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BIOMARKER DETERMINATIONS IN CRUDE OILS USING A TRIPLE-STAGE QUADRUPOLE MASS SPECTROMETER

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SUMMARY

Biomarkers are structurally complex organic compounds present in crude oils and source rocks that have been used extensively for a variety of purposes in petroleum exploration. Major applications include providing information on the nature of source materials, maturity, depositional environment and extent of biodegradation, and making oil–oil and oil–source rock correlations. The determination of biomarkers is generally accomplished using gas chromatography–mass spectrometry and ancillary techniques such as multiple-ion detection (selected-ion monitoring).

Hybrid and triple-stage quadrupole mass spectrometers, however, enable the use of alternative methods to determine the complex mixtures of isomers that occur within many classes of biomarkers. In general, the methods involve monitoring specific parent/daughter ion relationships which are characteristic of a specific compound or compounds. In this article examples of this approach will be given, using oils from various basins. In addition to demonstrating how these determinations are made, an example of how to obtain collision activated decomposition spectra of unknown compounds is described.

INTRODUCTION

Crude oils and source rock extracts are complex mixtures of organic compounds. A large proportion of the weight of the majority of oils consists of compounds with simple structures such as *n*-alkanes[1,2]. Also present are more complex compounds, for example steranes, terpanes, isoprenoids and porphyrins. The distributions of these compounds, called biomarkers, are widely used in petroleum exploration programs for a variety of purposes[3]. Applications include providing information on source material, nature of depositional environment, relative maturity and extent of biodegradation and undertaking oil–oil and oil–source rock correlations. The latter two applications provide information on the origin of the oil and its relationship to suspected source rocks and can lead to predictions about potential migration pathways. The geochemical information can, in turn, be correlated with seismic data to predict the presence of additional traps along the migration pathway, which may be filled with previously undiscovered oil accumulations.

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In general, the biomarkers of greatest value occur in low concentration and for most applications it is necessary to enhance their concentration prior to any analytical step by removing the *n*-alkanes. The method of choice for detection and determination of the biomarkers in crude oils and source rock extracts has been gas chromatography–mass spectrometry (GC–MS) and the associated techniques such as multiple-ion detection [MID; single-ion monitoring (SIM)][3,4]. The classes of biomarkers used most extensively for geochemical purposes and which are commonly analysed by GC–MS include sesqui-, di-, sester- and triterpanes, steranes, and isoprenoids. The ions commonly used to determine the distribution of these compounds are listed in numerous other publications[5]. Typical chromatograms for the distributions of steranes and terpanes in a crude oil are shown in Fig. 1a and b, respectively. The distributions of these biomarkers are complex and there are a number of co-eluting components that would provide a great deal of useful geochemical information if they could be resolved[5]. This is particularly difficult using MID since many of the components, which differ only in stereochemical configuration at certain carbon atoms, have the same molecular weight and major fragment ions. The advent of hybrid and triple-stage quadrupole mass spectrometers has provided a solution to this problem.

In this paper we discuss the use of a triple-stage quadrupole mass spectrometer, in the so-called MS/MS mode of operation, for analysing and resolving complex mixtures of the type found in crude oils and source rock extracts. There are a few other papers in the recent literature describing the use of tandem MS to resolve complex geochemical mixtures[6–9]. Warburton and Zumberge[6] described the use of metastable peak monitoring to resolve sterane distributions into individual C_{27}, C_{28} and C_{29} components. This approach was later extended to include C_{26} and C_{30} steranes which cannot be routinely monitored by GC–MS due to interferences from other steranes[7]. A similar approach involving metastable peak monitoring was used by Moldowan *et al.*[8] to determine the presence of tricyclic terpanes up to C_{45} in crude oils. Steen [9] used high-resolution selected metastable ion monitoring to determine the distribution of various biomarkers in oils and source rocks. This paper demonstrates how the triple-stage quadrupole mass spectrometer can resolve steranes and a wide variety of other components in complex mixtures during a single GC–MS/MS analysis.

EXPERIMENTAL

All analyses in this paper were performed using a Finnigan triple-stage quadrupole system (TSQ70). A Varian 3400 gas chromatograph equipped with a fused-silica capillary column (30 m × 0.25 mm I.D.) coated with DB5 (0.25-μm film thickness) was interfaced directly to the mass spectrometer. The column was operated in the split/splitless mode using helium as the carrier gas and temperature programmed from 40 to 300°C at 4°C min^{-1}. The mass spectrometer was operated in either the parent (PAR) or daughter (DAU) mode. The filament current was 200 μA and the electron multiplier set at 1600 V. Argon was used as the collision gas at a collision pressure of *ca.* 0.5 Torr and a collision offset voltage of −20 V. In general, each specific parent/daughter ion pair described in the discussion section was monitored for 0.02 s before switching to the next set of values, thus permitting a large number of experiments to be undertaken during each analysis.

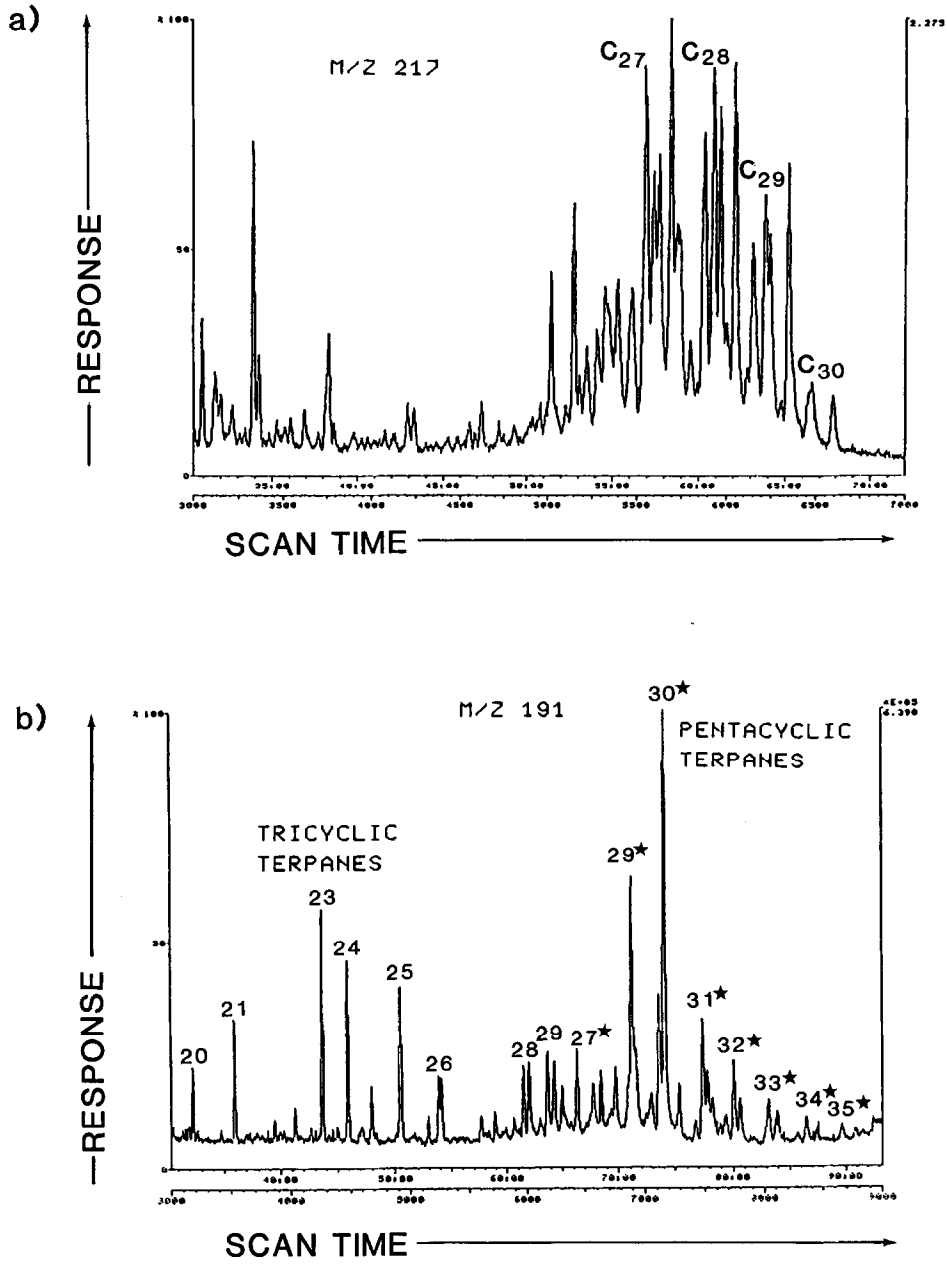

Fig. 1. Partial *m/z* 217 and 191 chromatograms obtained from GC–MS. The distribution of steranes may be investigated from the fragment ion at *m/z* 217 whilst that for terpanes is available from the fragment ion at *m/z* 191 (a and b, respectively). The peaks corresponding to the C_{27}–C_{30} steranes are indicated although some co-elution of regular and rearranged steranes occurs in the C_{27} and C_{28} region. In b the carbon number of tricyclic terpanes are indicated by 25 and pentacyclic terpanes by 30.

RESULTS AND DISCUSSION

The ability of the triple-stage quadrupole mass spectrometer to separate complex mixtures is based on the fact that specific parent/daughter ion relationships can be derived for different classes of compounds. In the majority of routine geochemical analyses, the classes of biomarkers commonly form pseudohomologous series. It was established from preliminary studies, that when molecular ions from these series of compounds are permitted to pass through the first quadrupole analyser (or "parent quadrupole") into the collision cell, characteristic daughter ions are formed by collision with argon atoms. These ions in turn can be monitored in the third or "daughter quadrupole" analyser. Hence, for each series of biomarkers a unique set of parent/daughter ions can be established. By rapidly switching between these relationships, each biomarker series can be continually monitored during the course of a GC–MS/MS analysis.

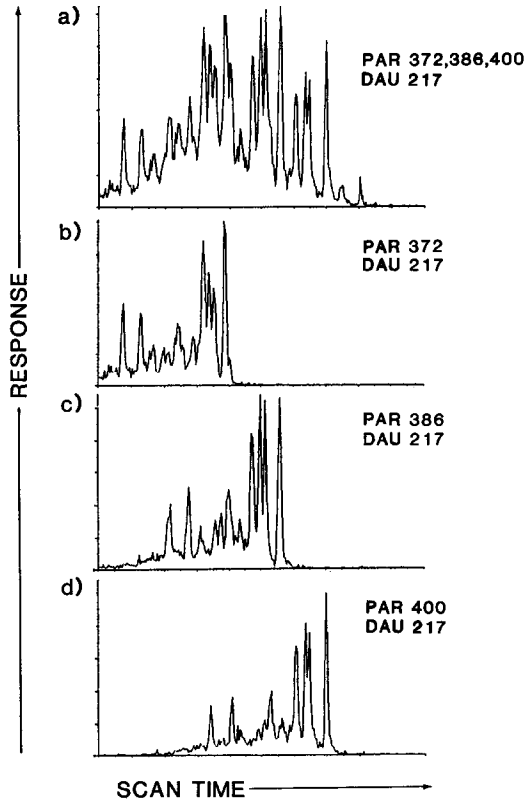

Fig. 2. (a) Partial chromatogram of the daughter ion at m/z 217 formed from the parent ions at m/z 372, 386 and 400, which correspond to the molecular ions of C_{27}, C_{28} and C_{29} steranes. Compare this distribution with that shown in Fig. 1a. (b) Partial chromatogram of the parent ion at m/z 372 producing the daughter ion at m/z 217 (C_{27} steranes). (c) Partial chromatogram of the parent ion at m/z 386 producing the daughter ion at m/z 217 (C_{28} sterane). (d) Partial chromatogram of the parent ion at m/z 400 producing the daughter ion at m/z 217 (C_{29} sterane). Thus the distribution of steranes for each carbon number may be studied.

Sterane parent ions (C_{27}, C_{28} and C_{29}) of the daughter ion at m/z 217 are shown in Fig. 2a. In this format the distribution is very similar to the m/z 217 chromatogram obtained, for the same sample, by "normal" GC–MS analysis (Fig. 1a). However, Fig. 2b–d show the individual distributions of the C_{27}, C_{28} and C_{29} steranes, which have been separated on the basis of their specific parent/daughter ion relationship. Since these steranes have now been separated on the basis of carbon number, it is possible to assess accurately their relative proportions. The relative proportion of steranes is necessary to describe the source materials contributing to an oil or extract[10,11]. Furthermore, maturity parameters based on the relative proportion of certain stereoisomers at each carbon number[4] can be accurately measured, without interference from co-eluting pseudohomologues of other carbon numbers.

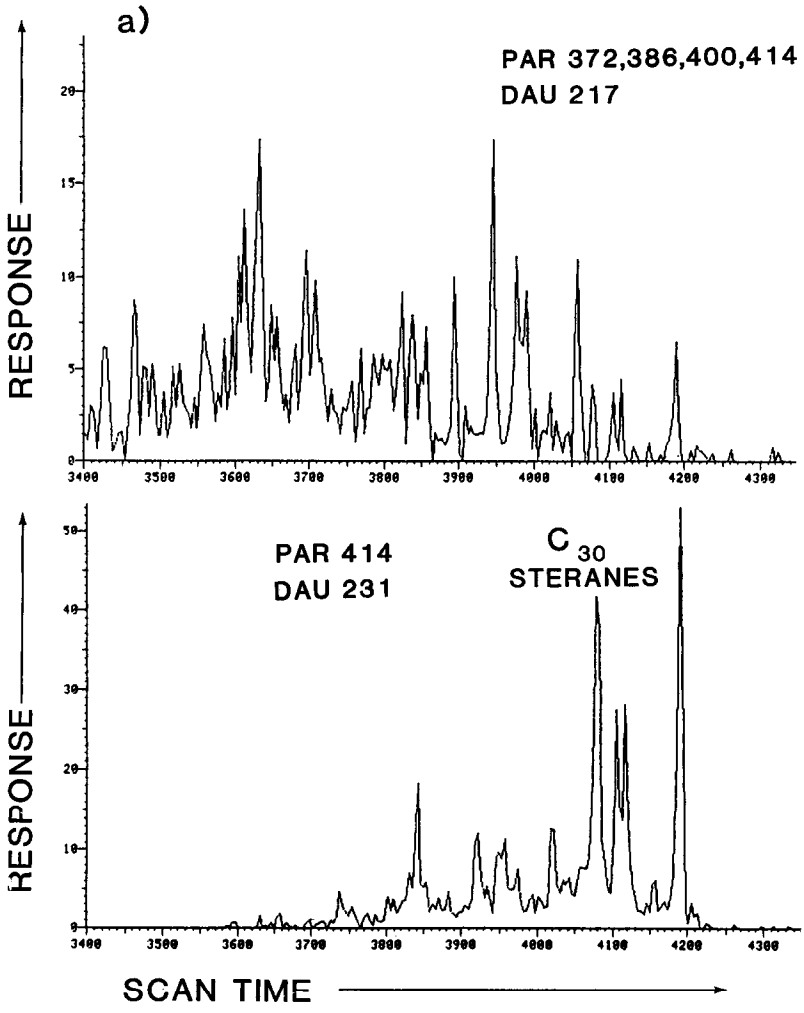

Fig. 3.

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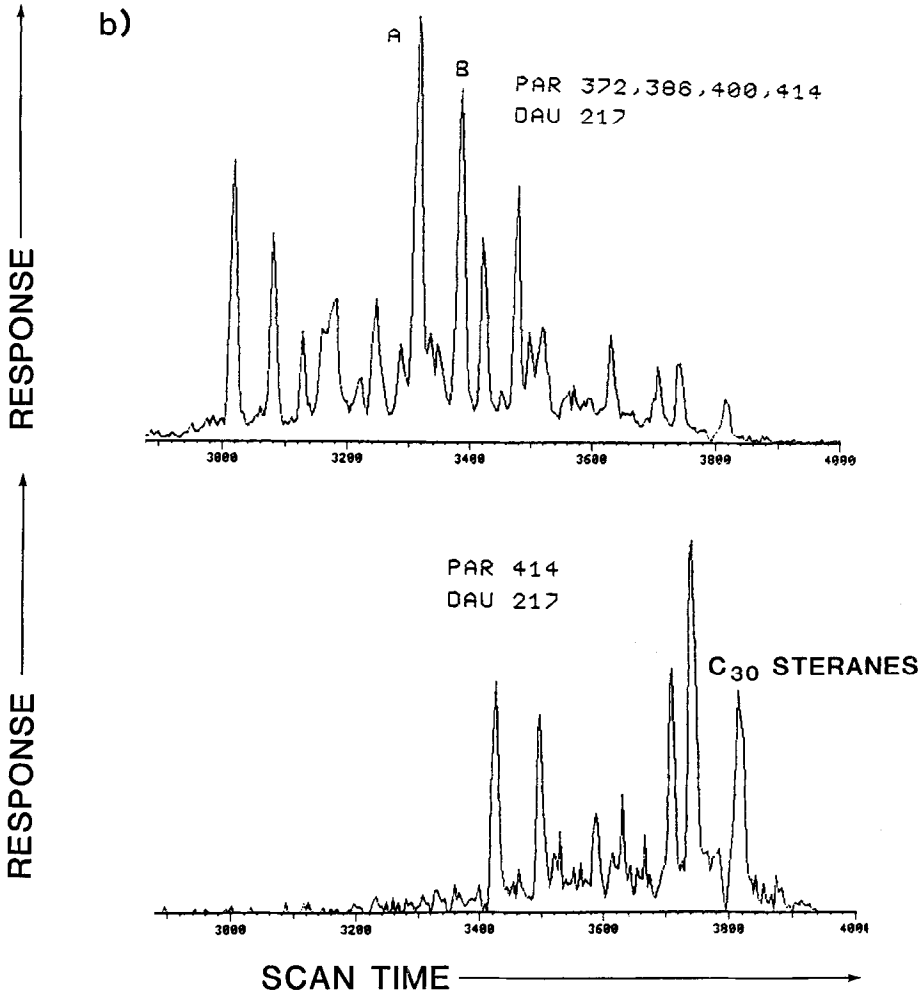

Fig. 3. (b) Partial chromatograms of the parent ions at m/z 372, 386, 400 and 414 and the parent ion at m/z 414 only which produce the daughter ion at m/z 217 (top and bottom, respectively). The C_{30} steranes (molecular ion m/z 414) are fragmenting to yield an intense ion at m/z 217, implying that the additional methyl group is on the side chain. (c) Partial chromatograms of the parent ions at m/z 372, 386, 400 and 414 which produce the m/z 217 daughter ion and the parent ion at m/z 414 which produces the m/z 231 daughter ion (top and bottom, respectively). The C_{30} steranes are fragmenting to yield an intense ion at m/z 231, indicating that the extra methyl group is in the A, B or C rings of the nucleus.

There has been considerable debate on the nature of the C_{30} steranes (parent: m/z 414) found in crude oils, in particular the position of the extra methyl group[12]. The position of this additional methyl group is important since it assists in differentiation of the type of source materials responsible for a particular sample. If the additional carbon atom is present as a methyl group in the A ring, the major fragment ion will be m/z 231 rather than m/z 217 because this fragment ion results from the cleavage of the A, B and C rings from the rest of the molecule[13]. This issue can be resolved by

performing two experiments, whereby the *m/z* 414/231 and *m/z* 414/217 (C_{30} sterane parent ion/daughter ion) relationships are monitored. For the sample illustrated in Fig. 3b the *m/z* 414/217 relationship was found to be far more intense than the *m/z* 414/231 relationship, which suggests that the additional carbon atom group is in the side chain of the molecule. On the contrary, the sample in Fig. 3a had a more intense *m/z* 414/231 relationship than *m/z* 414/217 relationship, indicating the presence of A ring methylated (4-methyl) steranes. The analogous experiment, using a double focusing magnetic sector mass spectrometer, has been performed by monitoring the first field free region metastable transitions *m/z* 414/217 and *m/z* 414/231 with similar results.

In the typical GC–MS analysis of geochemical samples for terpanes, using the ion at *m/z* 191, there is always a certain amount of overlap between the tricyclic and pentacyclic terpanes, particularly in the region of the chromatogram where the C_{27} hopanes elute (Fig. 1b). For a variety of geochemical reasons it is again desirable to separate these two classes of compounds. The only reported occurrence of such a separation in the past involved a time consuming column chromatographic fractionation prior to the GC–MS analysis[8]. The combined distribution obtained from monitoring parent/daughter ion relationships for tricyclic and pentacyclic terpanes is shown in Fig. 4a. The chromatogram resulting from the parent/daughter ion relationships of the tricyclic terpanes is shown in Fig. 4b and is completely devoid of any peaks corresponding to pentacyclic terpanes. Similarly, Fig. 4c shows the chromatogram for the parent/daughter ion relationships of the pentacylic terpanes and, again, is completely devoid of peaks due to the tricyclic terpanes. Hence, complete separation of pentacyclic and tricyclic terpanes can be achieved by the mass spectrometer even though they are not separated by GC.

Another example of the separation power of this approach is illustrated in Fig. 5. Oils derived from source rocks in hypersaline lacustrine environments commonly contain gammacerane (parent *m/z* 412)[14], a C_{30} triterpane, which on certain liquid phases co-elutes with the 22*R* epimer of 17α,21β-homohopane (parent *m/z* 426). This is a problem in many applications because the ratio of the (22*S*)- to the (22*R*)-17α,21β-homohopane is frequently used as a maturity indicator. Thus, many workers use a ratio based on the 17α,21β-bishomohopane (C_{32}) rather than the 17α,21β-homohopane (C_{31}) epimers. Fortunately, these two components have different parent ions but the same daughter ion at *m/z* 191. Hence, it is possible to resolve them mass spectrometrically using two parent/daughter ion relationships, namely *m/z* 412/191 and *m/z* 426/191 for 17α,21β-homohopane and gammacerane respectively. In Fig. 5a all of the *m/z* 191 parents for the C_nH_{2n-8} series (C_{27}–C_{31}) are shown (peaks 1–4 and 6). In Fig. 5b the *m/z* 412/191 relationship is shown, which separates the C_{30} hopane (peak 3) and gammacerane (peak 6) from the other triterpanes. Fig. 5c shows the *m/z* 426/191 relationship which results only from the 22*S* and 22*R* epimers of 17α,27β-homohopane (peaks 4 and 5), thus permitting their use in a maturity determination. Whilst it is realised that gammacerane can be resolved from the (22*R*)-17α,21β-homohopane fairly readily on fused-silica capillary columns, this provides a good example to illustrate the additional resolving power that can be achieved by using the MS/MS approach.

A number of oils derived from terrestrial source material, especially in south-east Asia, show several unusual peaks in the *m/z* 217 chromatogram (Fig. 6a)[15]. These

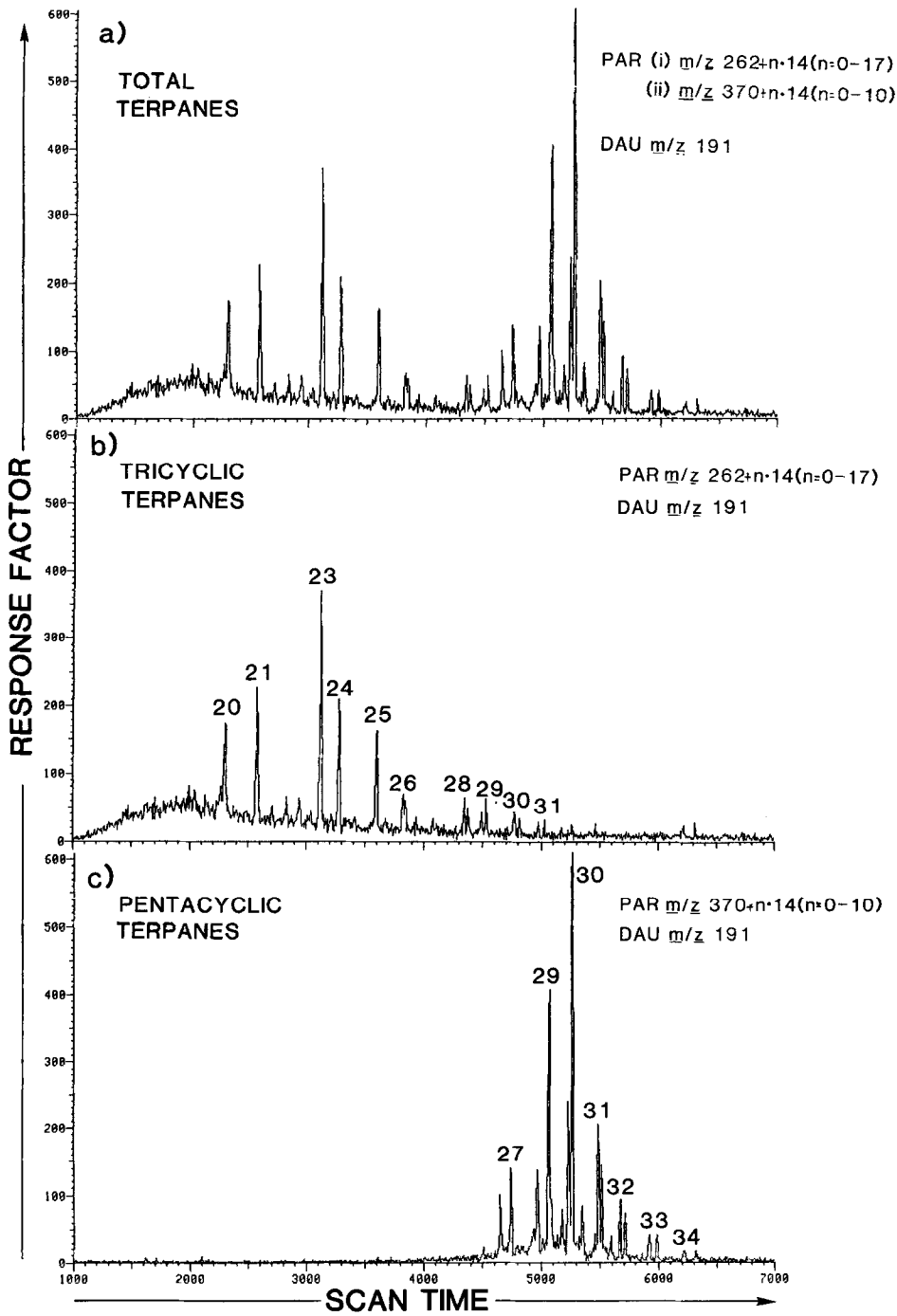

Fig. 4. (a) Partial chromatogram of the parent ions at m/z 262 + $n \cdot 14$ (n = 0–17) and at m/z 370 + $n \cdot 14$ (n = 0–10) which produced the m/z 191 daughter ions. This is analagous to a chromatogram of m/z 191 obtained from GC–MS. (b) Partial chromatogram of the parent ions at m/z 262 + $n \cdot 14$ (n = 0–17) which produced the daughter ion at m/z 191. This resolves the tricyclic terpanes from the pentacyclic terpanes. (c) Partial chromatogram of the parent ions at m/z 370 + $n \cdot 14$ (n = 0–10) which produce the daughter ion at m/z 191. This resolves the pentacyclic terpanes from the tricyclic terpanes.

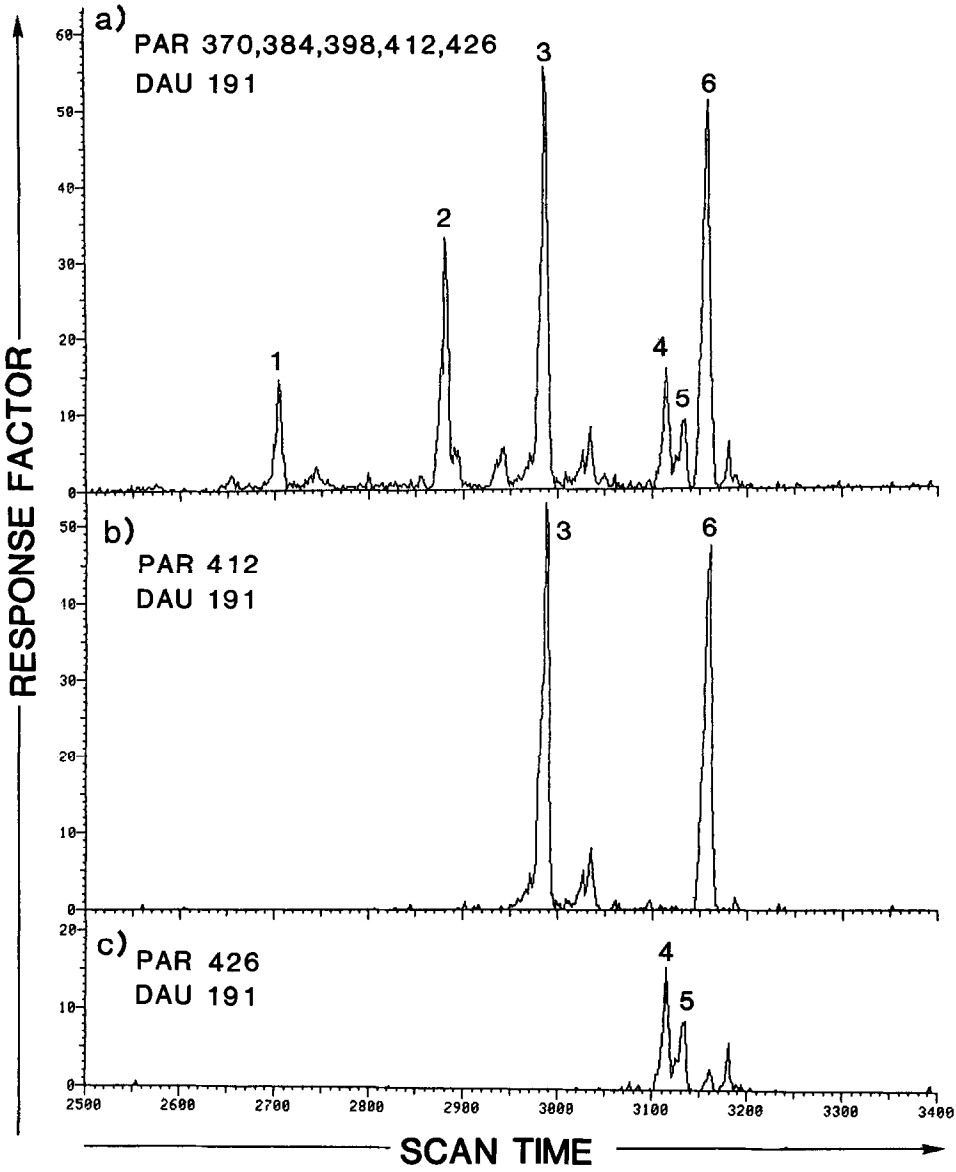

Fig. 5. Partial chromatograms of (a) the parent ions of m/z 370, 384, 398, 412 and 426, (b) the parents of m/z 412 only, and (c) parents of m/z 426 only which produce the daughter ion at m/z 191. Peaks 1, 2 and 3 are C_{27}, C_{29} and C_{30} hopanes, respectively, and gammacerane (peak 6) is co-eluting with (20*R*)-17α,27β-homohopane (peak 5). These latter two components may be mass spectrometrically resolved using the different parent/daughter relationships (m/z 412/191 and 426/191, respectively).

compounds do not appear to be steranes since all the major C_{27}–C_{30} steranes in that region of the chromatogram have been previously identified. This conclusion was confirmed when the MS/MS experiment was undertaken to examine the parent/daughter relationships ($C_nH_{2n-6}/217$; n = 27–29) for the C_{27}–C_{30} components. The

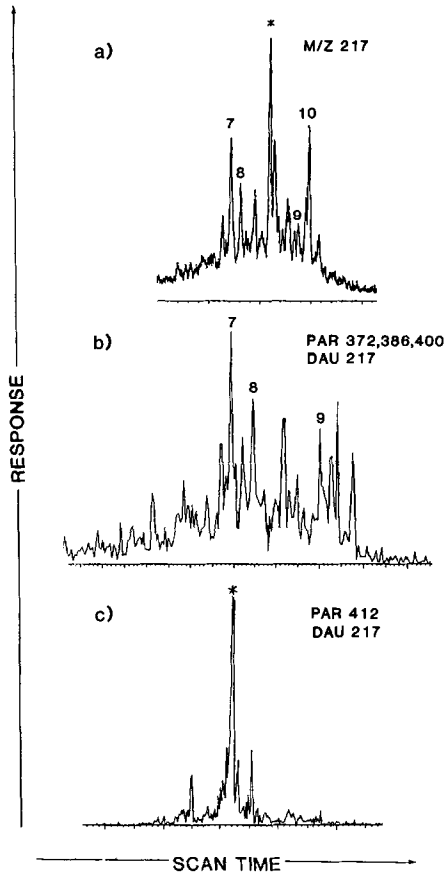

Fig. 6. (a) Partial m/z 217 chromatogram (GC–MS). Peaks 7, 8, 9 and 10 are steranes while the peak annotated with an asterisk (*) is an unknown component having an m/z 217 fragment ion but which is not a sterane. This analysis was performed under slightly different conditions than those for b and c, hence relative retention times differ between chromatogram a and those in b and c. (b) Partial chromatogram of the parent ions at m/z 372, 386 and 400 which produce the daughter ion at m/z 217, corresponding to the molecular ions of C_{27}, C_{28} and C_{29} steranes. Note that the peak annotated with an asterisk in Fig. 6a is absent. (c) Partial chromatogram of the parent ion at m/z 412 which produced the daughter ion at m/z 217. This implies that the daughter ion is derived from a C_{30} pentacyclic species.

resulting chromatogram showed that the major peak in the MID of m/z 217 chromatogram and annotated in Fig. 6a with an asterisk was absent (Fig. 6b), indicating that this component did not have a parent ion at m/z 372, 386 or 400 and, hence, was not a tetracyclic compound. Subsequently the experiment to measure the m/z 412/217 relationship was undertaken to determine whether this compound was a C_{30} pentacyclic compound having an intense m/z 217 fragment. As can be seen in Fig. 6c there is indeed an intense m/z 412/217 relationship. To obtain additional information on the structure of this compound it was necessary to operate the mass spectrometer in the daughter mode. In this mode only the m/z 412 parent ion formed in the ion source is allowed to enter the collision cell and a complete daughter spectrum is subsequently obtained by scanning the daughter quadrupole.

The chromatogram obtained from the daughter ion experiment is shown in Fig. 7a. The peaks in this chromatogram represent those compounds which have a molecular, or parent, ion at m/z 412 and from which complete daughter ion spectra have been collected. The major peak (marked with a star in Fig. 7a) is the unknown component with molecular weight 412 and a major fragment ion at m/z 217. In addition, a number of other peaks in this chromatogram can be identified as C_{30} terpanes, including 18α(H)-oleanane and 17α,21β-hopane (Fig. 7a, peaks C and D, respectively). Both of these components also have a molecular weight of 412 and hence will enter the collision cell in this particular mode of operation.

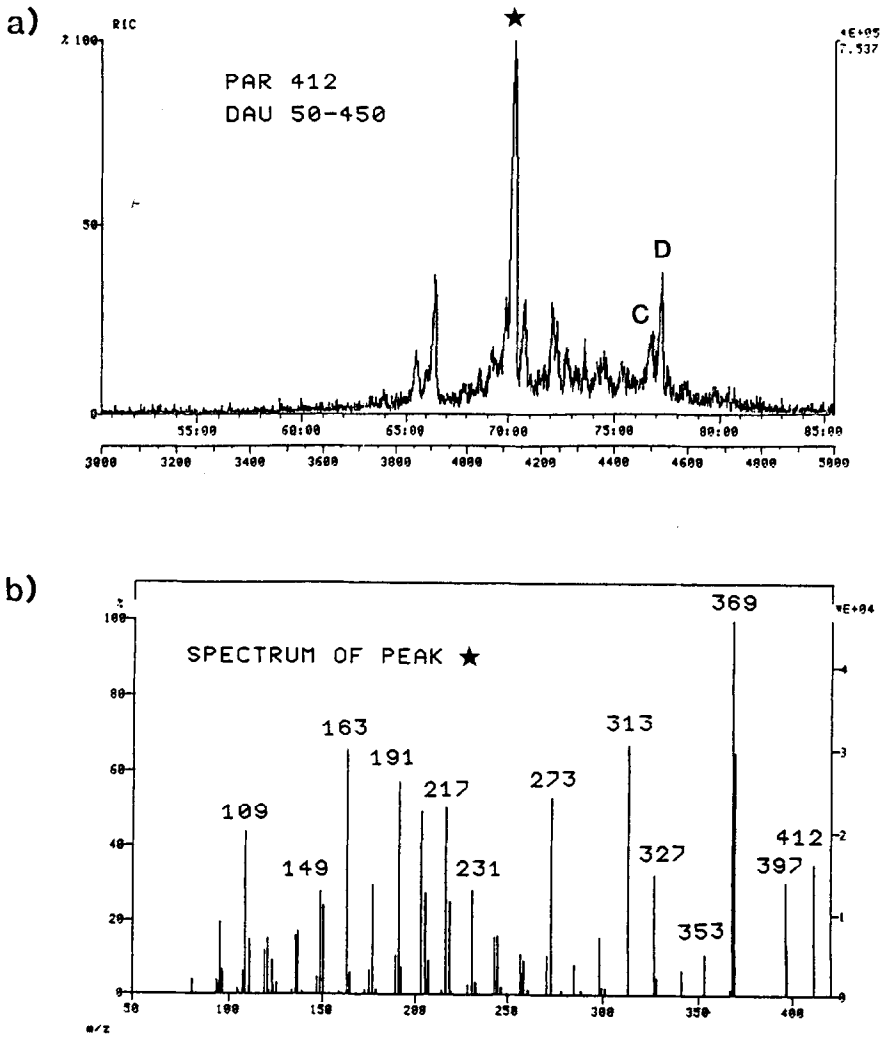

Fig. 7. (a) Partial chromatogram of the daughter ions (m/z 50–450) formed from the parent ion at m/z 412 whilst operating the mass spectrometer in the daughter mode. The peak annotated with a star (*) corresponds to the similarly annotated peak in Fig. 6a and c. (b) Daughter mass spectrum of the peak annotated with a star (Fig. 7a).

The collision spectrum of the unknown pentacyclic C_{30} component (star in Fig. 7a) is shown in Fig. 7b. Recently an electron impact (EI) mass spectrum of bicadinane, a C_{30}-pentacyclic hydrocarbon found in crude oils was published[16]. The masses of the major fragment ions in the published EI spectrum are generally similar to those shown

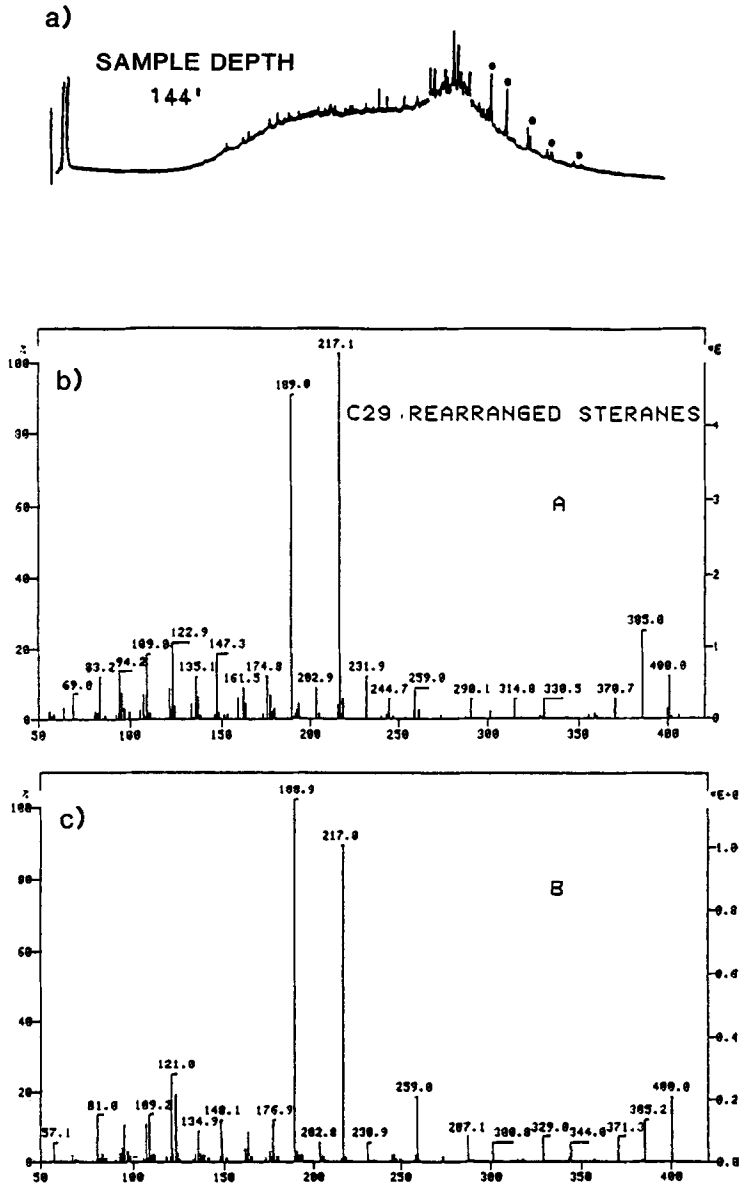

Fig. 8. (a) Gas chromatogram of the saturate fraction of a severely biodegraded tar sand bitumen. Daughter mass spectra formed from a parent ion at m/z 400 for peaks A and B in Fig. 3a (b and c, respectively). The small differences in the relative intensity of the ions at m/z 189 and 217 may reflect stereochemical differences between these components.

here for the collision spectrum. Both spectra have fragment ions at m/z 369 (M^+ – isopropyl), 313, 217, 191, 163, 149 and many others at lower m/z values although the relative intensity of these ions differ between the EI and collision activated decomposition (CAD) spectrum. On the basis of this similarity in the major fragments of the two spectra it was concluded that the unknown component in this oil was bicadinane.

A final example of operating the mass spectrometer in the daughter mode is taken from the analysis of a biodegraded tar sand whose gas chromatogram is shown in Fig. 8a. Few peaks in this chromatogram are clearly resolved from the extremely complex mixture. Analysis of this sample in the parent mode, searching for parent ions of m/z 217 produced the chromatogram shown in Fig. 3a. Since this sample is biodegraded, virtually all the regular steranes have been removed leaving only rearranged steranes. Once the molecular ion of these components has been established from the parent experiments it is possible to undertake the daughter experiments and obtain daughter spectra on each of them. Fig. 8b and 8c show the collision spectra of the peaks labelled A and B in Fig. 3a. From previous experiments it is known that these are rearranged steranes which have an intense ion at m/z 259 in their regular "normal" EI spectra. The collision spectra differ in that they have intense ions at m/z 189 and 217 of differing relative intensities possibly reflecting the different stereochemical configurations of these components. The reason(s) for the differences between the CAD spectrum and the "normal" EI spectra are not apparent at present; however, the point of this and the previous examples is to demonstrate the ability of the system to take an extremely complex mixture and obtain clean spectra of components without requiring background subtraction.

CONCLUSIONS

This preliminary investigation has shown some of the major applications of a triple-stage quadrupole mass spectrometer to the analysis of complex organic mixtures, such as those commonly obtained in samples of geochemical interest. Until now the commonly accepted method for the determination of biomarkers in these complex mixtures has been GC–MS with MID (SIM). MS/MS capability greatly enhances the ability to resolve individual series of compounds. This, in turn, greatly increases our ability to make accurate determinations of certain maturity measurements, such as those based on individual sterane isomers or on the relative proportions of tricyclic and pentacyclic terpanes. Finally, it is possible to take a complex mixture, with no clearly resolved chromatographic peaks and by using the triple-stage quadrupole mass spectrometer in the daughter mode, to obtain a clean spectrum of an unknown compound from the collision activated decomposition of its parent ion without the need for any background subtraction process.

ACKNOWLEDGEMENTS

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