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Origin and occurrence of 25-norhopanes: a statistical study

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Abstract—The alkane fraction of more than 200 rocks, biodegraded oils and non-biodegraded oils, have been analysed by means of computerized GC–MS, in order to investigate the effect of natural biodegradation on the occurrence of "demethylated hopanes", i.e. 17α-25-norhopanes. The results obtained indicate that 25-norhopanes are preexisting biomarkers the concentration of which is enhanced by selective biodegradation of more readily degradable homologs, i.e. regular hopanes, rather than by demethylation of hopanes in reservoirs. However, the use of 25-norhopane enrichment as a palaeobiodegradation indicator in apparently non-biodegraded oils is still valuable providing the initial background content in the corresponding source rocks is known. Furthermore, 25-norhopanes appear to be diagnostic of specific environmental conditions (marine and lacustrine source rocks, dysoxic and not very hypersaline). Lastly, one other (novel) bacterially resistant rearranged hopanoic compound, namely a C_{29} neohopane, is applicable for both biodegradation and maturation evaluation.

Key words—25-norhopanes, (palaeo-)biodegradation, 25-norhopane background in source rocks, statistical approach, environmental deposition, gammacerane, oleanane, 28,30-dinorhopane, 30-norneohopane

INTRODUCTION

During the last decade, organic geochemistry has become one of the main techniques used in petroleum exploration to study source rocks (in particular their generative potential and maturity), oil to source rock and oil-to-oil correlations. Improvements in analytic techniques have allowed extensive use of the molecular approach through the so-called biological markers, which can be considered as fossilized molecular remnants. Though a growing interest is observed in the field of macromolecular entities (e.g. asphaltenes, sulfur-rich polymers, kerogens), analysis of free molecules still remains the main target of the exploration (molecular) geochemist. Emphasis is laid on normal and branched alkanes, aromatics, and most of all, polycyclic compounds such as steranes and triterpanes. The latter indeed are essential complements of bulk analyses of reservoired oils such as API gravity, sulfur content, carbon isotopic composition or optical rotation.

However, compositional changes in petroleum induced by several phenomena such as maturation, deasphalting, water washing or biodegradation (Blanc and Connan, 1992) introduce complexities in the interpretation of molecular marker profiles. Biodegradation, in particular, is a relatively widespread phenomenon affecting a large number of reservoired-oils. The lack of reliability concerning the fate of some biomarkers during this degradation process makes it difficult to use them for maturity assessment, or oil to source rock correlation, in such a case.

There is general agreement between field observations and laboratory experiments which indicate the following order of increasing resistance of the main molecular families towards bacterial alteration: *n*-alkanes, isoprenoids, regular steranes, diasteranes, hopanes (Connan, 1984). The order of bacterial attack of regular steranes has even been defined to be: $\alpha\alpha\alpha R > \alpha\beta\beta R > \alpha\beta\beta S > \alpha\alpha\alpha S$ with $C_{27} > C_{28} > C_{29}$ (Rullkötter and Wendisch, 1982; McKirdy *et al.*, 1983; Volkman *et al.*, 1983; Goodwin *et al.*, 1983; Seifert *et al.*, 1984; Zhang Dajiang *et al.*, 1988; Chosson *et al.*, 1991, 1992). As far as hopanes are concerned, the rate of biodegradation seems to be $22R > 22S$ with $C_{35} > C_{30}$ (Goodwin *et al.*, 1983; Chosson *et al.*, 1992). However, discrepancies with this hierarchy of bacterial consumption of steranes and hopanes have been reported recently by Peters and Moldowan (1991) who tentatively showed hopane losses prior to any obvious sterane depletion, and resistance of C_{35} compared to C_{30}–C_{34} hopanes in a West Siberian oil (J. M. Moldowan, personal communication). The latter phenomenon was also observed by Requejo and Halpern (1989) in a California tar sequence, and the former by Cassani and Eglinton (1991) in extra-heavy oils from the Eastern Venezuelan basin. Furthermore, uncertainties still remain concerning the fate of these biomarkers through severe biodegradation.

Among polycyclic alkanes, "demethylated hopanes" have an unclear origin. Since their first detection by Reed (1977) in a weathered oil-impregnated sandstone from Utah, their presence in an oil has been regarded as indicative of heavy biodegradation. The elucidation of the exact site of demethylation (at C-4 or C-10) was not possible on the basis of mass spectra. Detailed investigation of biodegraded

asphalts from Madagascar and ^1H experiments showed demethylation to occur at C-10 and, hence, "demethylated hopane" to be 17α(H),21β(H)-25-norhopane (see Appendix) (Rullkötter and Wendisch, 1982). The presence of such compounds in apparently non-biodegraded oils, i.e. oils containing *n*-alkanes, has then been considered as diagnostic of a phase of palaeobiodegradation which has affected the oil prior to the latest phase or accumulation (Volkman *et al.*,1983; Philp, 1983; Howell *et al.*, 1984; Chosson *et al.*, 1992). Whatever their utilization in petroleum geochemistry could be, the problem of their origin is still a subject of controversy.

According to several authors (Seifert and Moldowan, 1979; Volkman *et al.*, 1983; Philp, 1983; Noble *et al.*, 1985; Peters and Moldowan, 1991; Cassani and Eglinton, 1991), 25-norhopanes are considered as demethylated products of hopanes in reservoired oils through biotransformation. This conclusion is mainly based on the preferential occurrence of these molecules in heavily biodegraded oils combined with their absence in pyrolysates of the associated asphaltenes or kerogens (Noble *et al.*, 1985; Cassani and Eglinton, 1986). However, the occurrence of demethylated hopanes in source rocks or shales (Noble *et al.*, 1985; Chosson *et al.*, 1992), their absence in biodegradation products of hopanes submitted to microbial strains under laboratory conditions (Rubinstein *et al.*, 1977; Connan *et al.*, 1980; Goodwin *et al.*, 1983; Chosson *et al.*, 1992), as well as their non-ubiquity in biodegraded oils (see ref. in Chosson *et al.*, 1992) are arguments against their neoformation through demethylation of hopanes during in-reservoir biodegradation processes. In fact, the degradation of αβ-hopanes to "demethylated hopanes", i.e. an alkane product, is very unlikely since biodegradation is a bioxidation process which should generate functionalized structures as seen for cholestane byproducts (S. Paulus and P. Chosson, personal communication).

Another pathway which explains the origin of "demethylated hopanes" is to consider that they occur as preexisting biomarkers in source-rocks and are subsequently concentrated in the associated crude oils by selective biodegradation of more readily degradable structures (i.e. steranes, regular αβ-hopanes, etc.) (Goodwin *et al.*, 1983; Howell *et al.*, 1984; Chosson *et al.*, 1992). The present paper (which is a more detailed presentation of the results related in Blanc and Connan, 1991) offers an attempt to highlight this hypothesis using a statistical approach of numerous GC–MS data. These data comprise 20 typical molecular ratios including the relative concentration of 25-norhopane. Comparison to source- or environmental-indicating biomarkers, as well as to literature data, enabled us to evaluate 25-norhopanes as potential diagnostic biomarkers of particular environmental conditions.

EXPERIMENTAL

Data bank

From 1650 analyses of crude oils, and rock extracts, on a worldwide basis, 213 samples have been found to contain the C_{29} demethylated hopane (abbreviated 29DH in our study), even at very low concentration. These 213 samples constituted our basic data bank. Among them, 62 are indigenous source rock extracts, 90 are non-biodegraded oils or impregnations, whereas 61 are biodegraded ones. Degrees of biodegradation have been assessed by the extent of the removal of normal alkanes. Each sample has been analysed by computerized GC–MS of its saturated hydrocarbon fraction.

Whenever possible, relative concentrations of 25-norhopanes have been calculated as a ratio of demethylated/regular hopanes (29DH/30H) and demethylated/norhopanes (29DH/29H) (for abbreviations see Appendix). It should be indicated here that "demethylated hopanes" are likely to exist as a complete family of homologs ranging from C_{28} to C_{34} (not necessarily completely paralleling the regular hopane series). In our samples from the data bank, the whole series is not alway observable. We chose the C_{29} compound since it is always present and the easiest to identify and quantify, on both the *m/z* 177 and *m/z* 191 fragmentograms (see Appendix).

The samples analysed cover a very wide maturation range, from immature to mature, as shown by classic molecular measurements (see for instance Fig. 1 for sterane ratios evolution). Geological data referring to

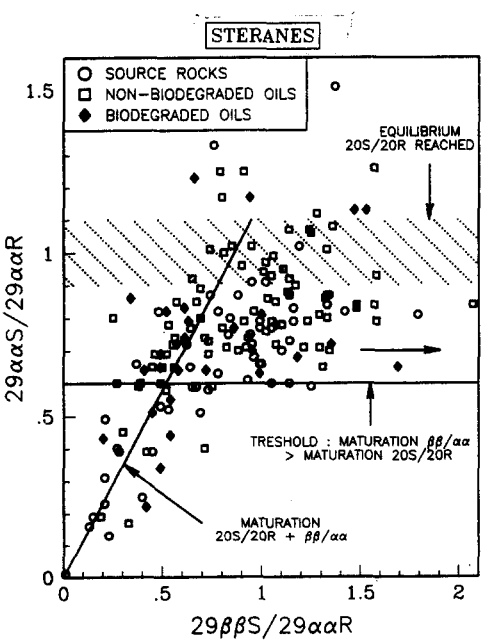

Fig. 1. Evolution of the 29ααS/29ααR ratio as a function of 29ββS/29ββR for the samples of the data bank: maturity assessment with steranes.

Table 1. Distribution of the origin and time of source rocks deposition (including corresponding source rocks of the oils and impregnations analysed)

Origin of O.M.	Marine		Lacustrine		Terrestrial	
%	45		39		16	
Time of deposition	Tertiary	Up.Cretaceous	Low.Cretaceous	Jurassic	Triassic	Ordovician
%	13	11	44	27	1	4

the age and origin of the organic matter are summarized in Table 1. Note that only 16% of the samples are of definite terrestrial origin, whereas 39% are lacustrine and 45% marine. It should be noted that these figures stem from the approximation that, in most instances, organic matter deposited under lacustrine condition (for instance) are predominantly (>50%) composed of remnants of lacustrine origin. We are aware of the fact that the situation is more complex, since terrestrial organic matter is likely to be deposited under lacustrine or marine environments. For example, Tertiary samples from Nigeria have been considered as terrestrial organic matter, though deposited under marine conditions. Other samples from Gabon or Congo (Lower Cretaceous) have been deposited under lacustrine environments but can be mixtures of organic matter of various origins. In such cases, "lacustrine origin" has been quoted. A noticeable fact is that almost none of the samples analysed derive from organic matter deposited under very confined or hypersaline environments. The distribution of the time of source rock deposition (including related oils and impregnations) is bimodal. A first mode ranges from Triassic to Tertiary, with a maximum during the Lower Cretaceous (almost half of the samples). The second mode (only 4%) concerns Ordovician source rocks which have features that are important in resolving our theory for the origin of 25-norhopanes.

Analytical procedure

Rocks have been Soxhlet-extracted with $CHCl_3$. Preparative medium pressure liquid chromatography was performed to recover alkanes (MPLC100, column NICOPREP filled with silica, grain size 40 μm, pressure 6–7 bar max., *n*-hexane as solvent). The recovered alkanes were analysed by gas chromatography and by computerized GC–MS.

Conditions for the gas chromatographic analyses were as following:

50 m × 0.21 mm i.d. columns, coated with OV1, SE30 or DB1; film thickness 0.11 μm; temperature program from 80 to 300°C at 1.6°C/min; H_2 carrier gas.

GC–MS analyses were performed using a FINNIGAN 4500 combined gas chromatograph/quadrupole mass spectrometer equipped with an INCOS data system. Gas chromatographic conditions were: capillary columns 60 m × 0.25 mm i.d., coated with DB5 or SE54; film thickness 0.10 μm. The oven temperature program was from 50 to 130°C at 9°C/min and from 130 to 300°C at 1.5°C/min. Acquisitions were in MID mode (16 typical ions, EI 70 eV, emission current 200 μA). Helium was used as carrier gas. Integrations of peak areas from *m/z* 177 and 191 fragmentograms have been used to assess the relative intensities of "demethylated" and regular hopanes (see examples in Appendix).

RESULTS AND DISCUSSION

Influence of biodegradation on biomarkers

The large number of samples used in our study allowed us to confer a statistical value to the results obtained. Histograms have been established, representing the distribution of a dozen molecular parameters for the three populations concerned, viz source rock extracts, non-biodegraded and biodegraded oils. These histograms reflect the behaviour of the different molecular markers when influenced by bacteria. Descriptions of each histogram would be too exhaustive; hence, we will concentrate on the main results, illustrating some of the more striking features.

Table 2 shows the behaviour of some molecular ratios, which increased (↗) or decreased (↙) during

Table 2. Positive (↗) or negative (↙) influence of biodegradation on typical molecular parameters, based on the results from the 213 sample data bank (see Appendix for the abbreviations)

Molecular Parameter	29DH/30H	29DH/29H	Gcrn/30H	Oln/30H	28DNH/29H	29/5/29H	Tm/Ts	30/3/29H	30/3/23/3	23/3/29H	TT/ST
Influence of Biodegradation	↗	↗	↗	↗	↘	↗	↘	↘	↘	↘	↗

biodegradation. Most of these results are in agreement with field observations or laboratory experiments, with a noticeable exception for the 30/3/29H ratio (defined in the Appendix). Though tricyclic terpanes seem to be degradable during weathering process, as has been shown in the severely biodegraded St Aubin asphalt (Connan, 1984), they are generally considered to be rather resistant to biodegradation (Seifert and Moldowan, 1979; Philp, 1983). However, Fig. 2, representing the distributions of the relative concentration of C_{30} tricyclopolyprenanes in the three populations, shows the 30/3/29H ratio is lower in biodegraded oils than in the two other populations, a result which tends to indicate that tricyclic terpanes would be less resistant than hopanes to bacterial degradation. Since this is not the general observation, the parameter considered could be misunderstood, or strongly influenced, by a source factor. Furthermore, evolution of sterane isomers, Fig. 1, shows that the three sample populations concerned (rocks, non-biodegraded oils, biodegraded oils) cover the same maturity range. Hence, difference of maturity only cannot explain this result.

A typical result is obtained with the triterpanes/steranes ratio (TT/ST, see Appendix), as shown in Fig. 3. Seventy-seven percent of the source rock extracts, and 73% of the non-biodegraded oils, have TT/ST values in the 0–5 range, against only 42% for the biodegraded oils. Further, 28% of the latter exhibit TT/ST ratios greater than 10, against 13% of the source rocks and 9% of the non-biodegraded oils. The wide diversity of the samples analysed suggest that steranes are more rapidly consumed than pentacyclic terpanes as indeed is seen in laboratory experiments.

According to Table 2, while gammacerane and oleanane are more resistant to biodegradation than hopane, 28,30-dinorhopane tends to be more affected than the 30-norhopane. The 29/5 compound is a C_{29}-pentacyclic constituent which was previously reported by Connan *et al.* (1990) as 17-methyl-28,30-dinorhopane; this compound appears to be more resistant than 30-norhopane towards bacterial degradation (see below).

As for 25-norhopanes, Fig. 4 shows histograms reflecting the distribution of the relative concentrations of the C_{29} homologue, with regard to regular hopanes (C_{29} and C_{30}). If all the data are taken into account, 11% of the biodegraded oils have their 29DH/30H ratios reaching values greater than 0.9 (9% for 29DH/29H), against less than 2% for the non-biodegraded oils and the source rocks (between 3 and 5% for 29DH/29H). Since we want to concentrate on samples containing real amounts of 25-norhopanes we have decided to discard samples in which 29DH/30H and 29DH/29H values are within the 0–0.2 range (i.e. very low concentrations). Histograms in Fig. 4 are plotted for values above 0.2. This represents subsets of 68 and 89 samples for 29DH/30H and 29DH/29H respectively.

The results obtained show that, while most source rocks are located in the 0.2–0.3 range, higher values are generally observed in oils. Most non-biodegraded oils exhibit values in the 0.4–0.8 interval, whereas biodegraded oils reach extremely high values [up to 8 or 9 for 29DH/30H in the case of North Sea samples, which are represented in 1.7–1.8 interval in Fig. 4(c)]. Nevertheless, very few biodegraded samples have relative concentrations of 25-norhopane in the 0.2–0.3 interval which is usual in non-biograded oils and source rocks. It is then clear that even if the number of samples appears weak for a full statistical treatment, the relative concentration of 25-norhopane is often enhanced by severe biodegradation, as seen in field observations.

The case of non-biodegraded oils is more complex, since they comprise oils enriched in 25-norhopane when compared with the source rock population. Generally, the other molecular ratios analysed exhibit similar distributions for the source rock extracts and

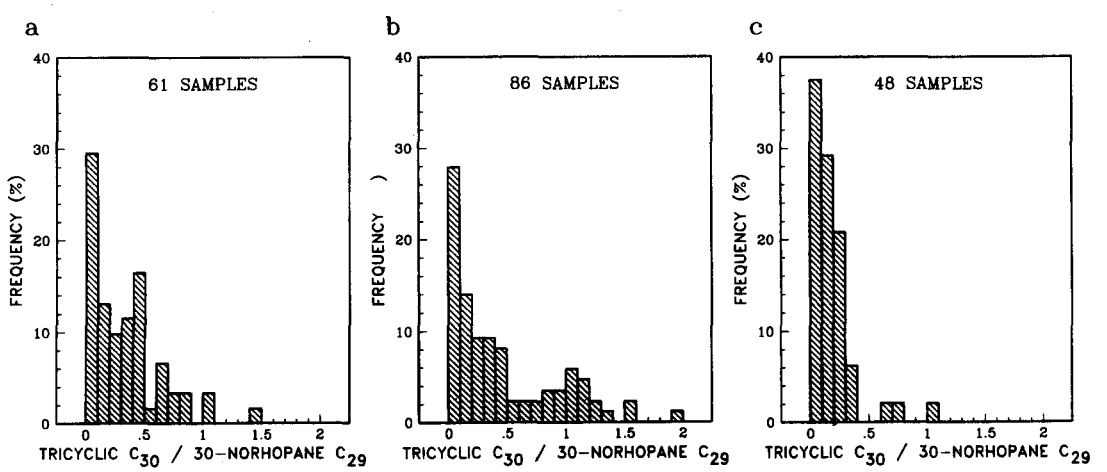

Fig. 2. Histograms representing the distribution frequencies of the C_{30}-tricyclopolyprepane/30-norhopane ratio for the populations of (a) source rock extracts, (b) non-biodegraded oils and (c) biodegraded oils.

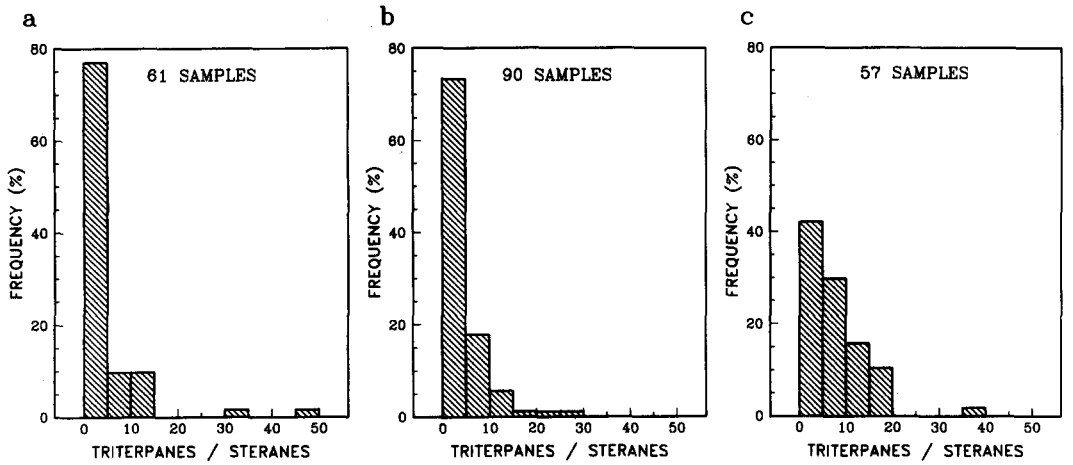

Fig. 3. Histograms representing the distribution frequencies of the triterpanes/steranes ratio for the populations of (a) source rock extracts, (b) non-biodegraded oils and (c) biodegraded oils.

the non-biodegraded oils. This can be seen in Fig. 2 for 30/3/29H and in Fig. 3 for TT/ST. Another convincing illustration is given by the gammacerane to hopane ratio (Gcrn/30H, cf. Fig. 5): while the histograms are very similar for source rocks and non-biodegraded oils, biodegraded oils exhibit higher values, thus meaning that gammacerane is more resistant than hopane when bacterial degradation occurs, a result which has been observed in natural cases (Blanc and Connan, 1992). The clear difference in distribution for both 29DH/30H and 29DH/29H ratios between source rocks and non-biodegraded oils (cf. Fig. 4) is therefore of particular importance. It shows that relatively high concentrations of 25-

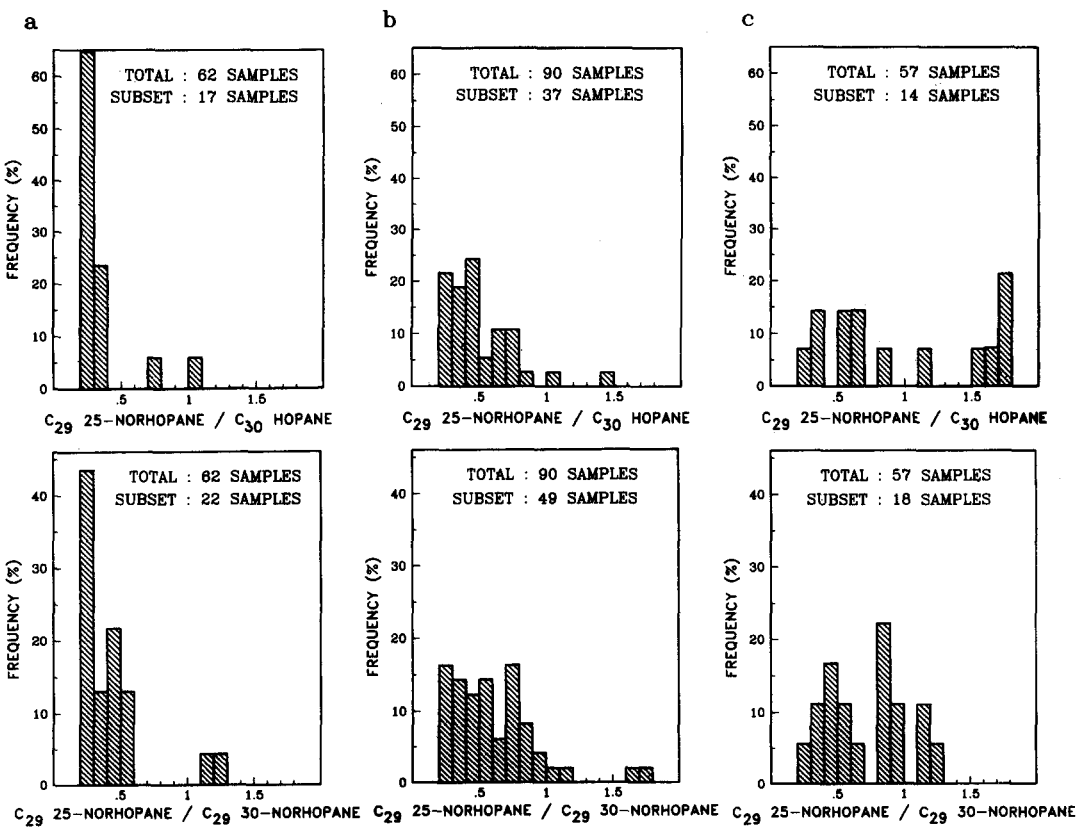

Fig. 4. Histograms representing the distribution frequencies of the 25-norhopane/hopane and 25-norhopane/30-norhopane ratios for the populations of (a) source rock extracts, (b) non-biodegraded oils and (c) biodegraded oils.

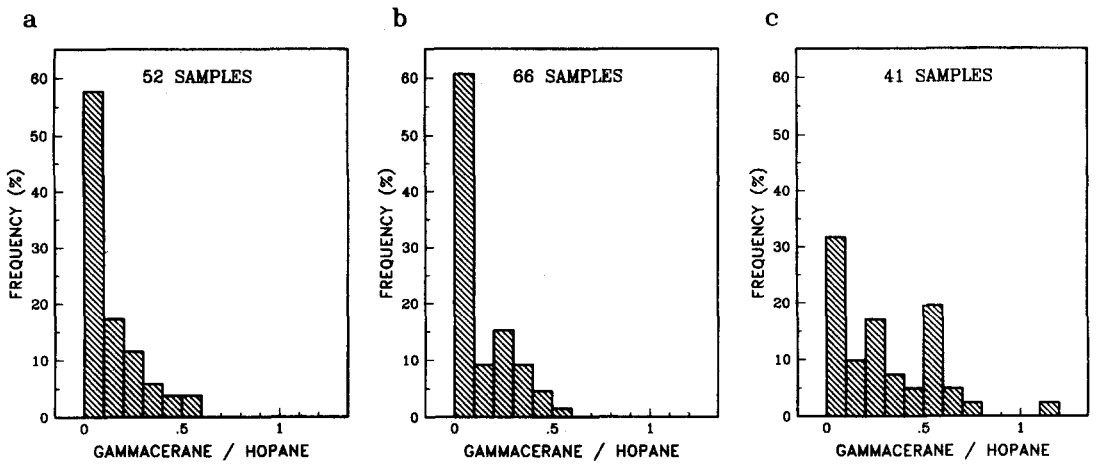

Fig. 5. Histograms representing the distribution frequencies of the gammacerane/hopane ratio for the populations of (a) source rock extracts, (b) non-biodegraded oils and (c) biodegraded oils.

norhopane not only appear in obviously biodegraded oils but also in non-biodegraded ones and as such are indicative of a palaeophenomenon which is not reflected by other usual molecular markers.

Palaeobiodegradation assessment using 25-norhopanes: concept of "background" level

To deal with the possibility of using 25-norhopanes as key indicators of palaeobiodegradation, the 29DH/29H ratio has been plotted as a function of the 29DH/30H one for the three types of sample (namely source rock extracts, non-biodegraded oils, biodegraded oils). This enabled an approach, leading to the concept of a "background" population or initial level in "demethylated hopanes" in source rocks.

Figure 6(a) represents this kind of plot for the rock extracts. Almost all the samples are located in a zone delimited by maximum values of 0.55 for 29DH/29H and 0.40 for 29DH/30H. (The two samples outside this zone are likely oil-impregnated rocks). This area defines what we called the "background" population, i.e. the reference level of 25-norhopane concentration in the source rocks analysed. Hence, the latter do contain "demethylated hopanes", though in rather low amounts, depending on their geographical location. This result agrees with the previous work of Noble *et al.* (1985) who reported the occurrence of partial 25-norhopane series in two Australian shales. Chosson *et al.* (1992) also illustrated that feature in an immature shaly source rock from Congo.

The same diagram applied to biodegraded oils [Fig. 6(b)] confirms that bacterial effects tend to enhance the relative concentration of 25-norhopane by selective destruction of more readily degradable terpanes (e.g. regular hopanes). But it should also be noticed that the majority of samples (including highly biodegraded ones) are still located in the background zone previously defined. Therefore, it can be said that

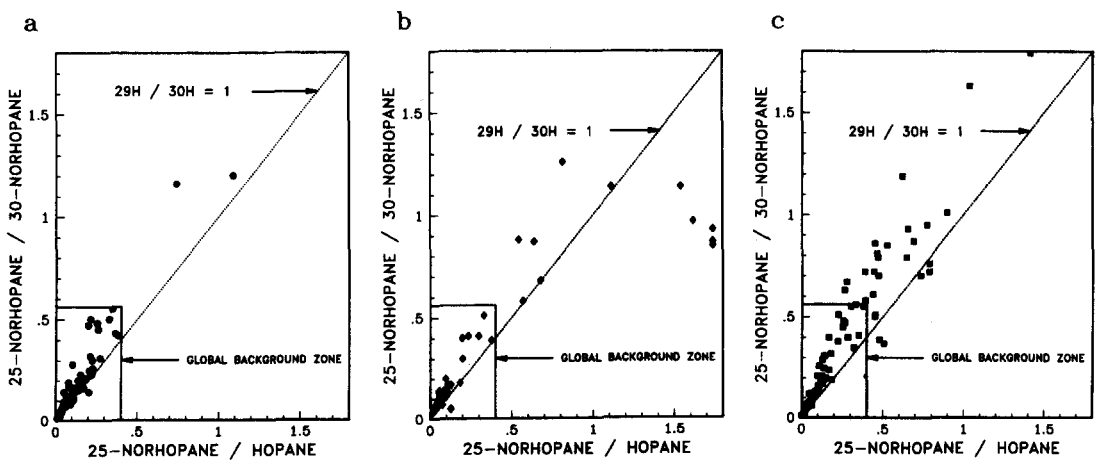

Fig. 6. Determination of the global background level in "demethylated hopane" [source rocks, Fig. 6(a)] and comparison with the cases of biodegraded [Fig. 6(b)] and non-biodegraded [Fig. 6(c)] oils from the data bank.

microbial degradation of reservoired oils does not necessarily imply a high ratio of "demethylated" to regular hopanes (Brooks *et al.*, 1988). This result does not favour the in-reservoir biotransformation of regular hopanes into demethylated ones. In fact, if the C_{29} 25-norhopane stems from bacterial demethylation of the C_{30} hopane, highly biodegraded oils should exhibit moderate to high relative concentrations in the former. In the meantime, the "unmasking" theory is still valuable in such cases since a low relative ratio of 25-norhopane in a severely biodegraded oil can be explained by a very low initial content of that compound in the related source rock.

The plot of 29DH/29H vs 29DH/30H for non-biodegraded oils [Fig. 6(c)] shows evidence for a palaeobiodegradation phenomenon. Whereas many oils are located in the "demethylated hopane" background area of source rocks, some others exhibit abnormal concentrations of the C_{29} 25-norhopane, outside the background zone. Since these oils do not appear to be clearly biodegraded on the basis of their *n*-alkane content, these data suggest that they result from the mixing of a highly biodegraded residual oil with a non-biodegraded more mature one. Hence, abundant amounts of 25-norhopane in these apparently non-biodegraded oils can be used as an indicator of "palaeobiodegradation".

It is important to keep in mind the fact that the palaeobiodegradation assessment of a reservoired oil, on the basis of an abnormally high concentration of 25-norhopanes, will not be valuable unless the corresponding "demethylated hopane" background zone has been defined for the related source rocks. We tried to operate in such a way whenever it was possible, and some convincing results have been obtained.

Analysis of samples from different sources and origins showed that the initial concentration level of C_{29} 25-norhopane in source rocks fluctuated depending on the geographical location. For instance, this background level is higher for source rocks from Angola, in comparison with source rocks from Gabon or Congo. In Angola, a large amount of non-biodegraded oil samples are located outside its related 25-norhopane background zone. Such oils can be considered as palaeobiodegraded. In contrast, biodegraded or unaltered oils from Gabon or Congo fit perfectly in the corresponding background zones. At first glance they have not been affected by palaeobiodegradation.

The most striking example, in our study, lies in the North Sea with the comparison between two neighbouring case histories from Norway and the United Kingdom. Figure 7(a) shows that the source rock samples from the Oseberg Field (Norway) are relatively rich in 25-norhopane, a result which leads to a relatively large "background area". Despite this, some biodegraded oils from the Frigg Field exhibit clear 25-norhopane concentration enhancements [Fig. 7(b)]. Meanwhile, non-biodegraded oils from Oseberg Field are perfectly located in the background zone previously defined from the corresponding source rocks [Fig. 7(c)]. No strong palaeobiodegradation phenomenon is apparent in that case. This result is in agreement with the study of Dahl and Speers (1986) who concluded that tar mat occurrence at the oil-water contact in Oseberg Field reservoirs was mainly due to a deasphalting phenomenon rather than biodegradation. The neighbouring Alwyn source rocks (U.K.) exhibit a low 25-norhopane/hopane ratio [see the small background zone on Fig. 8(a)]. However, the apparently non-biodegraded oils from the Alwyn Field are indeed enriched in C_{29} demethylated hopane, as seen by their location outside the corresponding background area [Fig. 8(b)]. These oils seem to have accumulated with a complex geochemical history including palaeobiodegradation.

These organic geochemical observations corroborate the differentiation which has been built up concerning reservoirs from the North Sea Viking

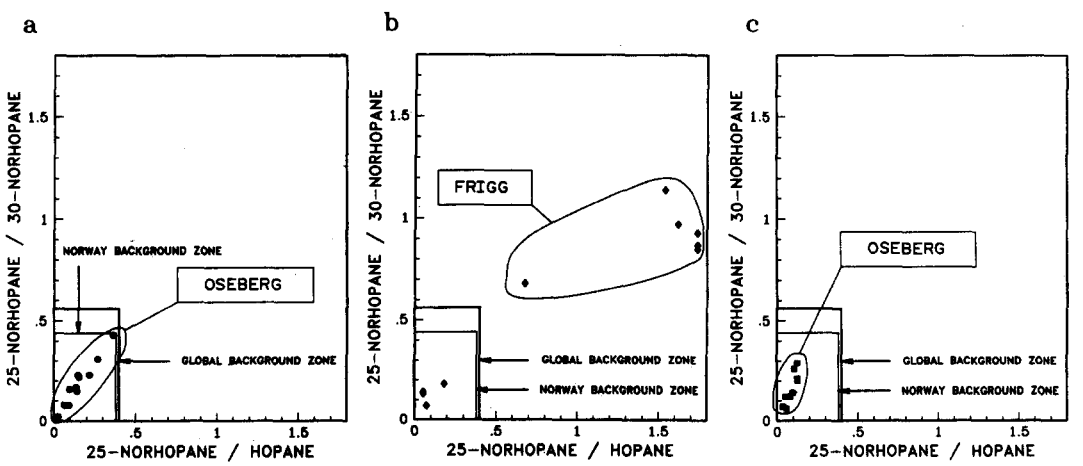

Fig. 7. Comparison of C_{29} 25-norhopane relative concentration in samples from the Norwegian North Sea (see Fig. 6 assessment of background zone).

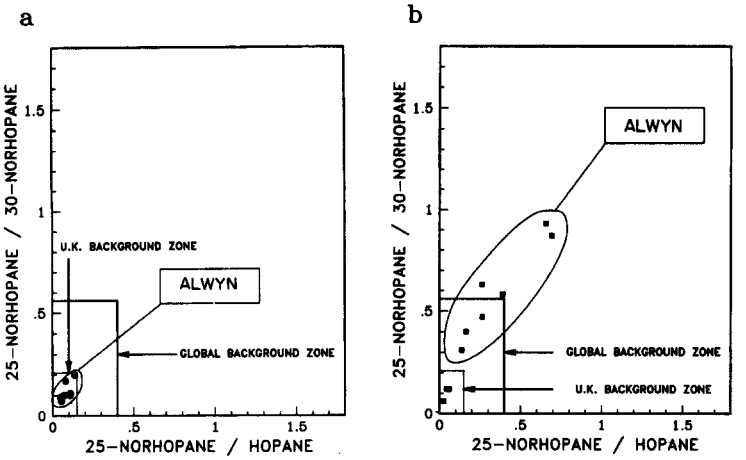

Fig. 8. Comparison of C_{29} 25-norhopane relative concentration in samples from the British North Sea: assessment of palaeobiodegradation in Alwyn Field on the basis of background level concept.

Basin, showing that two clearly different types of geological models can be applied to the two neighbouring Oseberg and Alwyn Fields (Thomas and Brévart, 1988). As a matter of fact, the two fields are located on oppposite sides of the Central Viking Graben and have undergone two different geological histories. In particular, the assessment of a palaeobiodegradation process, partly masked by accumulation of non-biodegraded oil for the Alwyn Field, is in agreement with the existence of at least two phases of hydrocarbon charging: the first during the Upper Cretaceous (75–65 My) from the central basin and a second from sub-basins in the western part during the Eocene (50–40 My). Moreover, hot, compaction-driven waters have circulated in Alwyn reservoirs, as early as the Lower Cretaceous (100 My), but also during the Upper Cretaceous and Paleocene (60 My), which could have affected hydrocarbon composition. In the meantime, the geological history of the Oseberg Field appears to be simpler, with a short distance from hydrocarbon generating basins. Our results, with others (Ducazeaux *et al.*, 1988), show the importance of integrating organic geochemical data (with inorganic geochemical data) in geological basin modelling.

A major improvement in using "demethylated hopanes" as a valuable geochemical tool will probably be brought about by applying quantitative GC–MS analysis. Preliminary results have been obtained from a set of samples from Venezuela: absolute amounts of 25-norhopane have been used to assess palaeobiodegradation (in agreement with the relative ratios 29DH/30H). It appears that 25-norhopanes are as abundant in palaeobiodegraded oils containing *n*-alkanes as in clearly biodegraded ones, and that they are not related to consumption of regular hopanes. These results are not in agreement with the previous work of Peters and Moldowan (1991) who applied quantitative GC–MS analysis to a biodegraded and non-biodegraded oil both supposed to issue from the same source rock (isotopic data and sterane distribution are identical). Nevertheless, these two oils exhibit two different hopane profiles. Assuming that steranes have not been affected by the biodegradation process, and that the lower hopane homologues have been attacked prior to the higher molecular weight compounds, the authors provide the C_{27} diasterane of the biodegraded oil with a concentration factor which, when directly applied to the 25-norhopanes, leads to the conclusion that the latter are necessarily formed by bio-demethylation of homohopanes. In fact, the non-biodegraded oil analysed seems to be completely devoided of 25-norhopanes.

Nevertheless, this study, which is based on only two samples, does not show complete evidence for a common and unique source rock with identical timing of generation for both oils. In particular, this assumption seems to be inconsistent with the behaviour of the C_{29} 30-norneohopane (Moldowan *et al.*, 1991, cf. later) in the two samples analysed. This compound is well represented in the non-biodegraded oil, but does not appear to have undergone "bio-demethylation" (absence of 18α(H)-25,30-dinorhopane, cf. Trendel *et al.*, 1990, in the biodegraded oil). In the meantime, it appears to have been diluted in comparison with regular hopanes. Since the latter are themselves supposed to decrease, this result highlights contradictions as far as a common and unique origin for both oils is concerned. Moreover, the bio-demethylation process does not support the fact that the relative depletion of the C_{34} hopane compared with the C_{35} homologue is not accompanied by a comparable enrichment of the C_{33} "demethylated hopane" in comparison with the C_{34} one. Furthermore, 25-norhopanes in the biodegraded oil could come from another source rock which does not feed the non-biodegraded reservoir, or from indigenous organic matter in the biodegraded reservoir, or, at last, from organic matter incorporated

during recovery of the sample. Also, Peters and Moldowan (1991) do not make any quantitative measurements of the 25,30-dinorhopane (C_{28}) or 25-norhopane (C_{29}): only extended hopanes are taken into account in the quantitative calculations. From this point of view, it seems that 25-norhopanes could eventually be considered as two separate groups: the C_{28} and C_{29} homologues on the one hand, the C_{30}–C_{34} ones on the other. As a matter of fact, while the former appear to be present in source rocks, the latter are either not observable or present at very low concentrations (cf. Appendix). A similar situation is also recorded for other hopanoid biomarkers, such as the 28,30-dinorhopane which has no observable higher molecular weight homologue, or the recently identified 18α(H)-norneohopane series (Moldowan *et al.*, 1991), for which just two compounds have been revealed (Ts and C_{29} 30-norneohopane, see later). Even the regular hopane series can be divided into two separate sets: the C_{27}–C_{30} compounds and the extended series (C_{31}–C_{35}), probably related to two different precursors (diploptene or diplopterol for the former and bacteriohopanetetrol, aminobacteriohopanetriol or other composite derivatives for the latter (Ourisson *et al.*, 1984; Rohmer *et al.*, 1992). This could favour a specific bacterial input leading to the predominance of the C_{28} and C_{29} homologues among the 25-norhopane series. The hypothesis of a reworking of hopane precursors at a stage of very early diagenesis is also consistent with our observations.

25,30-Dinorhopane and 25-norhopane could also exist as free hydrocarbons in sediments, like 28,30-dinorhopane and 25,28,30-trinorhopane (Noble *et al.*, 1985). However, the fact that 25-norhopanes seem not to be released from asphaltenes or kerogen on pyrolysis, even when the corresponding oils contain them, can be explained by three concomitant phenomena:

1—25-norhopane functionalized precursors are probably, initially, present in very low amounts in comparison with regular hopanoid precursors;

2—bound biomarkers are protected by the macromolecular network from alterations (e.g. maturation and biodegradation) (see Mycke *et al.*, 1987; Blanc and Albrecht, 1990, for instance);

3—the 25-norhopane skeleton is bacterially more resistant than the regular hopanoid one.

Therefore, while the free hydrocarbon profile from a biodegraded sample is likely to reflect a regular hopane consumption matching with a 25-norhopane concentration, pyrolysate profile reveals information which has not yet been affected by the unmasking process. Furthermore, even if hydrocarbons are thought to come from thermal cracking of kerogen and/or asphaltenes, there is no need for free hydrocarbons to be a precise image of their bound counterparts. In fact, previous studies have shown that some biomarkers could be selectively "quenched" in macromolecular and/or polar entities (Adam *et al.*, 1991), a result which could even lead to erroneous geochemical palaeoreconstruction (Kohnen *et al.*, 1991).

The final answer to the question of the origin of 25-norhopanes lies within the realms of microbiology. Until now, no 25-norhopanes or C-25 functionalized precursor has been found in microorganisms. Recently, De Lemos Scofield (1990) identified for the first time a C-25 functionalized hopanoic compound in Gaviota Beach rock from the Monterey Formation: 28,30-dinorhopan-25-oic acid. This sample also contains 28,30-dinorhopane and 25,28,30-trinorhopane. The acidic compound could be an intermediate between both structures. However, the 25,28,30-trinorhopane seems to have a closer genetic link with the 28-norhopanoic series than the 25-norhopanoic one and, therefore, to behave as a special case (Curiale *et al.*, 1985; Peters and Moldowan, 1991).

Assessment of environmental conditions using 25-norhopanes

It has already been noticed by Chosson *et al.* (1992) that "demethylated hopanes" could be encountered in some particular environmental conditions. Thus, screening from both the literature, and this study, revealed that 25-norhopanes have never been observed in carbonate evaporites. Correlation of the presence or absence of 25-norhopane with other typical biomarkers analysed in the present study brought some insight to this problem. Selected biomarkers were gammacerane, oleananes and 28,30-dinorhopane (see Appendix).

Gammacerane vs 25-norhopane

Since gammacerane was first found in Green River shale (Hills *et al.*, 1966), this compound was originally considered as a lacustrine indicator (Seifert and Moldowan, 1981). However, subsequent studies showed its presence in various environments, preventing gammacerane from being used as a discriminant indicator between non-marine and marine origins (Moldowan *et al.*, 1985). Moreover, its likely precursor, tetrahymanol, seems to occur ubiquitously in marine sediments, according to ten Haven *et al.* (1989) and Venkatesan (1989). Its origin could even be bacterial as suggested by Kleeman *et al.* (1990) who identified tetrahymanol in *Rhodopseudomonas palustris*. Furthermore, Harvey and McManus (1991) recently proposed bacterivorous marine ciliates as the dominant source of tetrahymanol, a feature which could explain the widespread distribution of gammacerane. Whatever its precise origin, several studies have used gammacerane as a good indicator of hypersaline environments whenever it was encountered in relatively high amount (Mello *et al.*, 1988a,b; Fu Jiamo *et al.*, 1990), though this assumption should also be taken with caution (ten Haven *et al.*, 1989).

Figure 9(a) cross-plots the relative concentrations of gammacerane and 25-norhopane for biodegraded

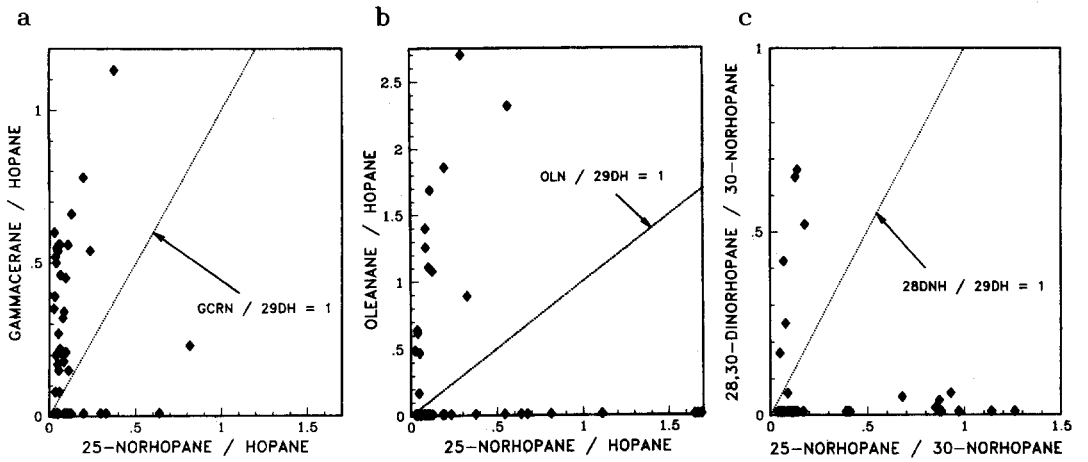

Fig. 9. Inverse correlations of 25-norhopane with (a) gammacerane, (b) oleanane and (c) 28,30-dinorhopane for the biodegraded oils from the data bank (relative concentrations with regard to regular hopanes).

oils. It appears that gammacerane-rich biodegraded oils are relatively depleted in C_{29} 25-norhopane. These highly biodegraded oils probably come from hypersaline environments, or marine carbonates, which usually do not contain "demethylated hopanes". It should however be noted that the simultaneous occurrence of gammacerane and 25-norhopane, in relatively high abundance in biodegraded samples, has been observed in the case of one oil from Colombia [see Fig. 9(a); Gcrn/30H = 0.23; 29DH/30H = 0.81]. Recently, we discovered a highly biodegraded oil from Gabon (E. Legendre, personal communication) containing both gammacerane and 25-norhopane with elevated relative concentrations (Gcrn/30H = 0.30; 29DH/30H = 0.92); the origin of this oil is nevertheless not clear. For the source rocks and non-biodegraded oils from our data bank, the situation is not clear, though there is no sample exhibiting both gammacerane and 25-norhopane in very high amounts.

Oleanane vs 25-norhopane

18α(H)- and 18β(H)oleananes are typical biomarkers of terrestrial input from angiosperms (Ekweozor and Udo, 1988; Riva *et al.*, 1988; Mello *et al.*, 1988a,b). The cross-plot of oleanane with 25-norhopane for biodegraded oils shows an inverse correlation between the two biomarkers [Fig. 9(b)]. It should be noted that the oleanane-rich samples all come from Nigeria. However, even when highly biodegraded, these samples do not exhibit high concentrations in 25-norhopane. This inverse correlation is also valid for non-biodegraded oils and source rocks analysed by us (data not shown).

28,30-dinorhopane vs 25-norhopane

The real origin of the 28,30-dinorhopane is not clearly understood. Seifert *et al.* (1978) had proposed a possible origin from ferns, which seems improbable nowadays. Other workers consider this biomarker as a typical molecule of anaerobic bacteria in highly anoxic environments (Katz and Elrod, 1983; Connan *et al.*, 1986); salinity seems also to play a role in its presence (Mello *et al.*, 1988a). Recently, Rullkötter and ten Haven (1989) even suggested the existence of a non-bacterial precursor of the 28,30-dinorhopane. However, more recently, De Lemos Scofield (1990) returned to the hypothesis of a bacterial origin, possibly *Methylococcus capsulatus*. It should be added that 28,30-dinorhopane can exist as three possible isomers, 17α,21β(H)-, 17β,21β(H)- and 17β,21α(H)-, the latter being the most abundant in mature petroleum (Moldowan *et al.*, 1984).

Graphical representation of both the 28,30-dinorhopane and the 25-norhopane for the biodegraded oils in our data bank shows that there is no sample containing both biomarkers in high amounts [Fig. 9(c)]. However, co-existence of the 28,30-dinorhopane and the 25-norhopane has been mentioned previously in Australian shales (Noble *et al.*, 1985), oil sands from western Canada (Brooks *et al.*, 1988) and a source rock in the Congo (Chosson *et al.*, 1992). As far as source rocks and non-biodegraded oils from our data bank are concerned, this co-presence has also been observed, but never with both compounds in high amounts. 28,30-Dinorhopane rich samples are 25-norhopane poor and vice versa.

The molecular correlations between 25-norhopane and other typical biomarkers, coupled with the geology of the samples, in particular the origin and condition of deposition of organic matter, lead us to conclude that "demethylated hopanes" appear to be typical of some marine and lacustrine environments, in particular, those that are dysoxic and not very hypersaline.

Extension : novel bacterially resistant rearranged hopanoic compound

From the results obtained on our 213 samples, it appears that small variations in the triterpane skel-

eton somehow alter the susceptibility of the molecule to bacterial attack. Lack of a methyl group at C-10 in hopanes is one example. Furthermore, analysis of the Tm/Ts ratio shows that Tm is more easily degradable than Ts (see Table 2); the two molecules only differ in a methyl rearrangement (shift from C-18 to C-17, Seifert and Moldowan, 1978). GC–MS analyses of a large set of samples showed the existence of a C_{29}-pentacyclic hopanoic compound abbreviated 29/5, the structure of which had, until recently, not been completely elucidated since it had not been isolated. This compound elutes just after the usually dominant 30-norhopane (29H) under current chromatographic conditions (see chromatograms in Appendix). Presumptions existed indicating this 29/5 could be a rearranged structure from 30-norhopane (i.e. Ts-type): 18α(H),21β(H)-30-norhopane II or, more simply, 30-norneohopane, previously referred by Connan *et al.* (1990) as 17-methyl-28,30-dinorhopane. Its identification has been elucidated by NMR and X-ray methods by Moldowan *et al.* (1991): they name it "C_{29} Ts".

Recent identifications of the 25,30-dinor-17α(H)- and the 25,30-dinor-18α(H)-hopanes in a biodegraded oil from the Congo (Loufika) by Trendel *et al.* (1990) already favoured such an hypothesis since these two molecules could appear as the demethylated homologues at C-10 of 29H and 29/5 respectively (mainly based on retention time comparison). Arguing this, we have plotted the evolution of the 29/5/29H ratio as a function of Ts/Tm, for all of the samples from the data bank (results expressed in % on Fig. 10). A general correlation exists between both parameters, confirming that, while 29H and Tm belong to the regular hopanoid series, 29/5 and Ts both belong to the rearranged one.

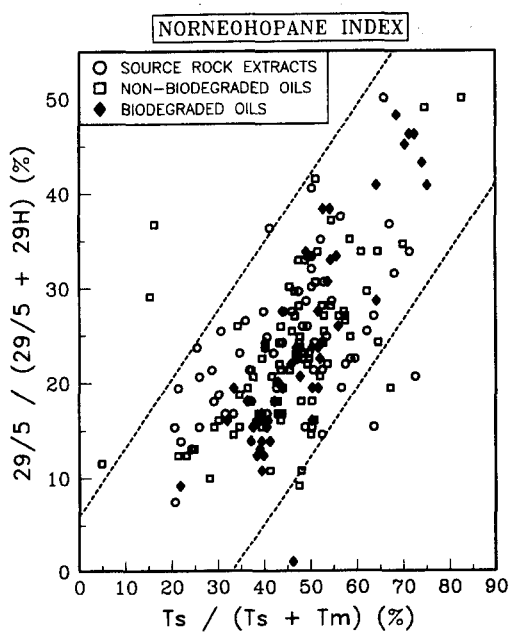

Fig. 10. Correlation between the 29/5/29H and Ts/Tm ratios for the samples from the data bank: assessment of a norneohopane index.

According to the histogram distributions (not shown) of the 29/5/29H ratio for source rock, non-biodegraded and biodegraded oil samples, 30-norneohopane has been found to be more resistant than its regular homolog towards bacterial degradation. Its behaviour with regard to C_{29} 30-norhopane parallels that of Ts compared with Tm (see Table 2). This is also clearly demonstrated in Fig. 10: samples with high Ts/Tm and high 29/5/29H ratios are principally biodegraded oils. This is also particularly well illustrated in a natural case history from the uraniferous basin of Lodève (Landais and Connan, 1986).

Moreover, biodegradation effects on these ratios compete with maturation phenomena. As a matter of fact, not only are Tm and 29H (i.e. regular hopanoid series) more easily biodegraded, but they also appear to be thermodynamically less stable than Ts and 29/5 respectively (i.e. rearranged hopanoid series). It should be added that recent molecular mechanics calculations support this assumption (Kolaczkowska *et al.*, 1990; Moldowan *et al.*, 1991). Therefore, it may be possible to use the 29/5/29H ratio as a maturity parameter. It has previously been used as such for crude oils from the Aquitaine Basin (Connan and Lacrampe-Couloume, 1992). In that respect, Fig. 10 represents an attempt to establish a "norneohopane index". This index measures the cumulative effect of maturation and, when appropriate, biodegradation.

CONCLUSION

Our study leads to the following conclusions concerning 25-norhopanes:

—they are likely to be encountered in rather small amounts in some marine and lacustrine source rocks that are dysoxic and not very hypersaline;
—their relative concentration can be enhanced during biodegradation, where they show a stronger resistance towards bacterial consumption than their regular homologues;
—they do not appear to be necessarily direct demethylation products of regular hopanes since they have merely been detected in some heavily biodegraded oils; in addition, biodegradation pathways would entail functionalization of alkanes which generates alcohols, ketones, aldehydes, acids, but no alkanes;
—their significant enrichment in apparently non-biodegraded crude oils still remains a tool to recognize palaeobiodegradation phenomena, providing their initial level in the corresponding source rocks is known.

In addition, the C_{29} 30-norneohopane, recently identified by Moldowan *et al.* (1991), has been clearly correlated in its geochemical activity with Ts. Both maturation and biodegradation phenomena

tend to enhance its relative concentration with respect to its regular analog.

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APPENDIX

Abbreviations Used in the Paper

Steranes

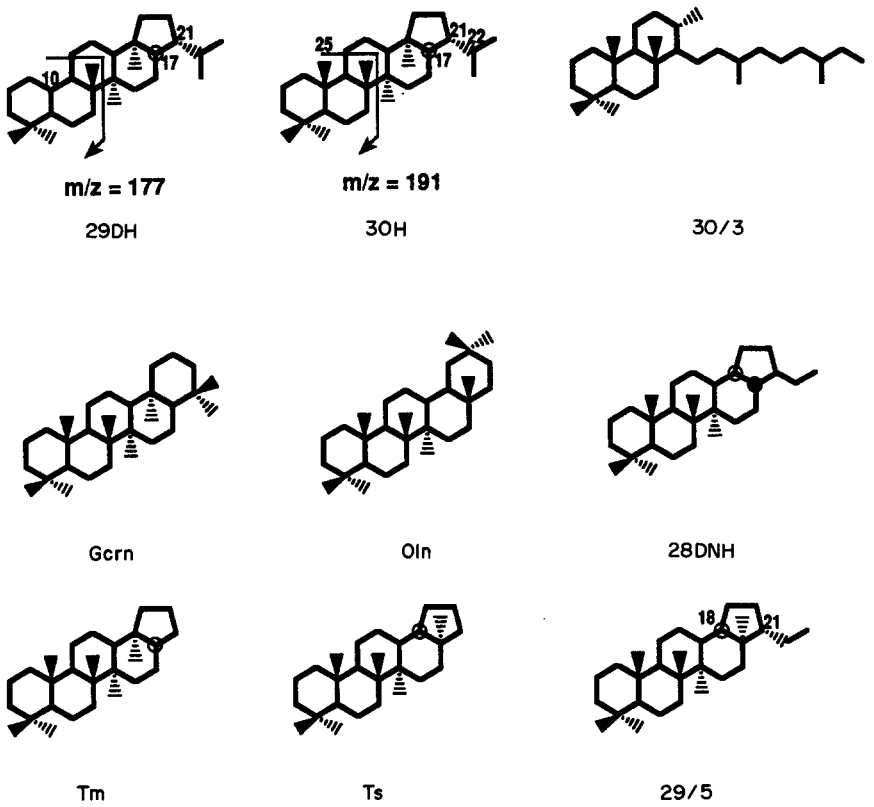
—ST: C_{27}–C_{29} steranes (monitored by the m/z 217 mass fragmentogram)
—29$\alpha\beta\beta S$: 5α(H),14β(H),17β(H)-20*S*-24-ethylcholestane
—29$\alpha\alpha\alpha R$: 5α(H),14α(H),17α(H)-20*R*-24-ethylcholestane
—29$\alpha\alpha\alpha S$: 5α(H),14α(H),17α(H)-20*S*-24-ethylcholestane
—29$\alpha\beta\beta R$: 5α(H),14β(H),17β(H)-20*R*-24-ethylcholestane

Terpanes

—TT: C_{27}–C_{35} triterpanes (mainly composed of hopanes and moretanes, monitored by the m/z 191 mass fragmentogram)
—29H: 17α(H),21β(H)-30-norhopane
—30H: 17α(H),21β(H)-hopane
—Tm: 17α(H)-22,29,30-trinorhopane (C_{27})
—Ts: 18α(H)-22,29,30-trinorhopane (C_{27})
—23/3: C_{23} tricyclopolyprenane
—24/4: C_{24} 17,21-secohopane
—30/3: C_{30} tricyclopolyprenane
—22*S*/22*R* C_{32}: %[17α(H),21β(H)-22*S*-30,31-bishomohopane]/[17α(H),21β(H)-(22*S* + 22*R*)-30,31-bishomohopane]
—29DH: 17α-25-norhopane ("demethylated hopane")
—28DNH: 28,30-dinorhopanes
—29/5: 18α(H),21β(H)-30-norneohopane
—Oln: 18α(H)- + 18β(H)-oleananes
—Gcrn: gammacerane

Appendix—*continued overleaf*

Principal Molecular Structures Encountered in the Paper



Examples of Detection of 25-Norhopanes with m/z 177 and m/z 191 Fragmentograms

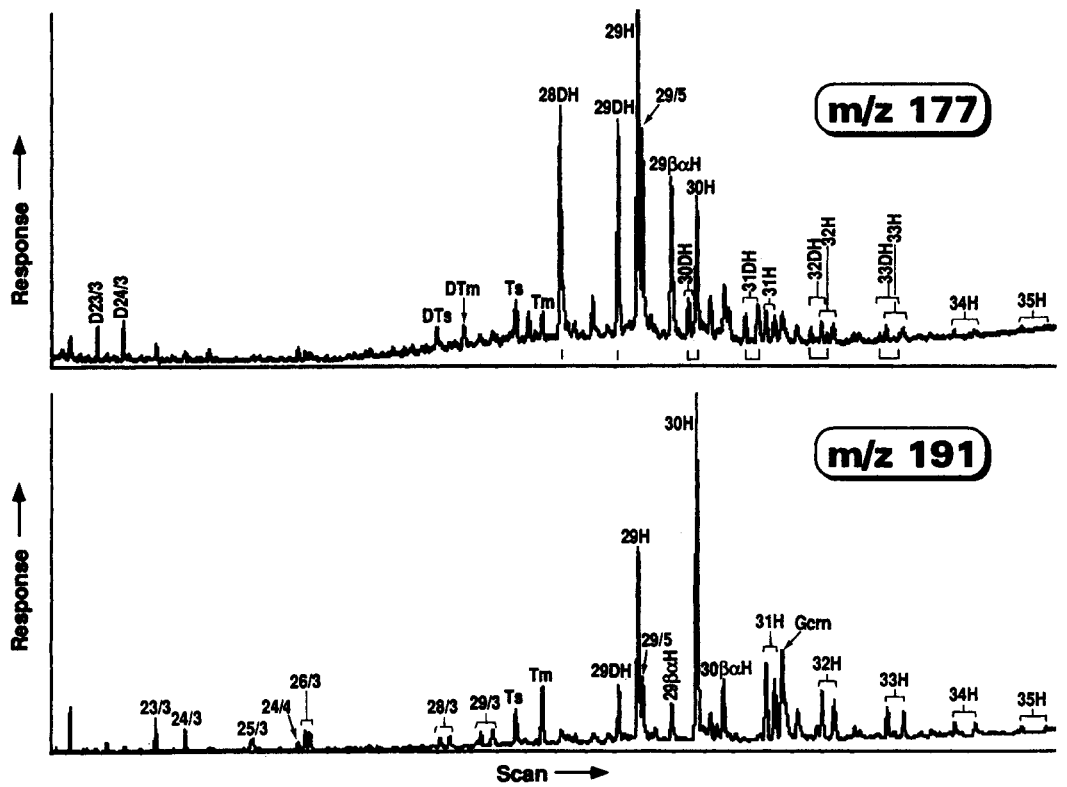


Fig. A1. Source rock from Gabon: 29DH/29H = 0.27; 29DH/30H = 0.15.

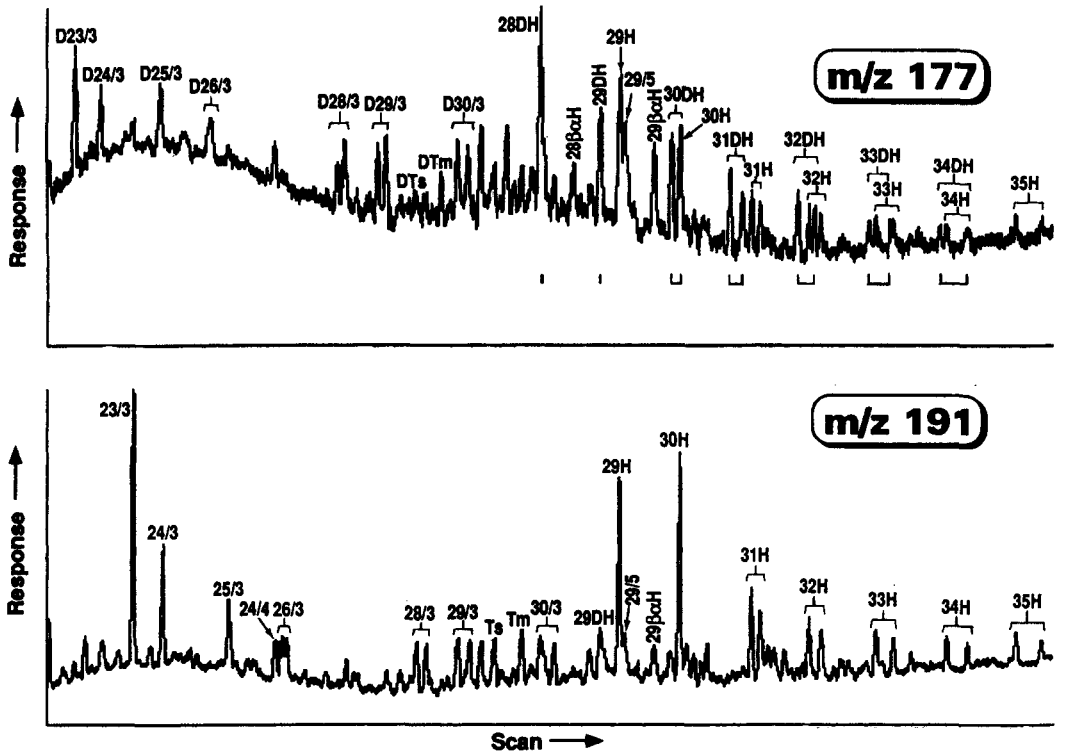


Fig. A2. Non-biodegraded impregnation from Angola: 29DH/29H = 0.51; 29DH/30H = 0.45.

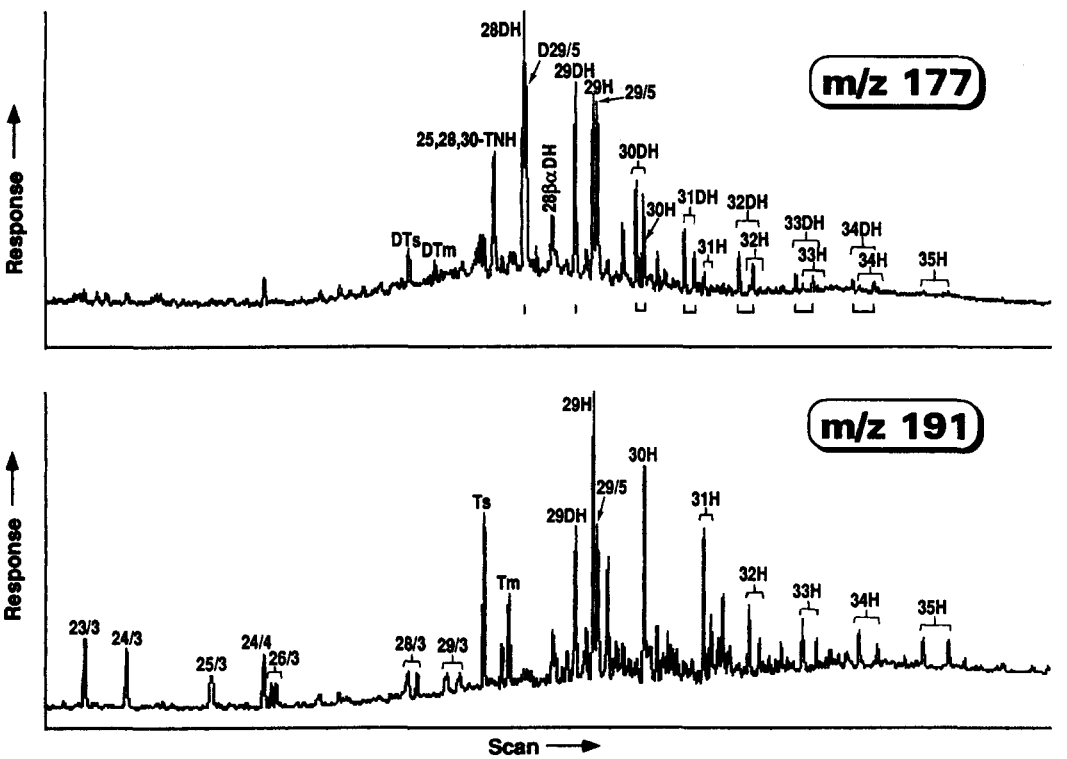


Fig. A3. Biodegraded impregnation from Norway: 29DH/29H = 0.58; 29DH/30H = 0.67.

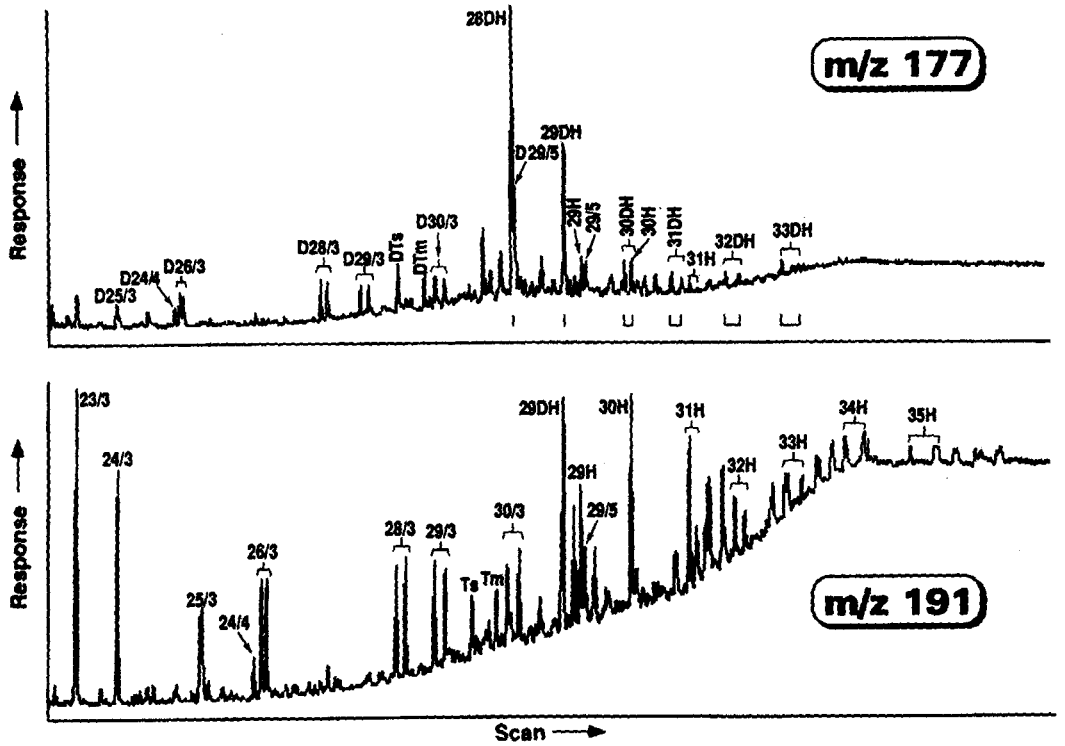


Fig. A4. Non-biodegraded oil from Angola: 29DH/29H = 1.63; 29DH/30H = 1.04.