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Biomarker generation from Type II-S kerogens in claystone and limestone during hydrous and anhydrous pyrolysis*

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Abstract—A claystone and a limestone containing immature Type II-S kerogen were thermally matured in the presence and absence of water, to study the influence of water and clay minerals on the generation of biomarkers. In contrast to hydrous pyrolysis, anhydrous pyrolysis of the claystone did not generate biomarkers, which resulted in the loss of important information. Desulfurization of the polar fraction of the claystone showed that anhydrous pyrolysis is not capable of converting S-bound biomarkers to free biomarkers. For the limestone, the differences between hydrous and anhydrous pyrolysis are less dramatic. Adsorption of the polar fraction of the claystone to smectite interlayers probably leads to cross-linking reactions, preventing the generation of free biomarkers. During hydrous pyrolysis, the smectite interlayers are occupied by water so that generation of biomarkers can take place. In addition, cross-linking reactions during anhydrous pyrolysis of the claystone may be enhanced because of the presence of S–S bonds in the organic matter of the claystone. These results show that water is important in closed system laboratory experiments designed to simulate natural maturation of sedimentary organic matter.

Key words—hydrous pyrolysis, anhydrous pyrolysis, Type II-S kerogen, clay minerals, smectite, biomarker generation, S-bound biomarkers

INTRODUCTION

Biomarkers are widely used to assess the source, depositional environment, level of thermal maturity and degree of biodegradation of organic matter in geological samples (Peters and Moldowan, 1993). The generation of biomarkers during the thermal maturation of kerogen has been viewed as a two step process (e.g. Lewan, 1985; Baskin and Peters, 1992; Koopmans *et al.*, 1996, 1997). As the kerogen first partially decomposes to a polar-rich bitumen, the bound biomarker moieties in the kerogen are maintained as bound moieties in the polar fraction of the generated bitumen. With increasing thermal maturity, the bound biomarker moieties are released from the polar fraction as free biomarkers as the bitumen decomposes to yield free hydrocarbons. In artificial maturation studies, this sequence has been most apparent when hydrous pyrolysis is employed to simulate natural thermal maturation (e.g. Lewan *et al.*, 1986; Eglinton and Douglas, 1988; Peters *et al.*, 1990; Koopmans *et al.*, 1996). However, the geological fate of biomarkers has also been studied by anhydrous pyrolysis under confined (e.g. Monthioux *et al.*, 1985; Landais *et al.*, 1989) and unconfined (e.g. Mackenzie *et al.*, 1981; Larcher *et al.*, 1987; Beach *et al.*, 1989) conditions.

The effects that different pyrolysis conditions have on the generation of biomarkers are not well understood. Specifically, the role of water (e.g. Lewan, 1997) and clay minerals (e.g. Eglinton *et al.*, 1986; Tannenbaum *et al.*, 1986; Huizinga *et al.*, 1987b) have been shown to be important. Monthioux *et al.* (1986) have argued that the presence of water does not play an essential role during pyrolysis, but Lewan (1997) showed that the total amount of pyrolysate generated by confined and unconfined anhydrous pyrolysis of Woodford Shale at 350°C is only half of that generated by hydrous pyrolysis. In general, attempts at comparing hydrous and anhydrous pyrolysis experiments have mainly concentrated on bulk fractions such as the residual kerogen (e.g. Michels *et al.*, 1995), whereas literature on comparison on a molecular scale is scarce (Comet *et al.*, 1986). Moreover, these studies have been mainly limited to biomarker ratios, so that the actual generation of individual biomarkers has not been monitored.

Here, we report on the generation of biomarkers from Type II-S kerogens on the basis of absolute

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concentrations. Experiments are conducted with a claystone and a limestone under both hydrous and anhydrous conditions, which enables us to evaluate the effect of water and clay minerals on the generation of biomarkers during closed system pyrolysis.

EXPERIMENTAL

Samples

The claystone is from the Gessoso-solfifera Formation (Upper Miocene, northern Italy). For an extensive description of the geology of this Formation, see Vai and Ricci Lucchi (1977). The sample has a TOC content of 2.0 wt%, and the organic matter is immature as determined by vitrinite reflectance measurements ($R_o = 0.25\%$; Kohnen *et al.*, 1991). Elemental analysis of the isolated kerogen indicates it is a Type II-S kerogen with an atomic H/C ratio of 1.44, an atomic O/C ratio of 0.17 and an atomic S_{org}/C ratio of 0.08. X-ray diffraction patterns on random powder packs of this sample indicate its mineral composition consists of quartz, smectite, illite, chlorite and dolomite. These data and petrographic examination of the sample in thin section allow the sample to be classified as an argillaceous claystone according to the classification of Lewan (1978).

The limestone is from the Ghareb Formation (Upper Cretaceous, Jordan). For a description of the geology of this Formation refer to Hufnagel (1984). The sample has a TOC content of 19.6 wt% and its organic matter is immature ($R_o = 0.39\%$; Koopmans *et al.*, 1998). Elemental analysis of the isolated kerogen indicates it is a Type II-S kerogen with an atomic H/C ratio of 1.38, an atomic O/C ratio of 0.07 and an atomic S_{org}/C ratio of 0.07. X-ray diffraction patterns on random powder packs of this sample indicate its mineral composition consists almost exclusively of calcite with only minor amounts (< 10 wt%) of quartz and apatite. These data and the petrographic examination of thin sections allow the sample to be classified as a biomicritic limestone according to the classification of Folk (1959).

Hydrous and anhydrous pyrolysis

In a series of separate experiments, rock chips of the claystone and the limestone were heated isothermally at temperatures between 200 and 360°C for 72 h under hydrous and anhydrous conditions. The experimental procedures for hydrous pyrolysis of these rocks have been described elsewhere (Koopmans *et al.*, 1996, 1998). For the anhydrous experiments, an additional amount of He was added before heating to ensure that the reactor pressure at the experimental temperature was similar to that for the hydrous experiments. None of the anhydrous experiments generated an expelled oil, in contrast to the hydrous experiments which generated an expelled oil at temperatures greater than or equal to 300°C for both rock types.

Extraction and fractionation

The work-up procedures for the claystone have been described in detail elsewhere (Koopmans *et al.*, 1996). Briefly, the rock samples were pulverised and ultrasonically extracted with a dichloromethane/methanol mixture (7.5:1 v/v). If an expelled oil was present after pyrolysis, it was combined with the extract. After precipitation of the asphaltenes, the resulting maltenes, to which a standard (6,6-d_2-3-methylhenicosane) was added for quantitative analysis, were fractionated using column chromatography with Al_2O_3. Elution with hexane and dichloromethane/methanol (1:1 v/v) yielded the total hydrocarbon fraction and the polar fraction, respectively. For some samples, a more laborious scheme was followed which included thin layer chromatography of the total hydrocarbon fraction to yield the saturated hydrocarbon fraction.

The limestone samples were extracted in a Soxhlet apparatus with an azeotropic mixture of benzene and methanol for 22 h. The extract was filtered through a 0.45 μm polytetrafluoroethylene filter. The expelled oils and the extracts were deasphaltened separately with *iso*-octane. Composited saturate plus aromatic hydrocarbon fractions were separated from the resulting maltene fractions on an Al_2O_3 column with an *iso*-octane/benzene mixture (3:1 v/v). These hydrocarbon fractions were spiked with two internal standards, i.e. squalane and 5β-cholane.

Raney Ni degradation

The polar fraction of the unheated claystone and the polar fractions after hydrous and anhydrous pyrolysis of the claystone were desulphurised with Raney Ni as described elsewhere (Koopmans *et al.*, 1996). A known amount of an internal standard [2,3-dimethyl-5-(1′,1′-d_2-hexadecyl)thiophene] was added for quantitative analysis.

GC and GC–MS

The experimental conditions during gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) of the saturated hydrocarbon fractions and the desulphurised polar fractions of the claystone have been described elsewhere (Koopmans *et al.*, 1996).

For the limestone, samples were injected on column into a HP 5890 GC equipped with a 60 m capillary column (DB 1701, ID = 0.31 mm). The initial oven temperature of 100°C was programmed at 50°C/min to 150°C and then at 3°C/min to 300°C for a soak time of 22 min. The eluting compounds were analyzed in the selected-ion-monitoring mode (i.e. m/z 57 and 217) by a double-focusing magnetic-sector VG 7024H mass spectrometer.

Quantitation

Individual biomarkers in the saturated hydrocarbon fractions and in the desulphurised polar fractions of the claystone were quantified by integration of their peak areas and the peak area of the standard in the FID trace.

For the limestone, individual biomarkers were quantified by integration of their peak areas and that of the standard in either the m/z 57 or the m/z 217 mass chromatogram. The concentrations for hydrous pyrolysis experiments that had both expelled oil and an extract were summed in proportion to their yields. Similar to the quantification of the claystone, all concentrations were calculated with respect to the original TOC of the unheated rock.

RESULTS

Free hydrocarbons in the saturated hydrocarbon fraction

Figure 1 shows the generation profiles of octadecane, phytane and (20*R*)-5α-24-ethylcholestane from hydrous and anhydrous pyrolysis of the claystone [Fig. 1(a)–(c)] and the limestone [Fig. 1(d)–(f)]. These generation profiles are typical for *n*-alkanes, acyclic isoprenoid alkanes, and steranes and terpanes, respectively.

For the claystone, the results are quite dramatic. At high maturation temperatures, hydrous pyrolysis generates a large amount of octadecane, whereas the amount present after the anhydrous experiments is low [Fig. 1(a)]. Similar results are found for phytane [Fig. 1(b)]. However, phytane could not be detected after anhydrous pyrolysis at 240°C, whereas octadecane is still present at that temperature, suggesting that phytane (or its precursor) is less stable under anhydrous conditions than octadecane. Although (20*R*)-5α-24-ethylcholestane was not detected in the unheated sample, large amounts of this sterane are generated during hydrous pyrolysis. During anhydrous pyrolysis, however, (20*R*)-5α-24-ethylcholestane is not generated at all [Fig. 1(c)]. The difference in generation profiles of octadecane, phytane and (20*R*)-5α-24-ethylcholestane during hydrous and anhydrous pyrolysis is also apparent from gas chromatograms of the saturated hydrocarbon fraction, which show that after anhydrous pyrolysis at 270°C mainly *n*-alkanes remain, whereas phytane and (20*R*)-5α-24-ethylcholestane are prominent compounds after hydrous pyrolysis at 280°C (Fig. 2).

For the limestone, approximately equal amounts of octadecane and phytane are generated during hydrous and anhydrous pyrolysis [Fig. 1(d)–(e)]. However, thermal destruction of these compounds at high maturation temperatures occurs at a higher rate during anhydrous pyrolysis, leading to lower concentrations in the anhydrous experiments at high temperatures [Fig. 1(d)–(e)]. The generation profiles of (20*R*)-5α-24-ethylcholestane during hydrous and anhydrous pyrolysis are covariant. At maximum generation, the concentration under anhydrous conditions, however, is diminished by *ca*. 30% [Fig. 1(f)]. Different thermal stabilities exist among the sterane isomers, as determined from higher ratios of (20*S*)- to (20*S* + *R*)-5α-24-ethylcholestane under anhydrous conditions at the same thermal stress levels. After anhydrous pyrolysis at 340°C, the (20*S*)/(20*S* + *R*)-5α-24-ethylcholestane ratio reaches 0.69, a value much higher than the equilibrium value of 0.55 reported in the literature (Seifert and Moldowan, 1986). For comparison, after hydrous pyrolysis at 340°C this ratio is 0.57.

S-bound hydrocarbons in the polar fraction of the claystone

The amounts of S-bound phytane and (20*R*)-5α-24-ethylcholestane in the polar fraction of the unheated claystone and in the polar fractions of the claystone after hydrous and anhydrous pyrolysis were determined by Raney Ni desulfurization (Table 1).

During hydrous pyrolysis, the relatively high amounts of S-bound phytane and (20*R*)-5α-24-ethylcholestane in the polar fraction of the unheated claystone are progressively thermally released at increasing temperatures. This coincides with the increasing amounts of free phytane and (20*R*)-5α-24-ethylcholestane in the saturated hydrocarbon fraction (Fig. 1). After anhydrous pyrolysis at 200°C, however, the polar fraction contains only a low amount of phytane and (20*R*)-5α-24-ethylcholestane is not detectable, while on the other hand their amounts in the saturated hydrocarbon fraction have not significantly increased. This is also evident from Fig. 3, which shows that desulfurization of the polar fraction of the claystone after anhydrous pyrolysis at 200°C yields mainly (low amounts of) *n*-alkanes and an unresolved complex mixture. These results strongly suggest that anhydrous pyrolysis is not capable of converting S-bound biomarkers in the polar fraction of the unheated claystone to free biomarkers.

DISCUSSION

Effect of water and clay minerals on biomarker generation during pyrolysis

The most conspicuous differences in hydrocarbon generation are observed during anhydrous pyrolysis of the claystone vs. anhydrous pyrolysis of the limestone (Fig. 1), which suggests that both the mineral matrix and the presence of water play a decisive role in the generation of biomarkers from these rocks. Laboratory pyrolysis experiments have shown that mineral matrices influence biomarker

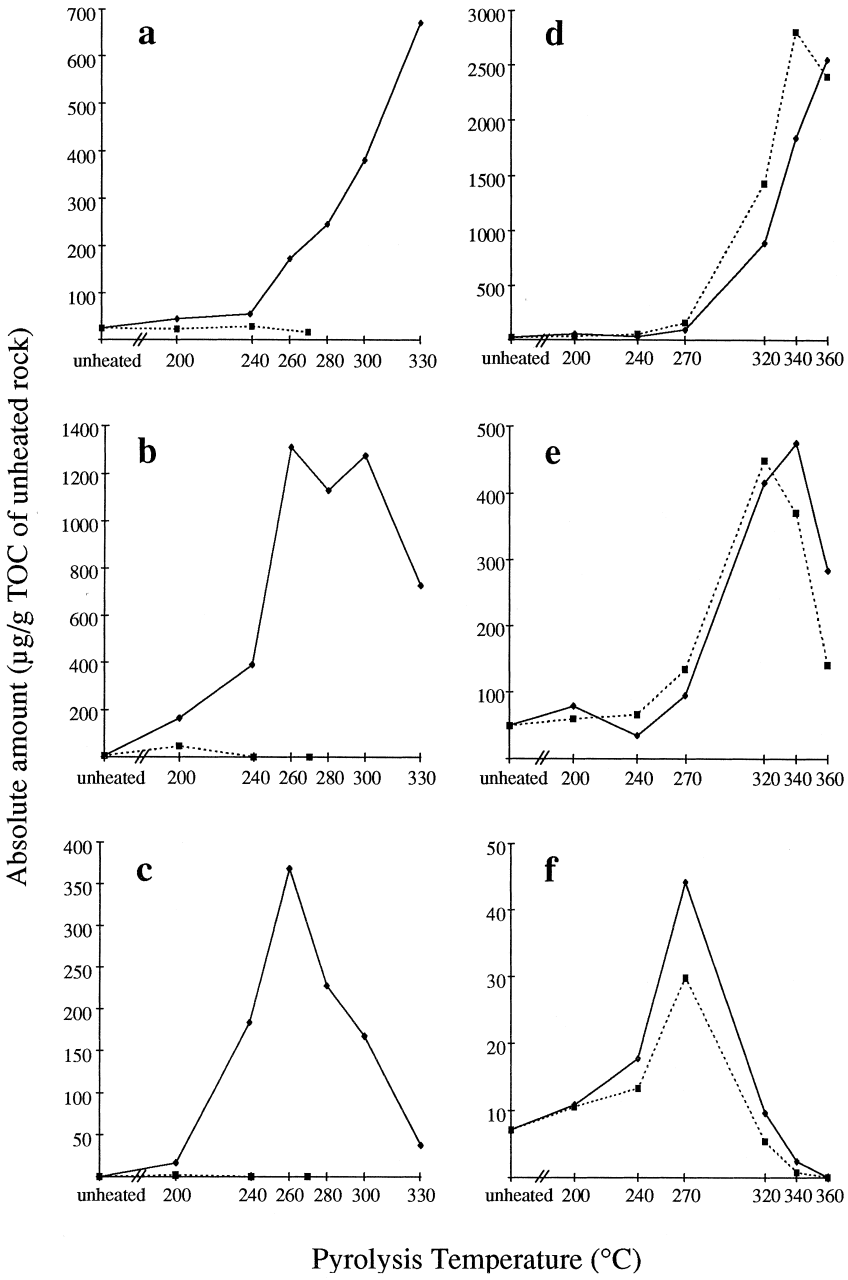

Fig. 1. Generation profiles of (a) octadecane, (b) phytane and (c) (20*R*)-5α-24-ethylcholestane for the claystone and (d–f) limestone from hydrous (solid line) and anhydrous pyrolysis (stippled line) for 72 h. Anhydrous pyrolysis of the claystone does not generate biomarkers.

maturity parameters (e.g. Eglinton *et al.*, 1986; Tannenbaum *et al.*, 1986; Lu *et al.*, 1989). Quantitative experimental studies by Huizinga *et al.* (1987a,b) showed that mineral matrix effects on hydrocarbon generation from powdered mixtures of kerogen and minerals were more extreme under anhydrous than hydrous pyrolysis. These experiments also showed that under anhydrous pyrolysis smectite and illite had a greater inhibitory effect on hydrocarbon generation than calcite. Anhydrous pyrolysis experiments with isolated Monterey and Green River kerogen showed that especially smectite inhibited generation of isoprenoids and longer chain *n*-alkanes (Huizinga *et al.*, 1987b). During anhydrous pyrolysis of isolated Monterey kerogen with added smectite, the generated bitumen was degraded much faster than for the Green River kerogen with added smectite, which was ascribed to the higher amount of S_{org} in the Monterey kerogen (Huizinga *et al.*, 1987a). Thus, the combined effects of smectite and a high S_{org} content for the claystone in the present study may explain the large differ-

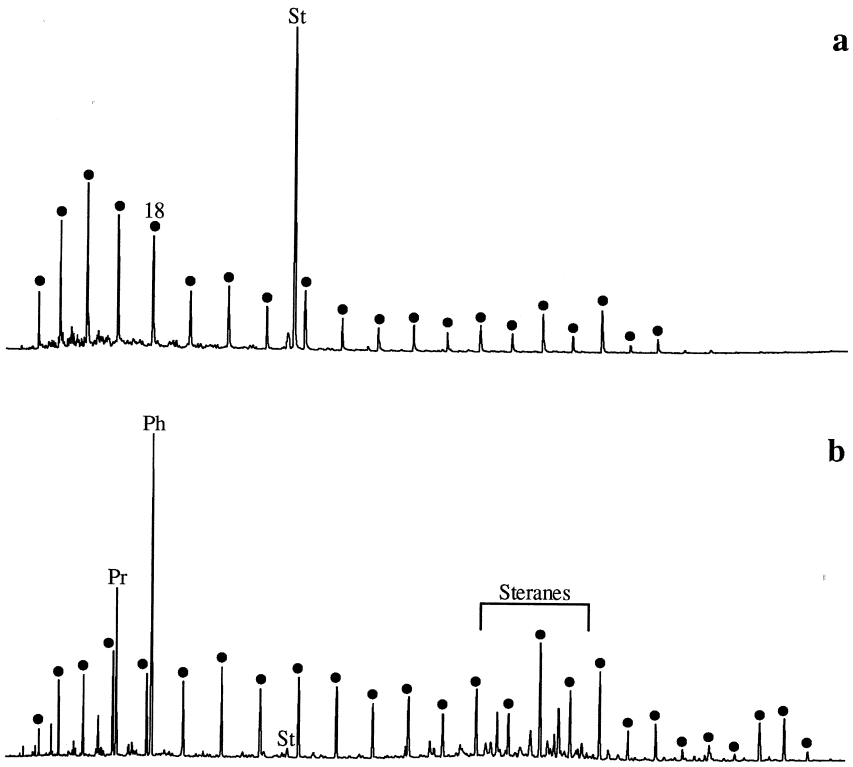

Fig. 2. Gas chromatograms of the saturated hydrocarbon fractions of the claystone (a) heated at 270°C under anhydrous conditions, and (b) heated at 280°C under hydrous conditions. Key: dots indicate *n*-alkanes (numbers represent total number of C atoms), Pr is pristane, Ph phytane and St standard. After anhydrous pyrolysis mainly *n*-alkanes remain. Note the different peak heights of the standard, indicating much lower amounts of *n*-alkanes after anhydrous pyrolysis.

ences observed between the claystone and the limestone. In addition, the relatively low TOC content of the claystone and the resulting high clay/TOC ratio probably enhances the destructive effects of the clay.

The actual mechanism and reactions responsible for clay minerals, especially smectite, inhibiting the generation of biomarkers under anhydrous conditions and not under hydrous conditions remain to be determined. One possible explanation may be related to the greater availability of clay mineral interlayers to bitumen under anhydrous pyrolysis than under hydrous pyrolysis, because under anhydrous pyrolysis these interlayers are probably less hydrated than under hydrous pyrolysis. Huizinga *et al.* (1987a) showed that adsorption of the polar constituents of bitumen to smectite and illite was enhanced during anhydrous pyrolysis. For our experiments, this would imply that the polar fraction of the claystone, which contains abundant S-bound biomarkers, would be adsorbed to these clay minerals during anhydrous pyrolysis. Once inside the clay mineral interlayers, the high contact area of mineral surface to organic matter may favor cross-linking reactions that form an insoluble pyrobitumen rather than cracking reactions that form lower-molecular-weight hydrocarbons. Thus, the biomarker thermal generation sequence suggested by Koopmans *et al.* (1996, 1997), i.e. (i) bound moiety in the kerogen, (ii) bound moiety in the polar fraction, (iii) free biomarker, is obstructed because no biomarkers are generated from the polar fraction. This explains the extreme differences in the amounts of S-bound biomarkers during hydrous and anhydrous pyrolysis of the claystone, and consequently also the differences in generation of free biomarkers

Table 1. Amounts (μg/g TOC of unheated rock) of phytane and (20*R*)-5α-24-ethylcholestane released upon desulphurisation of the polar fraction of the unheated claystone, and the polar fractions after anhydrous and hydrous pyrolysis at the temperatures indicated

	Temperature				
	Unheated	200°C	240°C	270°C	280°C
Phytane					
HP	410	340	210	na	120
AP	410	7	0	0	na
Sterane					
HP	340	240	79	na	5
AP	340	0	0	0	na

na means that no experiment was performed at this temperature.

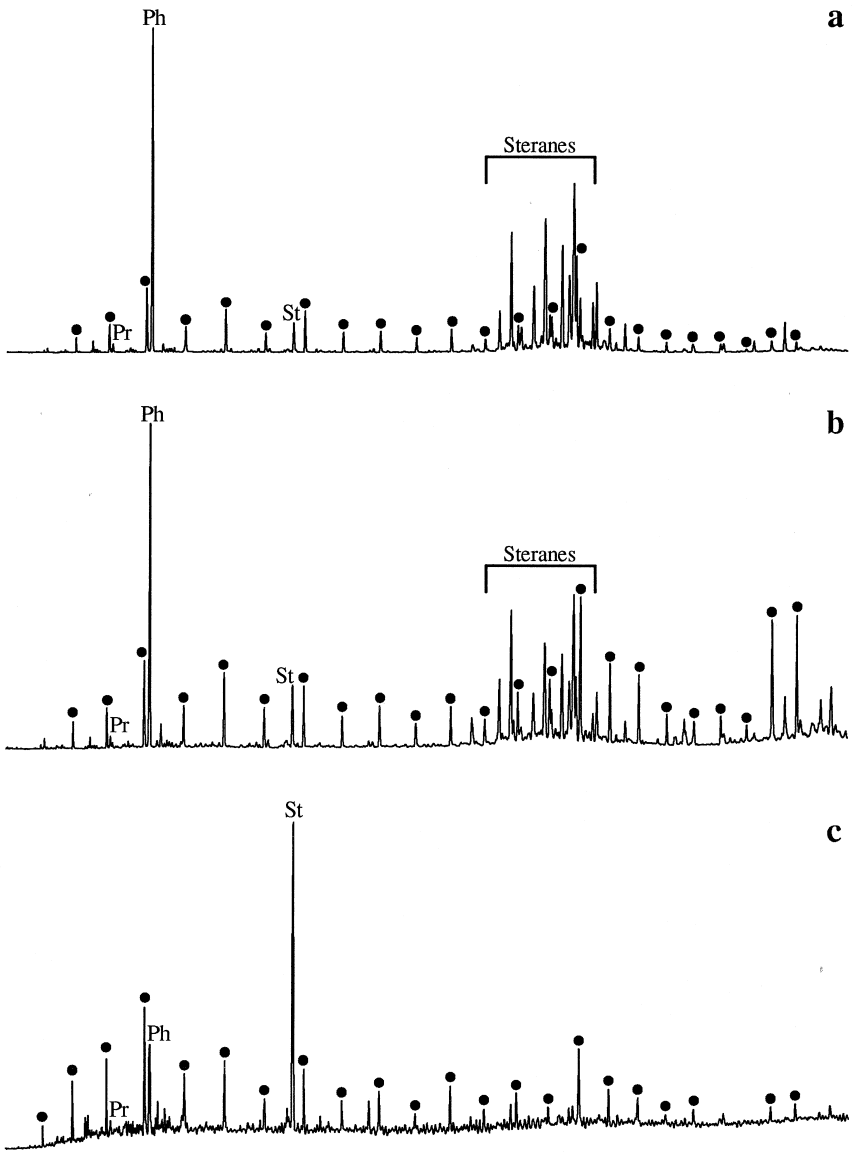

Fig. 3. Gas chromatograms of the desulfurized polar fractions of the (a) unheated claystone, and the claystone heated at 200°C for 72 h by (b) hydrous pyrolysis and (c) anhydrous pyrolysis. Key: dots indicate *n*-alkanes, Pr is pristane, Ph phytane and St standard. After anhydrous pyrolysis at 200°C only small amounts of *n*-alkanes and an unresolved complex mixture are released by selective cleavage of C–S bonds.

(i) between hydrous and anhydrous pyrolysis of the claystone, and (ii) between anhydrous pyrolysis of the claystone and the limestone.

Effect of organic matter type on biomarker generation during pyrolysis

An alternative explanation for the difference in biomarker generation between the claystone and the limestone under anhydrous pyrolysis may be the presence of S–S bonds in the organic matter of the claystone. Although both rocks have similarly high S_{org} contents, the claystone also contains a relatively high amount of S–S bonds (Kohnen *et al.*, 1991; Koopmans *et al.*, 1996), whereas the limestone does not (Koopmans *et al.*, 1998). Due to the weakness of the S–S bond, 1,2-dithianes with an octadecane, phytane and (22*R*)-17β,21β(H) C_{35} hopane carbon skeleton present in the unheated claystone were not detectable after hydrous pyrolysis at 200°C (Koopmans *et al.*, 1996). The low temperature degradation of S–S bonds probably provides a source for free thiyl radicals. During hydrous pyrolysis of the claystone, the thermally generated thiyl radicals are probably quenched by water-derived hydrogen (Lewan, 1997). During anhydrous pyrolysis of the claystone, however, the

thiyl radicals are not quenched but may initiate cross-linking reactions, leading to the formation of an insoluble pyrobitumen.

Implications

The realization that many of the commonly employed biomarker maturity parameters are a result of differences in the generation and thermal stability of biomarkers rather than specific reactions involving isomerization, aromatization, or side-chain cleavage (e.g. Abbott *et al*., 1990; Requejo, 1992; Bishop and Abbott, 1993; Koopmans *et al*., 1998) makes the employed pyrolysis conditions an important consideration in determining kinetic parameters. Anhydrous conditions will be heavily influenced by mineral matrix effects, which may yield faster rates. Conversely, hydrous conditions will be less influenced by mineral matrix effects, which will yield slower reaction rates.

Another implication is that hydrous pyrolysis provides the best means of revealing the complete biomarker potential of a rock for the interpretation of source and depositional conditions. During anhydrous pyrolysis, the presence of clay minerals will prevent full realization of the biomarker potential, resulting in loss of important information. It is conspicuous that the destructive effect of clay minerals on biomarker generation which probably accompanies confined pyrolysis experiments is not very well documented. Our results clearly show that water is important in generating biomarkers from rocks that contain clay minerals.

CONCLUSIONS

During anhydrous pyrolysis of the claystone, the polar fraction, which contains abundant S-bound biomarkers, is adsorbed to the clay minerals. This promotes cross-linking reactions that prevent generation of free biomarkers. During hydrous pyrolysis of the claystone, adsorption of the polar fraction to the clay minerals is diminished because the clay mineral interlayers are occupied by water, so that cross linking reactions are minimized and generation of biomarkers can take place. In addition, cross-linking reactions during anhydrous pyrolysis of the claystone are probably enhanced because of the presence of S–S bonds in the organic matter of the claystone. These results show that water is important in closed system laboratory experiments that aim at simulating the natural maturation of sedimentary organic matter, especially when clay minerals are present.

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