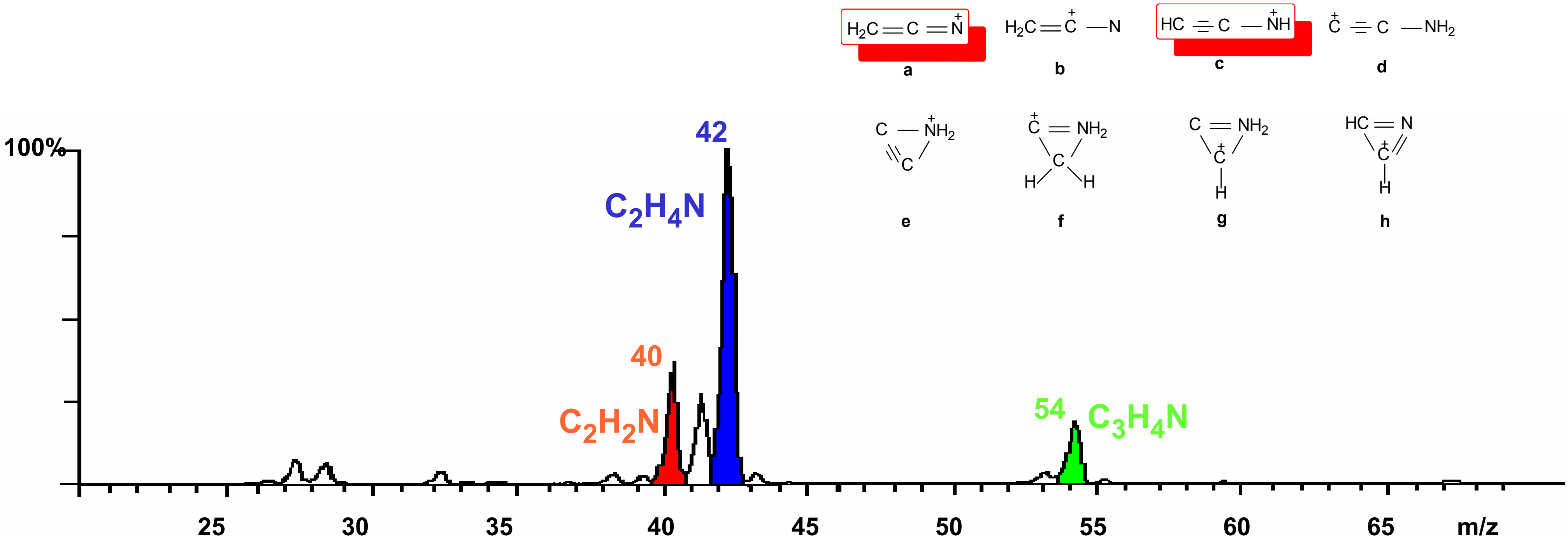
•C-Therefore,long-chained hydrocarbons can have (M+40) ions more intense than any other ions in the spectrum

•D - This means that even if these compounds are present in small quantities, they are identifiable because the (M+40) ions are very big.





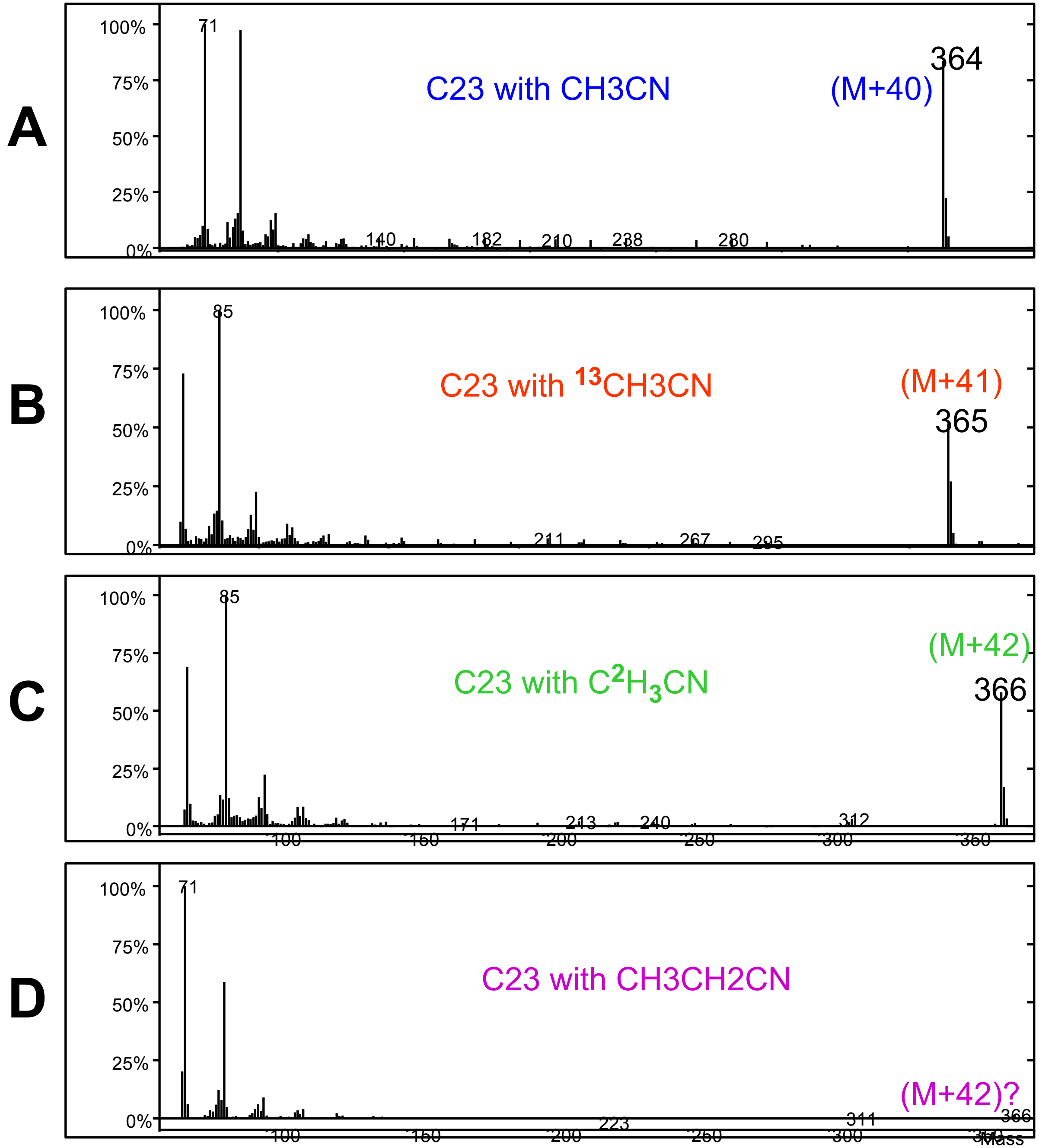
CHEMICAL IONIZATION WITH ACETONITRILE



- In CI, acetonitrile is introduced into the ion trap as the reagent gas. Together with the molecular ion of acetonitrile (m/z 42), the ions m/z 40 and m/z 54 are also present.
- The ion m/z 40 is the ion that forms an attachment with the hydrocarbons
- This ion can be written in eight structutally different configurations (a-h), but is probably present in the forms *a* or *c*.

Saturated hydrocarbon in CI with CH_3CN, $^{13}CH_3CN$, C^2H_3CN and CH_3CH_2CN

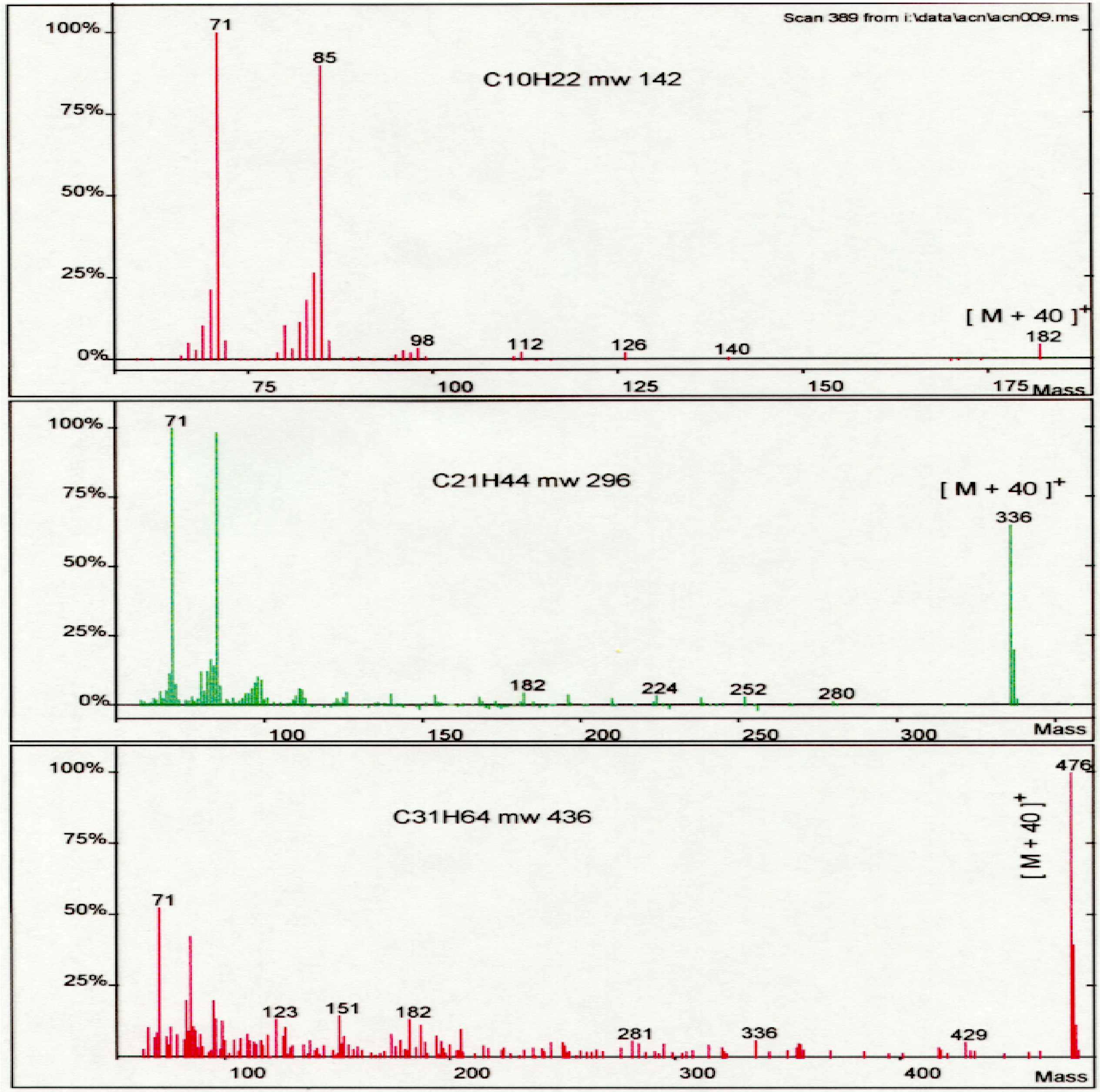
- We demonstrated that it was the acetenotrile reagent gas that formed the attachments with the hydrocarbons by using stable isotopes of acetenotrile like ^{13}C and ^2H as reagent gases.
- In the figures A-C the same C23 hydrocarbon forms attachments with the different reagent gases
- In D it can be seen that proprionitrile does not react with the hydrocarbon



SATURATED HYDROCARBONs IN CI WITH CH3CN: THE ABUNDANCE OF $(M+40)^+$ INCREASES AS CHAIN LENGTH INCREASES

- Chemical ionization of saturated hydrocarbons with acetonitrile reveals that the ion $(M+40)^+$ increases in intensity as the chain length of the hydrocarbon increases.

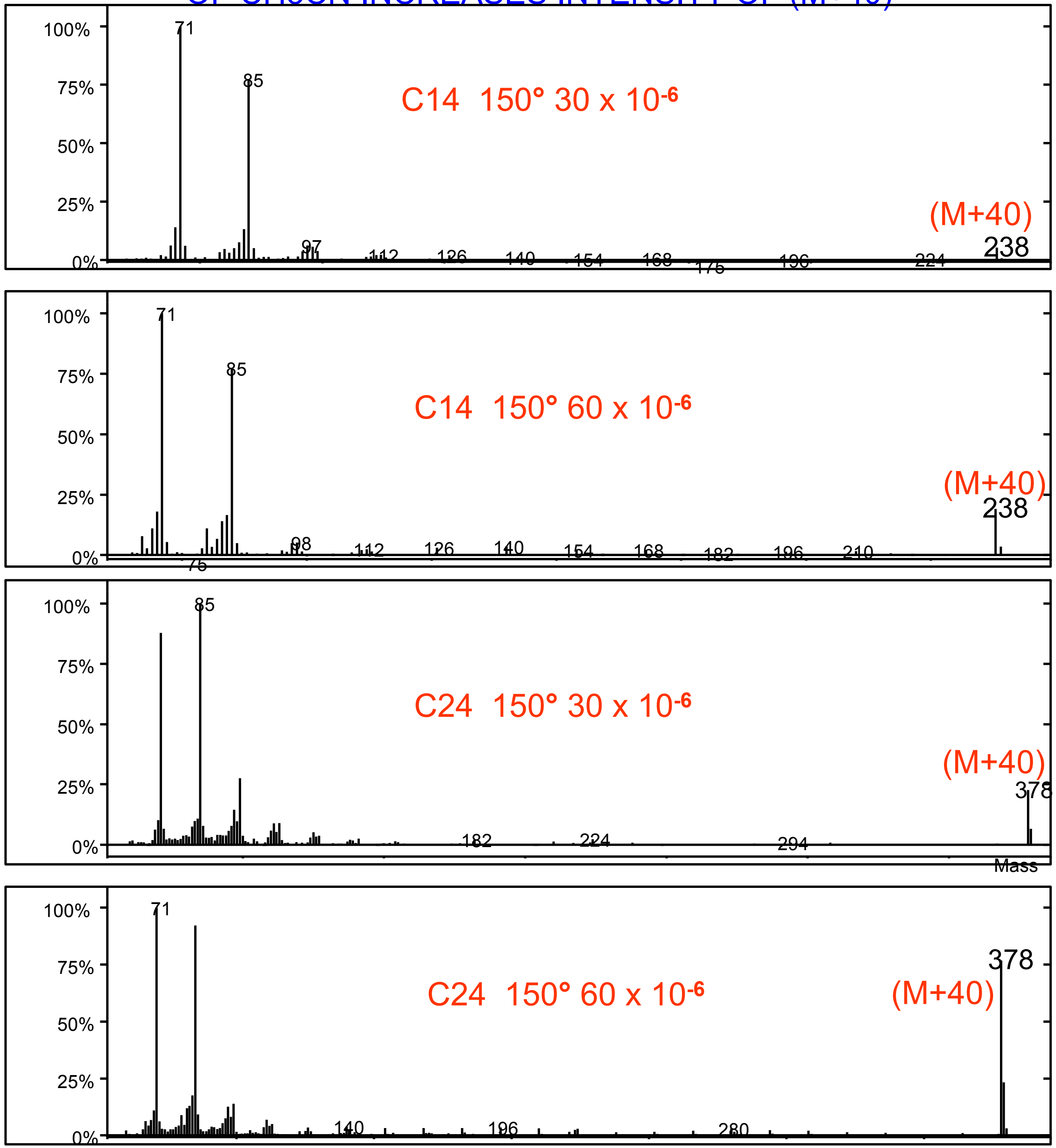
- Thus long chained hydrocarbons such as C31 have very visible $(M+40)^+$ ions and thus makes them very easy to identify.



SATURATED HYDROCARBON IN CI WITH CH3CN: INCREASE IN PRESSURE OF CH3CN INCREASES INTENSITY OF (M+40)

- We have also demonstrated that an increase in the pressure of the acetonitrile reagent gas increases the intensity of the $(M+40)^+$ ion at constant temperatures.

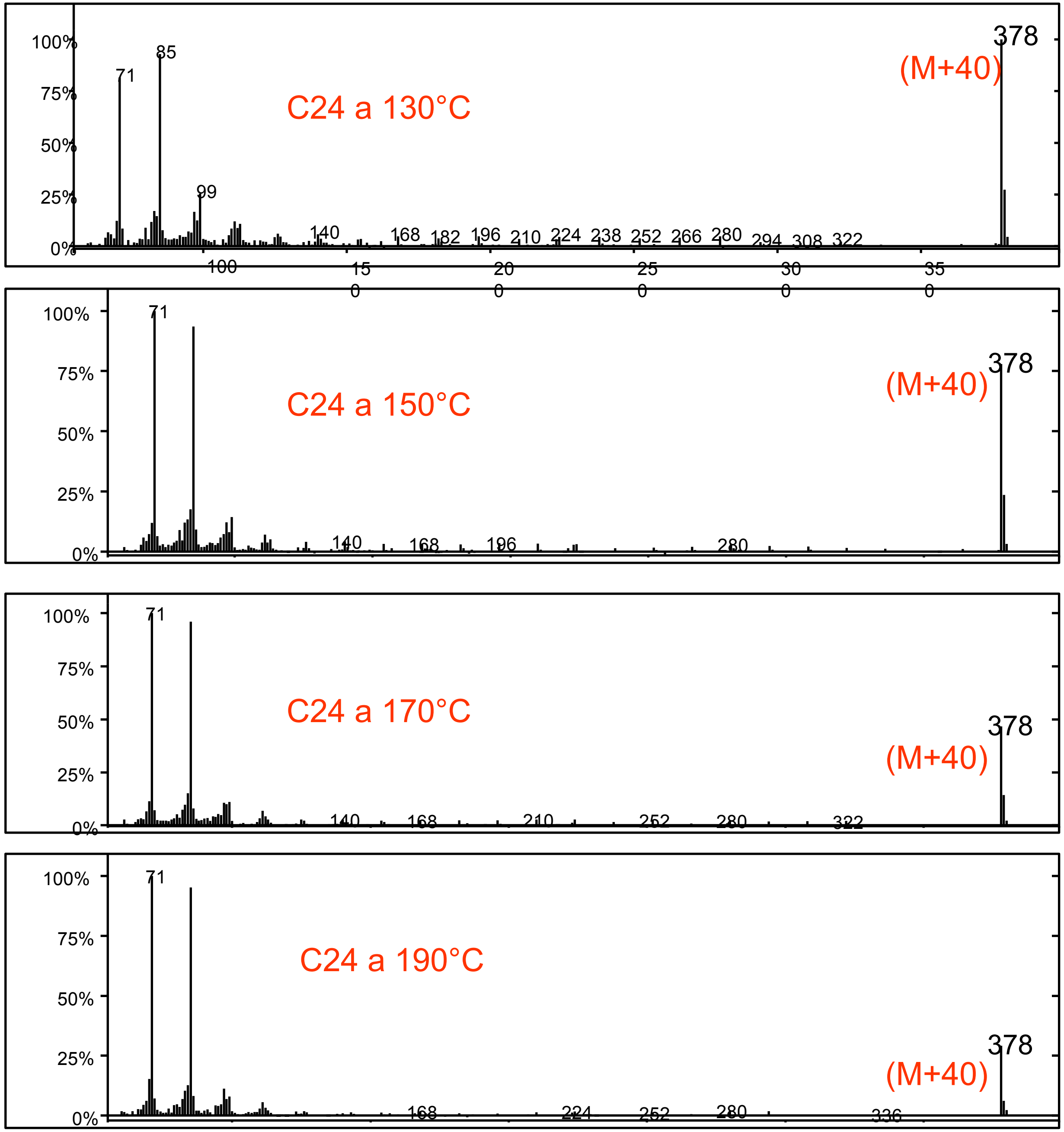
- Doubling the pressure of the gas doubles the intensity of the $(M+40)^+$



SATURATED HYDROCARBON IN CI WHIT CH3CN: AS INCREASE IN THE TEMPERATURE OF THE TRAP DECREASES (M+40)

- In contrast, when the temperature of the trap is increased the intensity of the $(M+40)^+$ ion decreases.

- Thus, the most suitable method for acquiring intense $(M+40)^+$ ions is at lower temperatures.

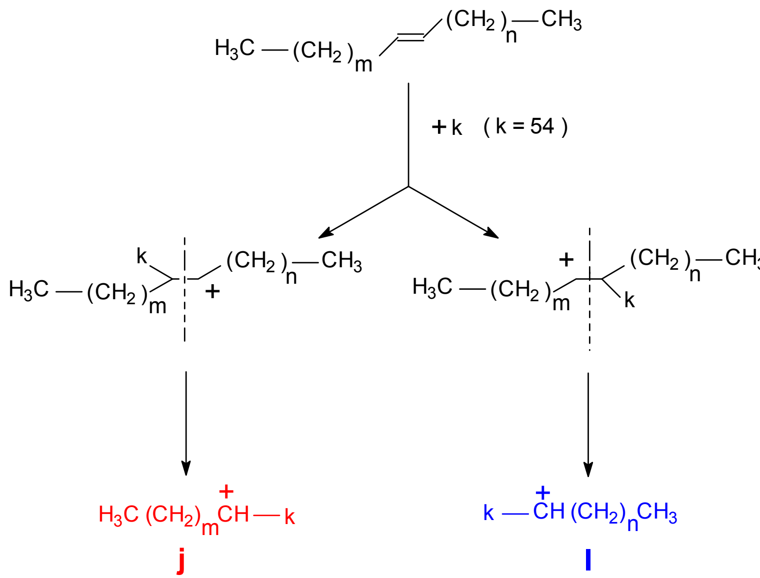


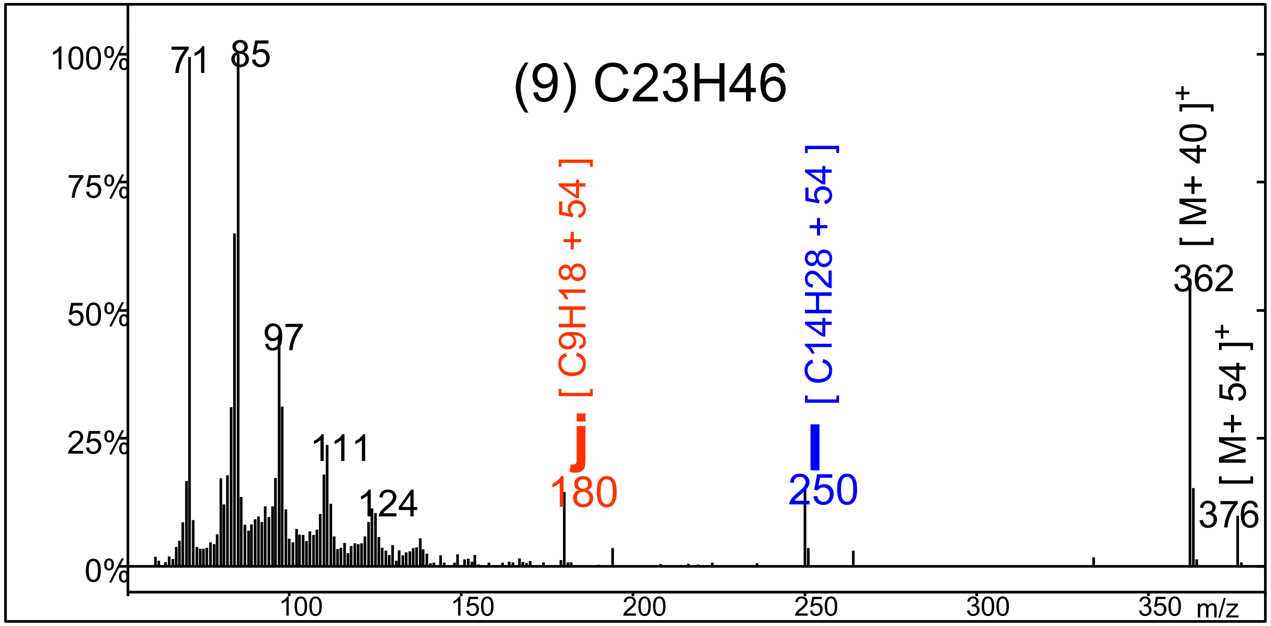
•Chemical ionization with acetonitrile also allows for the identification of mono-unsaturated hydrocarbons and the position of the double bond in these compounds.

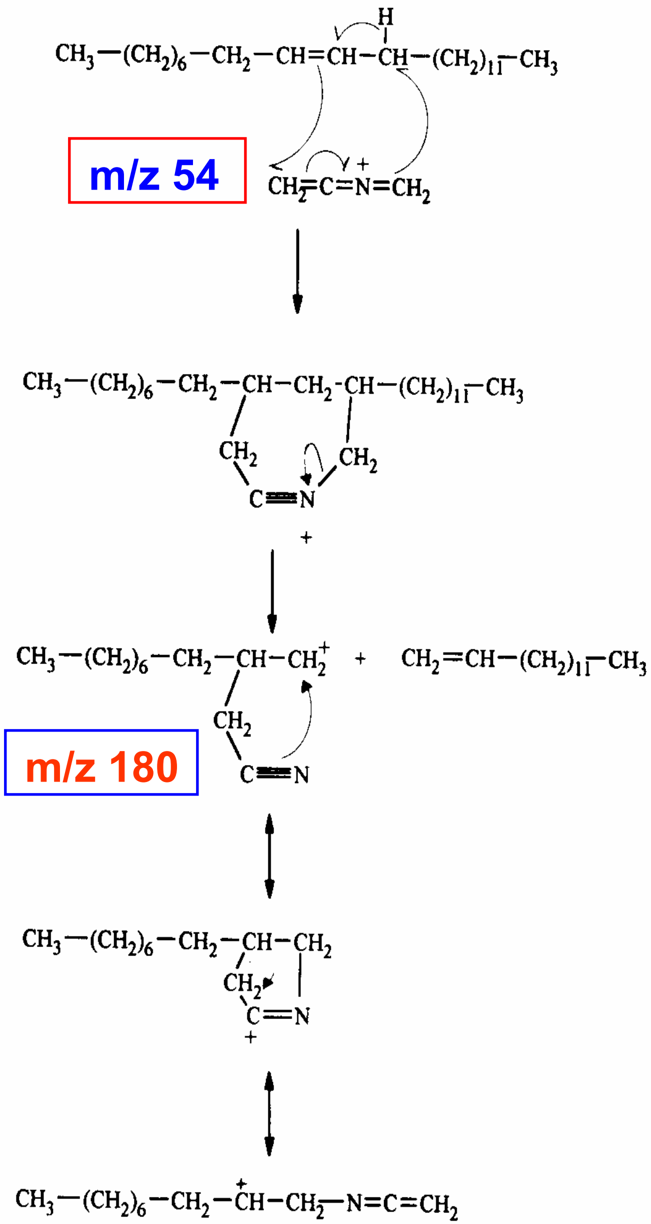
•In CI of these hydrocarbons a new reaction product is detected at m/z 356 corresponding to $(M+54)^+$. Thus mono-unsaturated hydrocarbons have both $(M+40)^+$ and $(M+54)^+$ ions while saturated ones have only $(M+40)^+$

•Furthermore, the presence of pairs of peaks of high abundance (e.g. m/z 180 and 250 in figure) displays the location of the double bond.

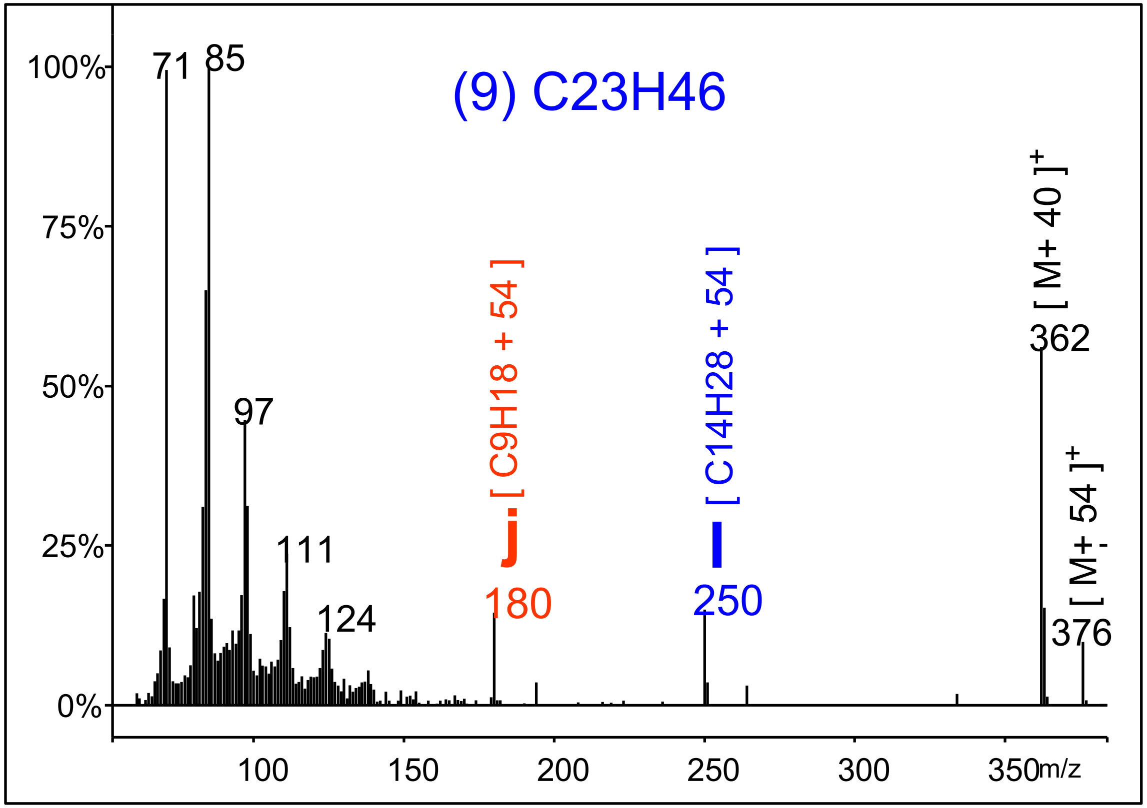
•These peaks can be explained by the scheme above. The reactive species at m/z 54 (k) can add to both CH groups of the double bond: by further cleavage of the addition products **j** and **l** are formed.







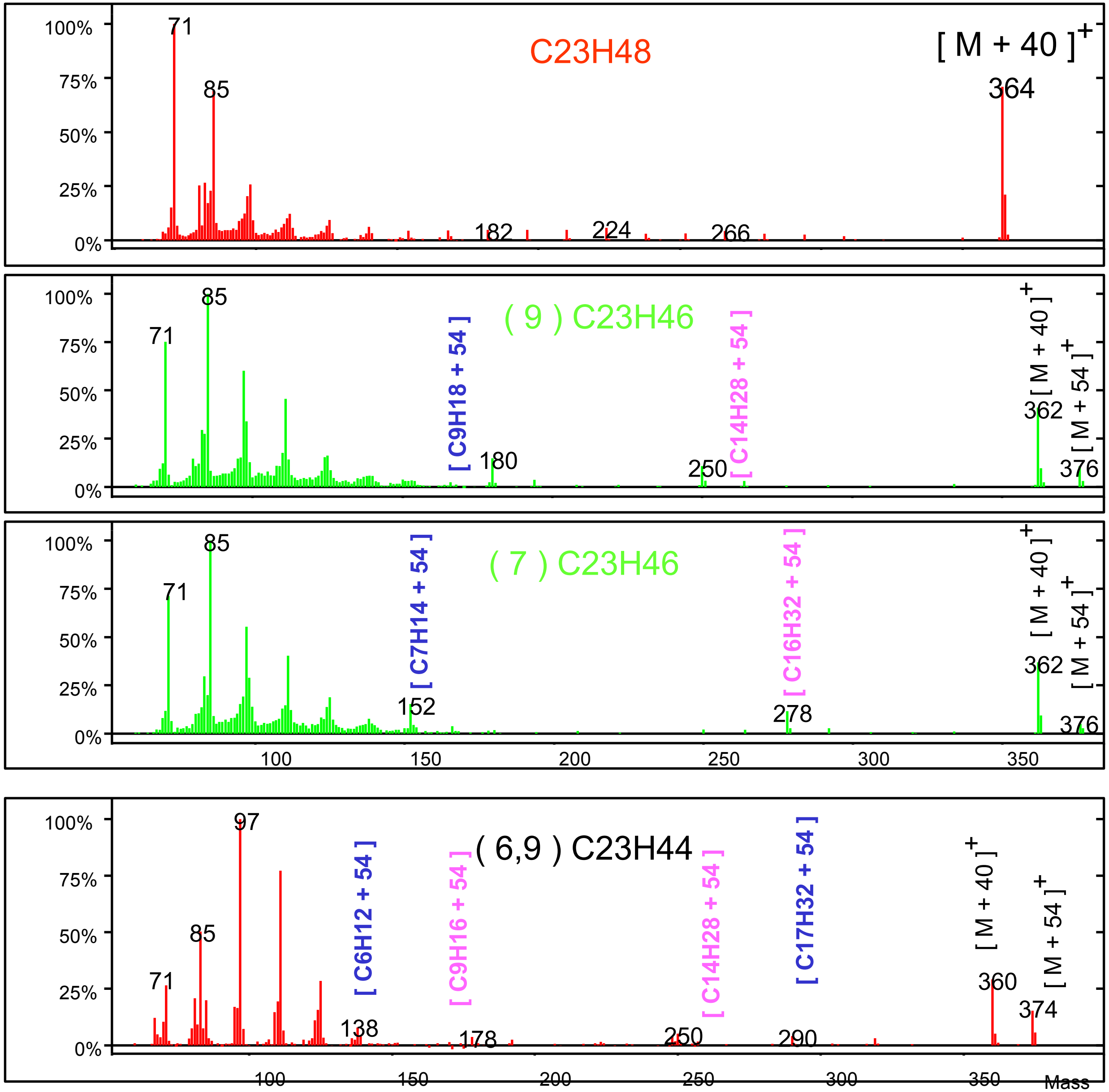
m/z 54
m/z 180



(9) C23H46
100%
75%
50%
25%
0%
71
85
97
111
124
[C9H18 + 54]
j
180
[C14H28 + 54]
l
250
[M+ 40]+
362
[M+ - 54]+
376
100
150
200
250
300
350
m/z

CI of ALKANES, ALKENES and ALKADIENES whit ACETONITRILE

- Chemical ionization with acetonitrile as the reagent gas thus allows for the identification of both saturated and unsaturated hydrocarbons of any chain length. For example:
- A - Alkanes only display $(M+40)^+$
- B and C - Alkenes possess both $(M+40)^+$ and $(M+54)^+$ ions. Peaks of high abundance display the position of the double bond: m/z 180 and 250 for (9)C23:1 (B) and m/z 152 and 278 for (7)C23:1 (C).
- D - Alkadienes possess also both $(M+40)^+$ and $(M+54)^+$ ions. Peaks of high abundance also allow for the determination of the position of the double bonds.



IDENTIFICATION OF ALKENES

- Another major advantage of chemical ionization with acetonitrile as the reagent gas is the fact that alkenes can be identified when they are present in very small amounts. This can be seen in the top figure.
- CI allows for the detection and identification of the alkenes (9)C24:1, (8)C24:1, and (7)C24:1, which are present in very low amounts.
- The added advantage is that derivatization does not have to be performed. Derivatization has been found to change the retention time of these compounds and thus the low abundances of the compounds makes them impossible to find.