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THÈSE
présentée par

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Contribution of molecular biomarkers to the knowledge of terrestrial plants development during the Palaeozoic

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To my little boy Carlos,
To my husband Frank,
To my family of my beautiful country Venezuela,
To my Venezuelan and French friends,
……with love….
Maria

Acknowledgements

Although three years have passed, I can still remember the first time that I arrived here to France with my family.... It was not easy.... but we did it......

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Abstract

The aliphatic and aromatic biomarker content from terrestrial and marine sediments of Late Ordovician to Early Carboniferous age have been related to their palynomorph assemblages (e.g. acritarchs, prasinophytes, chitinozoans, cryptospores, trilete spores and megaspores) in order to contribute to the knowledge of land plant evolution during the Palaeozoic. This investigation is therefore focused on the land-derived biomarkers and their attributions to specific kind of plants. The biomarker record of middle Silurian – lower Devonian sediments from southern Tunisia, Ghadamis Basin (Gondwana) reveals the presence of retene, cadalene, kaurane, norabietane, tetrahydroretene, C_{19} isohexylalkylnaphthalene and simonellite. The early Palaeozoic bryophytes and tracheophytes (e.g. *Cooksonia*, lycophytes and zosterophylls) may therefore be considered as potential precursors for retene and its related molecular compounds in sediments of Middle Silurian to Early Devonian age. In contrast, the Early Carboniferous flora formed by arborescent lycopods, sphenopsids and pteridosperms have been suggested here as a possible terrestrial source for phyllocladane, abietane, *ent*-beyerane, bisnorsimonellite, diaromatic totarane, diaromatic sempervirane and 2-methylretene in the Lower Carboniferous (Viséan) coal deposits at Dunbar (East Lothian, Scotland). Among the other biomarkers detected in our samples, ionene, alkyldibenzofurans, perylene and combustion-derived polycyclic aromatic hydrocarbons (PAHs) indicate pollen, lichens, fungi and vegetation fire contributions, respectively. Most of the biomarkers identified here had been so far generally associated to conifers, though conifers only evolved during Late Carboniferous. These compounds therefore are also characteristic of early land plants.

Keywords: Land plant biomarkers, Ghadamis Basin, The Midland Valley of Scotland, Silurian, Devonian, Early Carboniferous, terpenoids, retene, phyllocladane, kaurane, beyerane, alkyldibenzofurans, alkylphenanthrenes, cryptospores, trilete spores, megaspores.

Résumé

Le contenu en biomarqueurs aliphatiques et aromatiques de sédiments d'origine terrestre et marine de l'Ordovicien supérieur au Carbonifère inferieur a été comparé aux assemblages de palynomorphes (acritarches, prasinophytes, chitinozoaires, cryptospores, spores trilètes et mégaspores) afin de contribuer à la connaissance de l'évolution des plantes terrestres au cours du Paléozoïque. Cette étude est donc basée sur les biomarqueurs d'origine terrestre et leur attribution à une espèce de plantes. L'enregistrement des biomarqueurs dans les successions clastiques du Silurien moyen – Dévonien inferieur de Tunisie méridionale, dans le basin de Ghadamis (Gondwana) révèle la présence de rétène, cadalène, kaurane, norabiétane, tetrahydroretene, C_{19} isohexylalkylnaphthalene et simonellite. Les premières bryophytes et les trachéophytes du Paléozoïque (par exemple *Cooksonia*, lycophytes et zosterophylles) peuvent donc être considérés comme de potentiels précurseurs pour le rétène et ses composés moléculaires associés dans les sédiments du Silurien Moyen au Dévonien Inférieur. En contrepartie, la flore du Carbonifère inferieur formée principalement de lycopodes arborescents, sphenopsides et pteridospermes est proposée comme une possible source pour le phyllocladane, abiétane, *ent*-béyerane, bisnorsimonellite, totarane diaromatique, sempervirane diaromatique et 2-méthylrétène dans les dépôts de charbon du Carbonifère inférieur (Viséan) de Dunbar (Est Lothian, Ecosse). Parmi les autres biomarqueurs identifiés dans nos échantillons, ionène, alkyldibenzofuranes, pérylène et les hydrocarbures aromatiques polycycliques (HAPs) dérivés de combustion indiquent la contribution de pollens, lichens, champignons et végétation carbonisée, respectivement. La plupart des biomarqueurs identifiés ici ont été généralement associées aux conifères, qui ne se sont développés qu'après le Carbonifère supérieur. Il apparait donc que ces composés sont également caractéristiques des premières plantes terrestres.

Les mots clés: biomarqueurs de plantes terrestres, Basin du Ghadamis, Midland Valley of Scotland, Silurien, Dévonien, Carbonifère inférieur, terpénoïdes, rétène, phyllocladane, kaurane, béyerane, alkyldibenzofuranes, alkylphenanthrènes, cryptospores, spores trilètes, mégaspores.

Thesis structure

This PhD. thesis was performed in order to contribute for the project "ECLIPSE – Terrestrialization", INSU – CNRS. It has been financially supported by the FRE Géosystèmes of the Université Lille 1 and the Programme Alßan, a high level scholarship programme from European Commission specifically addressed to Latin America (scholarship No. E07D402105VE).

Chapter 1 entitled *Introduction*, describes a general review about the land plant evolution during the Palaeozoic ("terrestralization process"), the typical biomarkers often used as tracers of terrestrial organic matter and the scope and significance of this present study. A detailed description of investigated samples and the analytical methods are also given throughout this chapter.

Chapter 2 describes a first biomarker result obtained from MG1 core compared to its available palynological information. The first possibility that retene could derive from the maturation of kaurane-type compounds associated to the early Palaeozoic bryophytes is provided among this chapter. **Chapter 2** therefore shows my first article published in Organic Geochemistry:

Romero-Sarmiento, M.-F.; Riboulleau, A.; Vecoli, M.; Versteegh., G. J. M., 2010. Occurrence of retene in upper Silurian – Lower Devonian sediments from North Africa: Origin and implications. ***Organic Geochemistry 41***, 302 – 306.

Chapter 3 is mainly based on the biomarker occurrences and distributions from upper Ordovician to lower Devonian core samples from boreholes TT1 and MG1 (Ghadamis Basin, North Africa). The palynological contents of these two core sections have been previously defined by Vecoli et al. (2009) and Spina and Vecoli, (2009). A detailed comparison between the lipid biomarkers and palynomorphs was therefore performed. **Chapter 3** integrates the following manuscript to be submitted:

Romero-Sarmiento, M.-F.; Riboulleau, A.; Vecoli, M.; Versteegh. G. J. M. (In preparation). Aliphatic and aromatic biomarkers from Gondwanan sediments of Late Ordovician to Early Devonian age: An early terrestrialization approach.

Chapter 4 identifies and characterises the occurrence of land plant biomarkers in Lower Carboniferous (Visean) coals from Dunbar, East Lothian – Scotland. Based on the palynological information, these coal samples have been characterized by the presence of abundant and well-preserved miospore and megaspore assemblages (Spinner, 1969; Spinner and Clayton, 1973). The comparison of identified land plant biomarkers and land plant palynomorphs allows relating some biomarkers to specific plant taxa. This chapter corresponds to the third manuscript to be submitted:

Romero-Sarmiento, M.-F.; Riboulleau, A.; Vecoli, M.; Versteegh. G. J. M. (In preparation). Aliphatic and aromatic biomarkers from Carboniferous coal deposits at Dunbar (East Lothian, Scotland): Palaeobotanical and palaeoenvironmental significance.

Chapter 5 summarizes the major conclusions raised during the development of this scientific research.

Appendix 1 contains another manuscript submitted last year (2009) for publication in Organic Geochemistry. Several unknown compounds have additionally been detected in the aromatic fractions of some investigated samples. The most likely origin of these compounds is discussed in this article performed in collaboration with Australian researchers.

George, S.; Volk, H.; Romero-Sarmiento, M.-F.; Dutkiewicz, A.; Mossman. D. (Submitted). Di-*iso*-propylnaphthalenes: environmental contaminants of increasing importance for organic geochemists.

TABLE OF CONTENTS

Chapter 4: Aliphatic and aromatic biomarkers from Carboniferous coal deposits at Dunbar (East Lothian, Scotland): Palaeobotanical and palaeoenvironmental significance........ 101

LIST OF FIGURES

Chapter 1: Introduction

FIGURE NUMBER **Pag.**

Chapter 2: Occurrence of retene in upper Silurian – lower Devonian sediments from North Africa: Origin and implications

Chapter 3: Aliphatic and aromatic biomarkers from Gondwanan sediments of Late Ordovician to Early Devonian age: An early terrestrialization approach

Chapter 4: Aliphatic and aromatic biomarkers from Carboniferous coal deposits at Dunbar (East Lothian, Scotland): Palaeobotanical and palaeoenvironmental significance

Chapter 5: Conclusions

LIST OF TABLES

Chapter 1: Introduction

TABLE NUMBER **Pag.**

Chapter 3: Aliphatic and aromatic biomarkers from Gondwanan sediments of Late Ordovician to Early Devonian age: An early terrestrialization approach

Chapter 4: Aliphatic and aromatic biomarkers from Carboniferous coal deposits at Dunbar (East Lothian, Scotland): Palaeobotanical and palaeoenvironmental significance

Chapter 1

Introduction

1.1. The evolution of land plants during the Palaeozoic

The origin and early diversification of land plants took place during the Palaeozoic, and constitute one of the major events of innovation in the history of life on Earth. Moreover, the evolution of land plant had a profound impact on several physical processes and biogeochemical cycles acting at the biosphere-geosphere interface (Algeo et al., 2001; Berner, 2001). At the same time, the terrestrial vegetation provided new habitats for the colonization of the land by animals, thus fostering further biological innovations in the animal kingdom. The process of progressive development of life on emerged land is generally known as the “terrestrialization” process.

Evidence on the origin and early diversification of land plants has come mainly from dispersed spores (e.g., Strother et al., 1996; Steemans et al., 1996; Steemans, 1999; Edwards and Wellman, 2001). These spores are the only evidence of terrestrial vegetation during the first 60 million years of their existence (from Middle Ordovician to mid-Silurian times, when the first plant macrofossils occur; Edwards and Wellman, 2001). In this context, the main theme of the present research is to investigate if the origin of land plant populations and their progressive spread on the continents over time is also reflected on the molecular composition of the organic matter present in Palaeozoic sediments by means of, for instance, variations in the assemblages of plant-derived biomarkers (e.g. van Aarssen et al., 2000).

While bacteria, algae, lichens and fungi were probably the first inhabitants of terrestrial environments (Kenrick and Crane, 1997; Retallack, 2000, 2008), bryophytes (mosses, hornworts and liverworts) and tracheophytes (lycopods, horsetails, ferns and seed plants) represent the major accepted Palaeozoic land plants. The classification of major plant groups and the geological interval followed in this study are shown in figure 1.1. The colonization of land masses by these plants probably took place in freshwater or marginal marine environments under tropical or subtropical conditions with abundant sunlight and frequent dry periods (Gensel and Andrews, 1987).

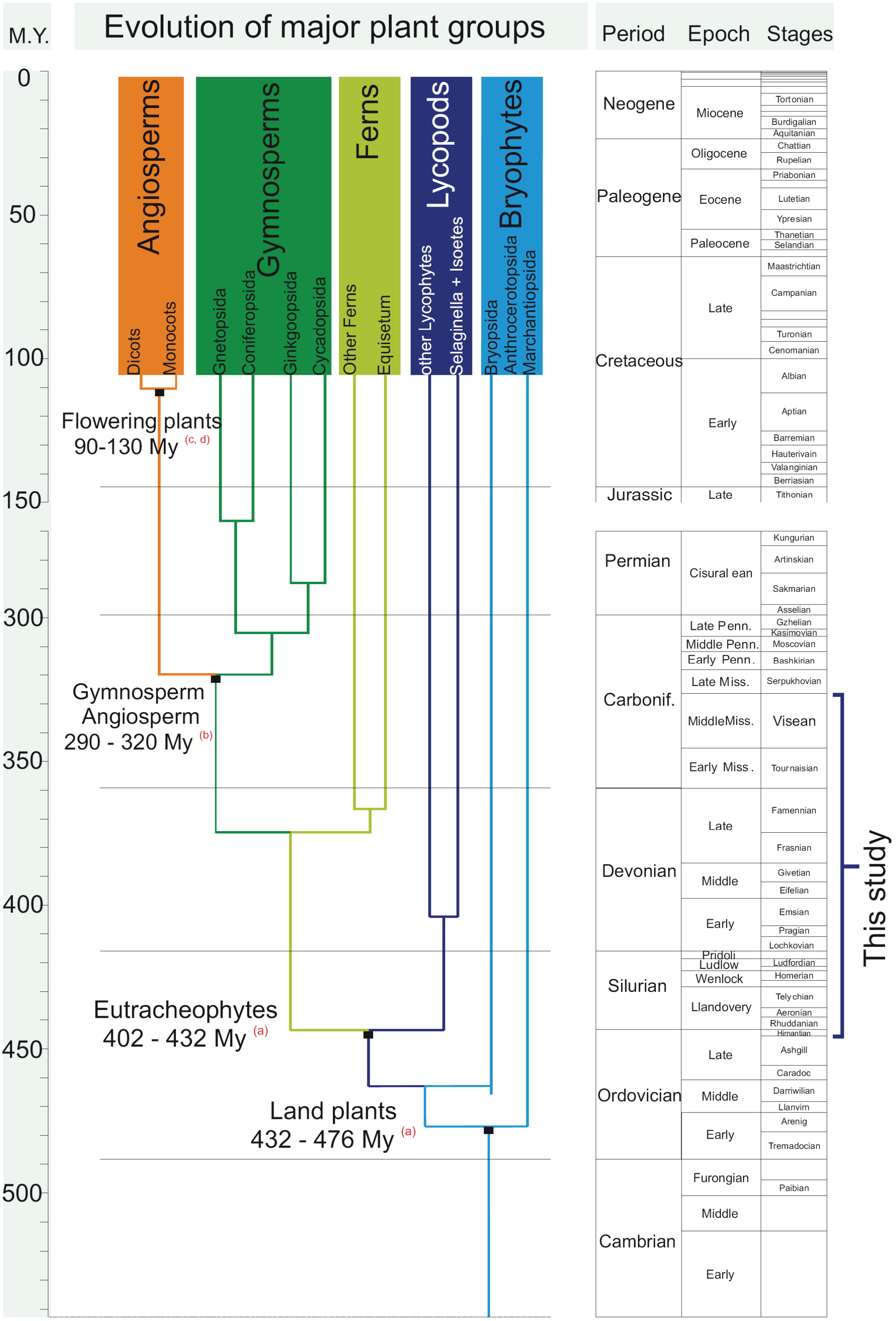


Figure 1.1: The evolution of major plant groups and the geological interval covered in this study. Tree and evolutionary information modified from Martone et al. (2009); (a) Kenrick and Crane (1997); (b) Bowe et al. (2000); (c) Schneider et al. (2004) and (d) Crane et al. (1995). Geologic time scale from the International Commission on Stratigraphy (ICS)

The oldest uncontroversial land-plant-produced dispersed spores ("cryptospores") have been reported from middle Ordovician strata in Saudi Arabia (Darriwilian; Strother et al., 1996; Fig. 1.2). These early spores are generally believed to have been produced by the Early Palaeozoic bryophytes, based on comparative morphology (Gray, 1985, 1991). Cryptospores are the most abundant plant remains recovered from the upper Ordovician to lower Silurian sediments (Steemans, 2000). Cryptospores become very rare after the Lochkovian and are superseded by trilete spores, which dominate the terrestrial palynomorph record during Devonian times (Steemans, 2000).

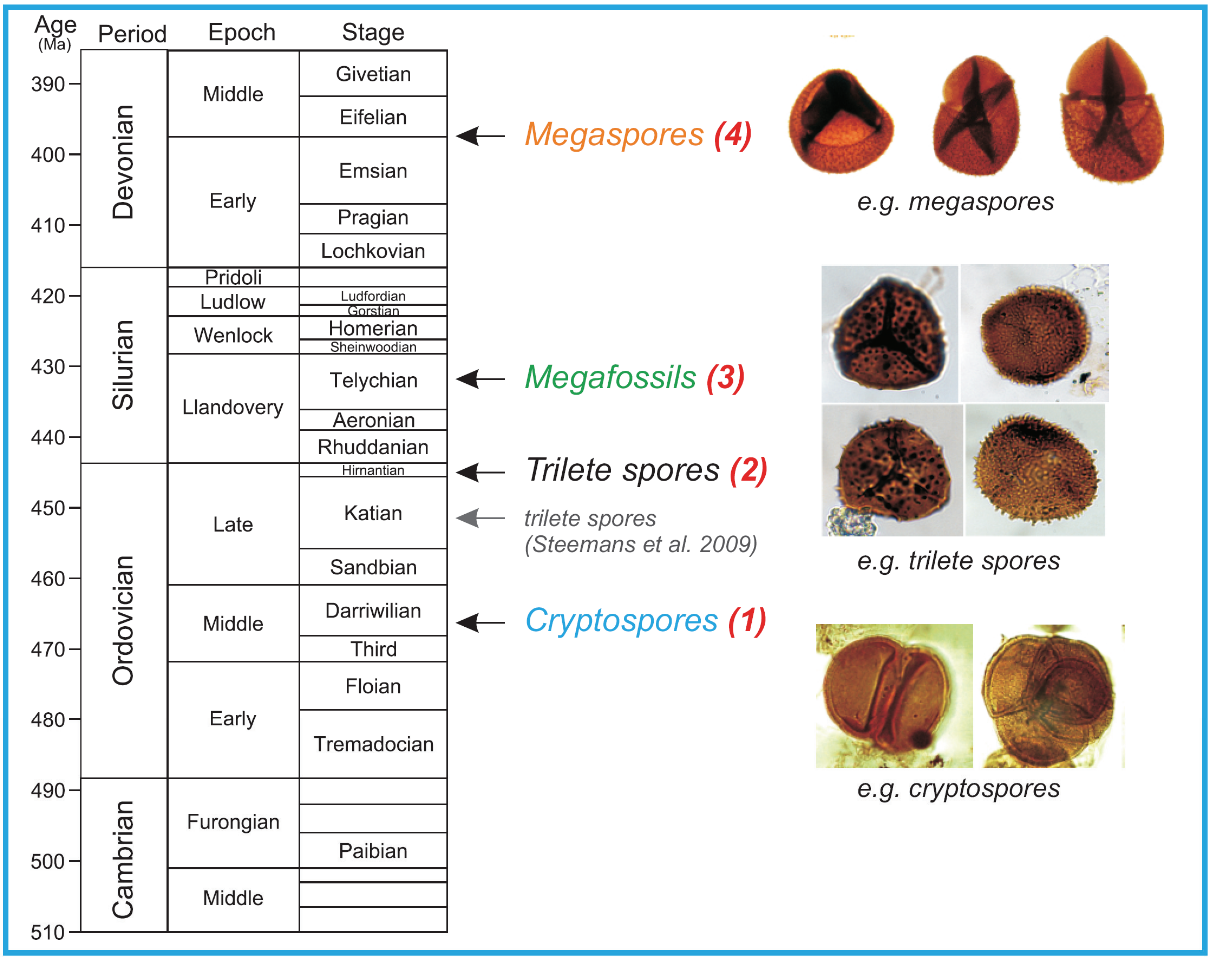


Figure 1.2: Stratigraphic position of (1) the oldest cryptospores (Strother et al., 1996); (2) the previous oldest trilete spores from Turkey (Steemans et al., 1996); (3) the oldest megafossils of the vascular plant lineage (Edwards and Wellman, 2001) and (4) the oldest megaspores from Lower – Middle Devonian (Chaloner, 1967)

The fist land plants were probably very small without water conducting organs such as trachea or roots, similar to the modern Bryophytes, which are the most primitive land plants living today (Wellman et al., 2003). More than 20,000 species of bryophytes are currently known in the world (Asakawa, 2001). Bryophytes could be divided morphologically into three classes (Fig. 1.1): Marchantiopsida (liverworts 6000 species), Anthocerotopsida (hornworts 300 species) and Bryopsida (mosses 14,000 species). According to Asakawa (2001), the bryophytes could contain a large number of phenolic and terpenoids compounds, the liverworts are also rich sources of a number of different skeletal diterpenoids and the mosses contain many triterpenoids (Asakawa, 2001).

Since recently, the first stratigraphic occurrence of trilete spores (monads formed by the dissociation of spore tetrads) was fixed in latest Ordovician (Hirnantian) strata from Turkey (Steemans et al. 1996; Fig. 1.2). These early trilete spores are supposed to be mainly produced by sporangia of tracheophytic land plants (Fanning et al., 1991; Edwards and Richardson, 1996). However, it should be borne in mind that also a small proportion of extant bryophytes can produce trilete spores (Steemans et al., 2009). The recent report of morphologically complex trilete spores from mid- to late Katian sediments of Saudi Arabia potentially push back the origin of vascular plants by several million years (Steemans et al., 2009; Fig. 1.2). Trilete spores-producing land plants become progressively abundant in the late Llandovery (late Early Silurian; Wellman and Gray, 2000).

The earliest reported vascular plant megafossil in Middle Silurian (Edwards and Wellman, 2001; Fig. 1.2) is coincident with a peak in diversification of trilete spores (Steemans, 2000). Tracheophytes are the only group of land plants with a well-documented fossil record and were affected by adaptive radiations and extinctions (Gray, 1993). The early tracheophytes presumably evolved from charophytes (green algae) and are characterized by their capacity to produce sporopollenin, cutin, phenolic compounds and the glycolate oxidase pathway (Graham, 1985). Vascular tissues were already developed by tracheophytes allowing them the adaptation to different habitats (Gray and Shear, 1992). The major vascular land plants which have dominated the vegetation since the middle part of the Silurian and some extinct transitional lineages are given in figure 1.3.

Based on the major changes in spore assemblages and the appearance of vascular plant megafossils, vascular plants are considered to have originated and adaptively radiated from the Early Silurian (Steemans et al., 2009). Land plant macrofossils become abundant in continental sediments of Lower Devonian age (Zhu and Kenrick, 1999). These terrestrial assemblages are mostly herbaceous vascular plants which are most closely related to living lycopsids (e.g. Niklas and Banks, 1990; Gensel, 1992; Hueber, 1992; Zhu and Kenrick, 1999). Lycophytes were generally small, short-living trees with a determinate growth in peat swamps with a low taxonomic diversity (Gray, 1993). While an increase in plant size, including arborescence in some lycopods mainly occurs in Middle Devonian, a diversification from the primitive types of lycopsids, sphenopsids and ferns takes place during the Late Devonian (Stewart and Rothwell, 1993).

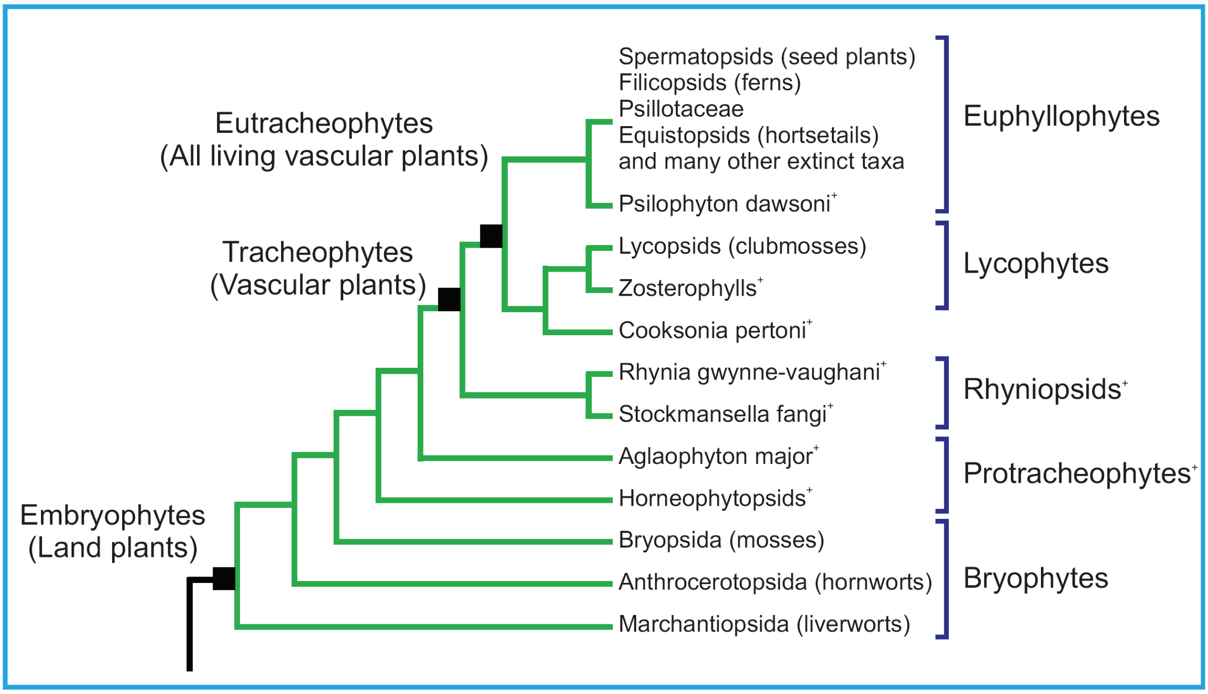


Figure 1.3: A detailed evolutionary relationship of the vascular land plant (modified from Kenrick and Crane, 1997)

The Carboniferous (Mississipian and Pennsylvanian) has been characterized by a great diversification of cordaites, seed ferns in great variety, herbaceous and arborescent lycopsids, sphenopsids and ferns (Stewart and Rothwell, 1993). Actually, arborescent lycophytes are considered the main contributor to the biomass of Lower and Middle Pennsylvanian coals (e.g. Collinson et al., 1994). Wood was a relatively minor component of the biomass except in swamps with abundant cordaites or calamites (Cross and Phillips, 1990). Furthermore, five kinds of tropical to subtropical trees have been described in the Euramerican Upper Carboniferous coal-forming floras: (1) lycopods, (2) tree ferns, (3) calamites, (4) seed ferns, and (5) cordaites dominated (Cross and Phillips, 1990).

Living ferns and horsetails (Figure 1.3) are considered the sister group to lycopsids (Kenrick and Crane, 1997). It has been also proposed that horsetails and ferns (tracheophytes) are the ancestors of seed plants (Pryer et al., 2001; Fig. 1.3). The first megaspores were observed around the Lower – Middle Devonian boundary, around 397.5 Ma and constitute the first evidences for the heterospory (Chaloner, 1967; Fig. 1.2). The oldest seed or proto-seed were exactly found in the Ronquières locality from Belgium (Givetian to Earliest Frasnian; Gerrienne et al., 2004). However, the major diversification of seed plants (e.g. gymnosperms) took place during the Carboniferous and the Permian (Hart, 1987; Kerp, 1996).

Cycadales, ginkgoales, gnetales and conifers are considered as the major orders among the class gymnospermopsida (gymnosperms) (Stewart and Rothwell, 1993; e.g. Fig. 1.1). However, the phylogeny of gnetales has also been related to angiosperms (Schmidt and Schneider-Poetsch, 2002) and/or to conifers (Chaw et al., 2000). Conifers have been also divided into many families such as *Podocarpaceae*, *Pinaceae*, *Cupressaceae*, *Auaucariaceae*, *Taxodiaceae* and *Chephalotaxaceae* (Stewart and Rothwell, 1993). The oldest direct evidence for the existence of the conifers is represented by small leaves and a leafy shoot from Upper Carboniferous (Scott, 1974)

Most families of the plant kingdom had evolved at the end of the Palaeozoic (Fig. 1.1). Diversification and expansion of angiosperms took place during the Mesozoic. The oldest unambiguous angiosperm fossil is dated of the early Cretaceous, around 132 Ma (Crane et al., 1995). The typical age-biomarker oleanane is suggested to derive from β-amyrin, which is a constituent of angiosperms (Moldowan et al., 1994). The abundant presence of oleanane, and therefore of angiosperms is restricted to periods of the Cretaceous and younger (Moldowan et al., 1994). However, the diversification of angiosperms based on DNA-analysis and findings of oleanane in Pennsylvanian coals, has been recently reported during the Late Carboniferous (Martin et al., 1989; Moldowan et al., 1994).

In summary, spore assemblages demonstrate that higher plant life probably began millions of years before the Late Silurian (Gray, 1993). From the earliest land plant megafossils appeared in Middle Silurian (Edwards and Wellman, 2001), a substantial evolutionary diversification of land plants followed during Devonian and a wide range of flora evolved throughout Carboniferous (Stewart and Rothwell, 1993). Finally, it has been commonly accepted that land plants first evolved on the Gondwana plate, in the North Africa – Middle East area (Steemans et al., 2009).

1.2. Terrestrial plant biomarkers

Biomarker compounds are organic molecules that can be detected by gas chromatography and mass spectrometry techniques in the hydrocarbon fractions obtained from the extracts of recent and ancient sediments. These molecules which are characteristic of the organism from which they originate may be used to interpret the terrestrial, marine and/or microbial input involved during the rock formation.

In the first part of this study, a detailed review of published record of typical land plant biomarkers was performed. This has permitted to develop a database of target biomarkers of land plant origin. A vascular or land plant biomarker is defined as a well recognized molecular compound present in the geosphere whose carbon skeleton can be traced to an obvious land plant precursor. Such compound therefore can be used to make palaeobotanical and palaeoenvironmental reconstructions in sedimentary basins (e.g. Fleck et al., 2001, 2002; Hautevelle et al., 2006).

Like other chemical fossils, the biogenic precursors of terrestrial plant biomarkers are transformed into their geological counterparts by various chemical processes which take place in the water or sediment columns under the influence of microbial activity or mineral catalysis (e.g. Eglinton and Calvin, 1967). These transformations lead to a complete or partial preservation of the biogenic carbon skeleton.

Each target biomarker that will be mentioned throughout this chapter is identified by means of a number into parentheses. Terrestrial plant biomarkers can be subdivided into to the following families:

1.2.1. Aliphatic biomarkers

The aliphatic hydrocarbons which have been generally considered as markers of terrestrial organic matter are mainly the long-chain *n*-alkanes, C_{29} steroids, bicyclic alkanes and tricyclic and tetracyclic diterpenoids.

1.2.1.1. Long chain *n*-alkanes

Short and long chain *n*-alkanes occur widely in crude oils and sediments (Noble, 1986). In general, the short chain *n*-alkanes are interpreted to be derived from bacterial and/or algal debris (Simoneit, 1978). Conversely, higher plant lipids, which consist of a high proportion of waxes and wax esters, have been typically accepted as the biological sources of *n*-alkanes with more than 23 carbon atoms (>C_{23}) (e.g. Fig. 1.4). These long chain *n*-alkanes **(1)** are characterized by an odd-over-even carbon number predominance and a maximum at C_{29} or C_{31} carbon atoms (Eglinton and Hamilton, 1967; Caldicott and Eglinton, 1973; Tissot and Welte, 1984). However, some studies have suggested that the distribution of long *n*-alkanes might have changed in relation to plant evolution. For instance, Disnar and Harouna (1994) proposed that the occurrence of linear *n*-alkanes with a maximum at C_{20} and/or C_{23} homologues without any remarkable odd/even preference in Lower Carboniferous coals could derive from non-flowering plants.

(1)

Long-chain *n*-alkanes (e.g. C_{29} *n*-alkanes)

Figure 1.4: Chemical structure of long-chain *n*-alkanes

1.2.1.2. C_{29} steroids

The relative abundance of C_{27}, C_{28} and C_{29} steroids **(2)** is frequently used to differentiate depositional settings (Peters et al., 2005). Huang and Meinschein (1979) proposed a useful ternary diagram to identify the source of the organic matter which can be constructed by the distribution of C_{27}, C_{28} and C_{29} sterols. These authors demonstrated that the presence of C_{29} sterols in sediments is mainly derived from terrestrial plants, whereas zooplankton and phytoplankton are the dominant source of C_{27} and C_{28} sterols, respectively (Huang and Meinschein, 1979). These interpretations were supported by the detected large amounts of C_{29} relative to C_{27} steranes in crude oils containing organic matter derived from land plants (Mackenzie et al., 1982). On the other hand, it has been also demonstrated that C_{29} sterols can be particularly produced by certain unicellular algae (e.g. Volkman, 1986). Figure 1.5 shows a simplified scheme the transformation pathway for C_{29} sterol after its deposition in sedimentary environments.

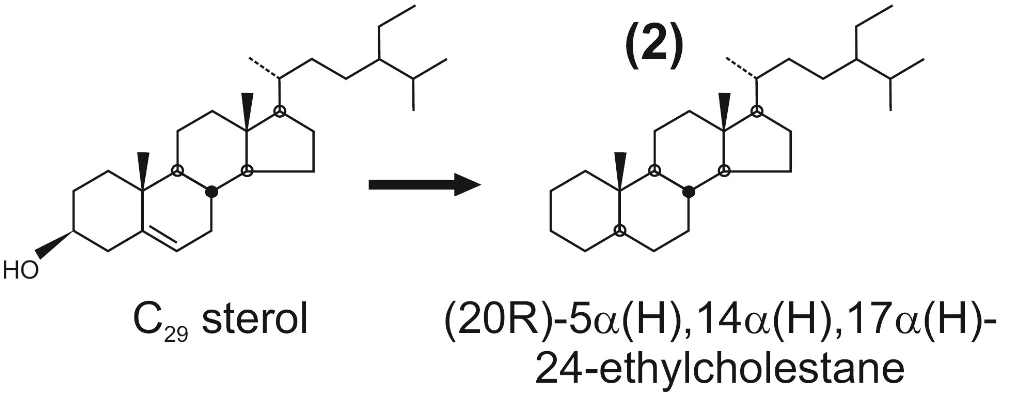


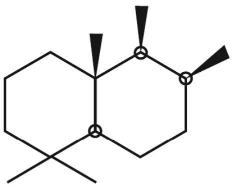
Figure 1.5: A simplified scheme showing the transformation of C_{29} sterol to C_{29} sterane

1.2.1.3. Bicyclic alkanes

Bicyclic alkanes have been reported to occur in crude oils and sediments from a wide range of geological ages and locations (Alexander et al., 1984; Noble et al., 1987 and references therein). The widespread occurrence of 8α(H)- and 8β(H)-drimanes **(3-4)**, as well as 8α(H)- and 8β(H)-homodrimanes **(5-6)** in sedimentary organic matter, and their presence in crude oils and ancient sediments which predate the evolution of land plants, indicates that drimanes and homodrimanes are not derived exclusively from sesquiterpenoids of land plant origin (Alexander et al., 1984; Noble et al., 1987). These compounds are more likely formed by biological alteration of hopanoids precursors during diagenesis (Alexander et al., 1984). The proposed bacterial origin for these compounds is also attested by their occurrences in Proterozoic sediments (Dutkiewicz et al., 2007).

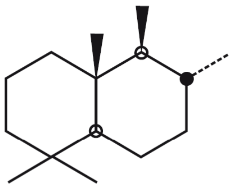
Bicyclic alkane with the eudesmane skeleton [4β (H)-eudesmane **(7)**] was only observed in samples containing plant-derived organic matter, and it has been proposed as a sedimentary marker for higher plants (Noble, 1986). Large amounts of 4β (H)-eudesmane were detected in Late Carboniferous coals from Pennsylvania (Dzou et al., 1995). This terrestrial biomarker was also reported by del Rio et al. (1994) in Late Carboniferous oil shales from Spain. Accordingly, the apparent absence of this compound in samples older than Devonian, has been interpreted as evidence for a land plant origin for the bicyclic alkane with eudesmane skeleton (Philp, 1994). Furthermore, the occurrence of eudesmane skeleton, for instance, in samples of Permian age or younger has been frequently associated to more evolved forms of plant life such as some angiosperms and gymnosperms (Noble, 1986). This molecular compound has been also reported in a few sponges (Gross and König, 2006), but also in some liverworts (Toyota and Asakawa, 1993). These ambiguous reports demonstrate that 4β (H)-eudesmane cannot be fully considered as a specific land plant biomarker. The chemical structures of some bicyclic alkanes are given in figure 1.6.

(3)



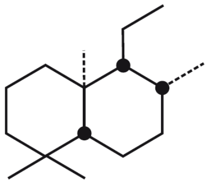
8α(H)-drimane

(4)



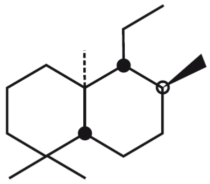
8β(H)-drimane

(5)



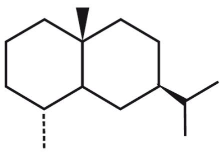
8α(H)-homodrimane

(6)



8β(H)-homodrimane

(7)



4β-eudesmane

Figure 1.6: Chemical structures of bicyclic alkanes

1.2.1.4. Tricyclic and tetracyclic diterpenoids

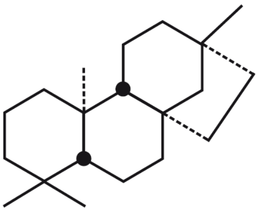
Numerous studies indicate that saturated diterpenoids occur frequently in coals, fossil resins, crude oils and lacustrine sediments which have received influxes of terrigenous organic matter (e.g. Noble et al., 1985; Otto and Simoneit, 2001; among others). Accordingly, aliphatic diterpenoids form a large group of natural products which are widely distributed in the plant kingdom (Noble et al., 1985). The tricyclic and tetracyclic diterpenoids represent the most common aliphatic diterpenoids present in sediments. They are generally abundant in higher plants and in particular, are the major constituents of conifer resins (e.g. Noble et al., 1985; Otto and Simoneit, 2001; among others). Typical structures of these tricyclic and tetracyclic diterpenoids are summarized in figure 1.7.

The biogenic precursors of the tetracyclic diterpenoids *ent*-beyerane **(8)**, 16β(H)- and 16α(H)-phyllocladane **(9-10)**, *ent*-16β(H)- and *ent*-16α(H)-kaurane **(11-12)** are mostly produced by land plants. The widespread occurrence of these compounds in modern conifers suggests that they are markers for conifer resins (Noble et al. 1985; Schulze and Michaelis 1990; Otto and Simoneit, 2001).

The biomarkers *ent*-beyarane, 16β(H)- and 16α(H)-phyllocladane have been often related to all conifer families except *Pinaceae* (Noble et al., 1985; Schulze and Michaelis, 1990). The precursors of kaurane are abundant in many conifer species, and in particular, those of the *Araucariaceae*, *Podocarpaceae* and *Taxodiaceae* families (Thomas, 1969; Noble, 1986). However, compounds with a kaurane skeleton are also widespread among bryophytes, and in particular liverworts (Chopra and Kumra, 1988; Asakawa, 2004). The precursors of *ent*-beyerane are less widely distributed than those of phyllocladane and kaurane, nevertheless are abundant in some species of the *Cupressaceae*, *Podocarpaceae* and *Araucariaceae* families (Aplin et al., 1963; Noble, 1986).

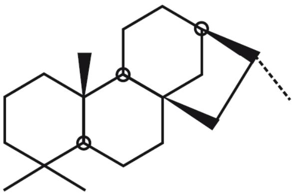
Saturated diterpenoids based on the beyerane and phyllocladane skeleton are not commonly associated to most primitive land plants such as pteridophytes and bryophytes (Ourrison, 1973; Asakawa, 1982; Noble, 1986). Accordingly, the land plant evolution through the sedimentary record can be characterized by the relative presence or absence of these tetracyclic diterpenoids (Alexander et al, 1987).

(8)



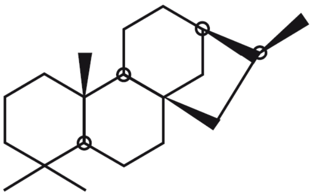
ent-beyerane

(9)



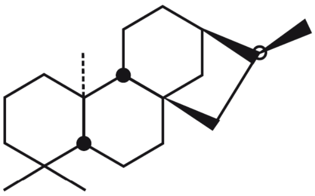
16β(H)-phyllocladane

(10)



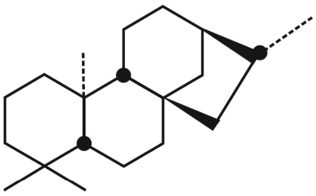
16α(H)-phyllocladane

(11)



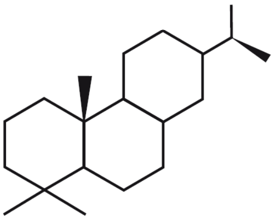
ent-16β(H)-kaurane

(12)



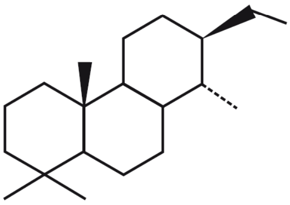
ent-16α(H)-kaurane

(13)



norabietane

(14)



pimarane

Figure 1.7: Chemical structures of tricyclic and tetracyclic diterpenoids

Phyllocladane, *ent*-beyerane and *ent*-kaurane are commonly reported in Permian to Late Carboniferous coals (Noble et al., 1985; Schulze and Michaelis, 1990; Fleck et al., 2001; Fabianska et al., 2003). The occurrence of these tetracyclic diterpenoid hydrocarbons is consistent with the relatively evolved flora which existed in the Late Carboniferous. In addition, significant concentrations of tetracyclic diterpanes, particularly *ent*-kaurane and *ent*-beyerane were traced in Middle Devonian humic coals (Sheng et al., 1992). This latter is the oldest report of *ent*-beyerane in Middle Devonian coals, which can be associated to Early Palaeozoic pteridophytes (Sheng et al., 1992).

The tricyclic diterpenoids norabietane **(13)** and pimarane **(14)** as well as abietane, fichtelite, norpimarane, among others, represent similarly saturated diterpanoids which are characteristic of the conifer resins (Noble et al., 1985; del Rio et al., 1994; Otto and Wilde, 2001). In general, these compounds can be associated to conifer groups but not to individual plant families (Otto and Simoneit, 2001; Tuo and Philp, 2005). It therefore seems that the relative proportions of saturated diterpenoids preserved in sediments can reflect the land plant palaeobiodiversity involved during the sedimentary rock deposition (e.g. Hautevelle et al., 2006).

1.2.2. Aromatic biomarkers

A significant part of the organic matter in terrestrial sediments is represented by aromatic hydrocarbon compounds (Tissot and Welte, 1984). These aromatic biomarkers can be summarized in the following groups:

1.2.2.1. Naphthalene and related compounds

Naphthalene **(15)** and its related compounds are generally ubiquitous constituents of sedimentary organic matter and are thought to be derived from terrestrial sources (Radke et al., 1994). Although some cyclic sesquiterpenoids from resinous constituents of conifers are potential precursors of alkylnaphthalenes (Pentegova et al., 1968), the methylated naphthalenes in sediments has been widely associated to sesqui- and triterpenoids derived from microbial and land-plant sources (Püttmann and Villar, 1987; Killops, 1991; Bastow et al., 1998; van Aarssen et al.; 1999; among others).

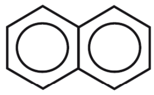
For instance, the aromatic biomarkers 1,6-dimethylnaphthalene **(16)** and 1,2,5-trimethylnaphthalene **(17)** are considered as indicators of terrestrial organic matter (e.g. Strachan et al., 1988; van Aarssen et al., 2000). Due to the fact that these aromatic biomarkers can also be formed through other processes, they cannot be associated to specific kind of land plants (e.g. van Aarssen et al., 2000). Some chemical structures of naphthalene and related compounds are shown in figure 1.8.

Cadalene **(18)** has long been recognized as a terrestrial biomarker for all vascular plants (van Aarssen et al., 2000; Hautevelle et al. 2006). Cadalene is thought to derive from cadinenes and cadinols which are ubiquitous in plants, bryophytes and fungi (Bordoloi et al., 1989). Cadinanes have been widely found in bryophytes (Chopra and Kumra, 1988; Asakawa, 2004), gymnosperm resins (Simoneit, 1986) and in particular conifer resins (Bordoloi et al., 1989; Otto and Wilde, 2001) and angiosperm resins (van Aarssen et al. 1990). However, the earliest reported occurrence of cadalene in sediments corresponds to Lower Carboniferous, specifically Visean age (Armstroff et al., 2006).

Few reports describe the occurrence of aromatic compounds with methylated-tetrahydronaphthalene skeletons (e.g. Achari et al., 1973; Wang and Simoneit, 1990). They are usually regarded as non-specific biomarkers for land plants, derived from sesquiterpenoids (Wang and Simoneit, 1990, van Aarssen et al., 2000).

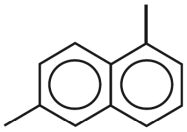
Ionene [1,1,6-trimethyltetralin or 1,2,3,4-tetrahydro-1,1,6-trimethyl-naphthalene **(19)**], 1,1,5,6-tetramethyltetralin **(20)** and pentamethylindane are the most common methylated-tetrahydronaphthalene aromatic compounds identified in sediments and brown coals (e.g. Wang and Simoneit, 1990). Ionene is probably derived from degraded and cyclized carotenoids which have generally been proposed to constitute sporopollenins derived from some land plant pollen (Wang and Simoneit, 1990). In addition, large amounts of ionene were particularly detected in pyrolysates of sporopollenins derived from some higher plant pollen and spore coal (Achari et al., 1973).

(15)



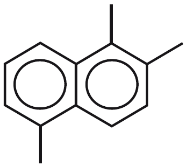
naphthalene

(16)



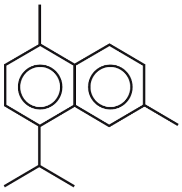
1,6-dimethylnaphthalene

(17)



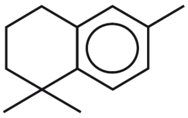
1,2,5-trimethylnaphthalene

(18)



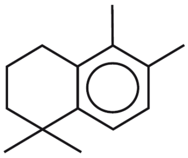
cadalene

(19)



ionene

(20)



1,1,5,6-tetramethyltetralin

Figure 1.8: Some chemical structures of naphthalene and its related compounds

1.2.2.2. Phenanthrene and related compounds

Phenanthrene **(21)** and most of the related compounds are observed in petroleum and sediment extracts. Among these alkylphenanthrenes, some isomers are mainly produced by the aromatisation of biological diterpenoids, containing an abietane and pimarane skeleton (Simoneit et al., 1986). These latter diterpenoids are typical constituents of ambers and resins present in vascular plants (Thomas, 1969; Simoneit et al., 1986). According to Radke et al.

(1998), phenanthrene **(21)** and methylphenanthrenes such as 1-methyl-phenanthrene **(22)** are generally abundant in organic solvent extracts of bituminous coals and sedimentary rocks containing a mixture of terrestrial and marine organic matter. The biomarker 1,7-dimethylphenanthrene **(23)** probably derives from aromatisation of diterpenoids with pimarane structure (Simoneit et al., 1986). However, it has been also considered as a potential decomposition product of retene **(24)** (Simoneit et al., 1986).

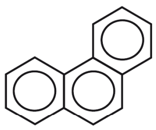
Retene [1-methyl-7-isopropylphenanthrene **(24)**] is the most studied land-plant derived aromatic hydrocarbon which has generally been considered as a conifer biomarker (e.g. van Aarssen et al., 2000). Retene and related aromatic hydrocarbons such as simonellite **(25)**, diaromatic totarene **(26)**, diaromatic sempervirane **(27)** and tetrahydroretene **(28)** occur in recent and ancient sediments (Otto et al., 1997; Otto and Simoneit, 2001; van Aarssen et al., 2000; Tuo and Philp, 2005; Hautevelle et al., 2006; among others).

Retene **(24)** has been identified in pine forest soils (Simoneit, 1986), Carboniferous coals (e.g. del Río et al., 1994; Fabianska et al., 2003; Armstroff et al., 2006), humic coals (Radke et al., 1982), nonmarine petroleum source rocks (Cheng, 1988), deep-sea sediments (Simoneit, 1977), Tertiary sediments and lignites (Simoneit et al., 1986; Wang and Simoneit, 1991) and Late Devonian (Frasnian) cannel coals (Armstroff et al., 2006). Nevertheless, retene has been also detected in sediments of pre-Devonian age (Zhang et al., 1999) and pyrolysates of algal and bacterial organic matter (Wen et al., 2000).

Retene **(24)**, simonellite **(25)** and tetrahydroretene **(28)** are frequently derived from the partial degradation of abietic acid and/or all abietane class bioditerpenoids which are the major constituents of conifer resins (Otto and Simoneit, 2001; Hautevelle et al., 2006). However, Alexander et al. (1987) demonstrated that the presence of retene **(24)** and simonellite **(25)** in Miocene coals can also derive from maturation of phyllocladanes **(9 – 10)** and kauranes **(11 – 12)**.

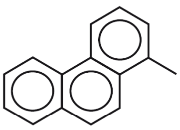
Bastow et al. (2001) detected a new aromatic diterpenoid derived biomarker, named 2-methylretene **(29)** in crude oils and rock extracts. These authors suggested that this biomarker is formed from the aromatisation of diterpenoid natural products with the abietane or phyllocladane skeleton, similar to retene. It could therefore be considered as a potential terrestrial mark in samples containing higher plant derived material. Figure 1.9 summarizes the typical structures of phenanthrene and related compounds.

(21)



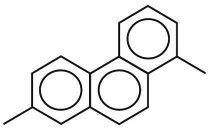
phenanthrene

(22)



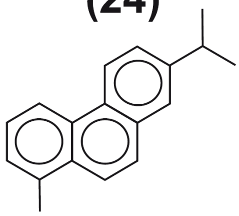
1-methyl-phenanthrene

(23)



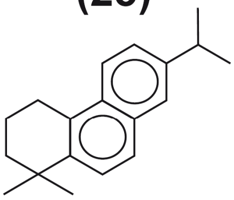
1,7-dimethylphenanthrene

(24)



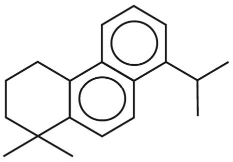
retene

(25)



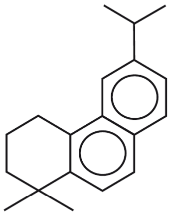
simonellite

(26)



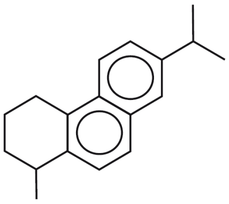
diaromatic totarene

(27)



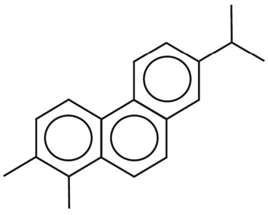
diaromatic sempervirane

(28)



tetrahydroretene

(29)

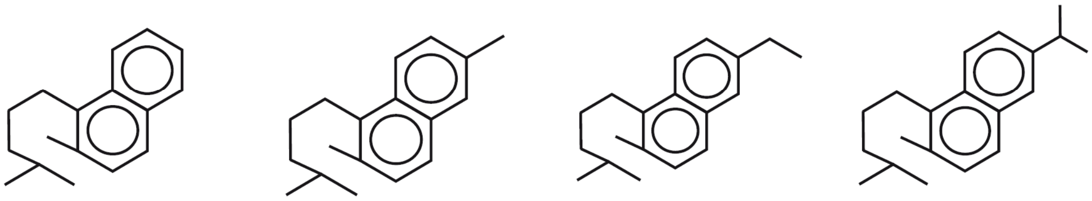


2-methyl-retene

Figure 1.9: Some chemical structures of phenanthrene and related compounds

Series of C_{17} to C_{20} isohexylalkylnaphthalenes **(30 – 33)** (Fig. 1.10) have been detected in crude oils of Permian to Tertiary age (Ellis et al., 1996). These classes of biomarkers frequently occur in conjunction with typical land plant-derived saturated biomarkers. It appears that isohexylalkylnaphthalenes are mainly derived from terrestrial terpenoid precursors by aromatisation of ring A opening (Ellis et al., 1996). Due to the presence of the isohexyl radical, it has been suggested that isohexylalkylnaphthalenes are more specific biomarkers for terrestrial input than 1-methyl-phenanthrene **(22)** and 1,7-dimethyl-phenanthrene **(23)** which can be randomly produced by typical aromatisation processes (Ellis et al., 1996).

(30) **(31)** **(32)** **(33)**



C_{17} iHMN C_{18} iHMN C_{19} iHMN C_{20} iHMN

Figure 1.10: Isohexylalkylnaphthalene (iHMN) structures

1.2.2.3. Alkyldibenzofurans

Dibenzofuran **(34)** and methyldibenzofuran isomers such as 4-methyl-dibenzofuran **(35)** and 1-methyl-dibenzofuran **(36)** are markers of terrestrial organic matter (Radke et al., 2000). The latter demonstrated the potential of this biomarker family as indicators of terrestrial organic matter independent of lithology. In particular, alkyldibenzofurans may provide evidence for the occurrence of lichens in ancient sediments (Radke et al., 2000). It appears that lichens are the sole organisms known to produce dibenzofuran derivatives under normal natural conditions. Dibenzofuran (DBF) and some methyldibenzofuran (MDBF) structures are given in figure 1.11.

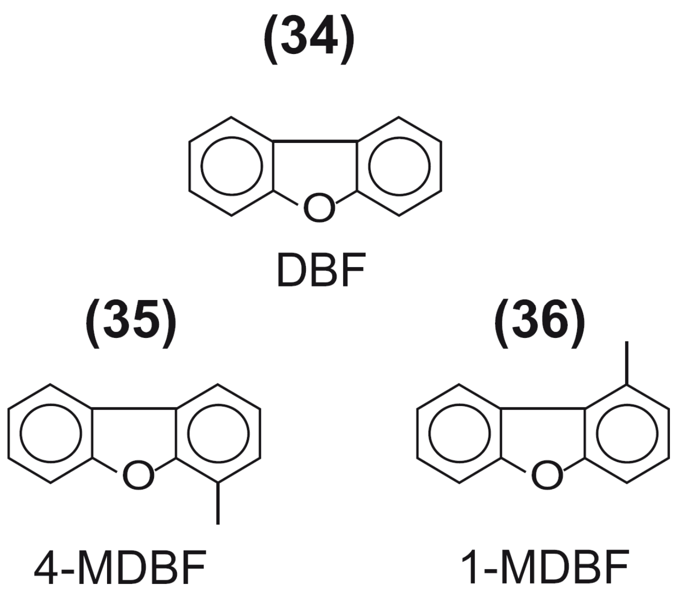


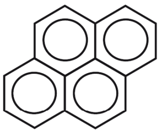
Figure 1.11: Dibenzofuran (DBF) and methyldibenzofuran (MDBF) structures

1.2.2.4. The combustion-derived polycyclic aromatic hydrocarbons

Although the combustion-derived polycyclic aromatic hydrocarbons (PAHs) are not specific land plant biomarkers, combustion of organic materials such as fossil fuels and plant remains has been proposed as the major source for these PAHs in sediments (e.g. Blumer, 1976; Laflamme and Hites, 1978; Jiang et al., 1998 and references therein).

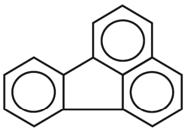
The combustion-derived PAHs are represented by pyrene **(37)**, fluoranthene **(38)**, benzo[a]anthracene **(39)**, benzo[b]fluoranthene **(40)**, benzo[k]-fluoranthene **(41)**, benzo[e]pyrene **(42)**, benzo[a]pyrene **(43)**, benzo[ghi]perylene **(44)** and coronene **(45)** (Jiang et al., 1998 and references therein). These compounds are produced by incomplete biomass burning during ancient vegetation fires (Killops and Massoud, 1992; Jiang et al., 1998; Hautevelle et al., 2006). They could be introduced into the organic matter during or before early diagenesis (Radke et al., 1982). The presence of combustion-derived PAHs in recent sediments is attributed to their sorption onto combustion-generated airborne particulate matter which is subsequently deposited into sediments (Blumer, 1976; Laflamme and Hites, 1978). Figure 1.12 shows some structures of the typical combustion-derived PAHs.

(37)



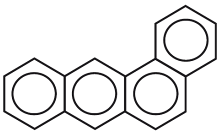
pyrene

(38)



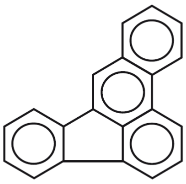
fluoranthene

(39)



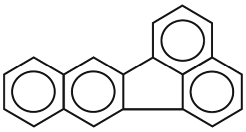
benzo[a]anthracene

(40)



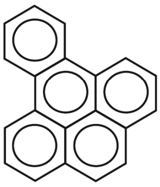
benzo[b]fluoranthene

(41)



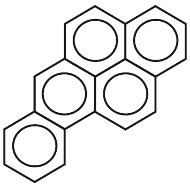
benzo[k]fluoranthene

(42)



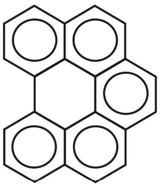
benzo[e]pyrene

(43)



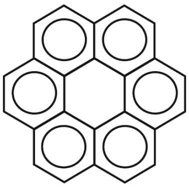
benzo[a]pyrene

(44)



benzo[gui]perylene

(45)



coronene

Figure 1.12: Examples structures of the combustion-derived polycyclic aromatic hydrocarbons

1.2.3. Summary

The frequency of occurrence of terrestrial terpenoid biomarkers associated to a land plant origin is summarized in table 1.1. The tabulated palaeobotanical classification is mainly divided into five families in evolved order: Bryophytes (mosses and liverworts), lycophytes (e.g. lycopsids), pteridophytes (e.g. ferns), conifers and angiosperms (flowering plants). Most of the biological precursors for these land plant biomarkers are widespread in conifers and angiosperms (Table 1.1). It appears that these groups of plants are the major source of terrestrial aliphatic and aromatic biomarkers in sediments. Few selected biomarkers have been reported to derive from bryophytes, lycophytes and pteridophytes (Table 1.1). Kaurane and cadalene are the more frequently traced biomarkers deriving from these plants (Table 1.1)

Table 1.1: Occurrence of the biological biomarker precursor in land plants

Legend: XXX widespread; XX common; X infrequent; - rare or unreported

	Type of plants [a]				
Biomarkers [b]	Bryophytes (e.g. mosses and liverworts)	Lycophytes (e.g. lycopsids)	Pteridophytes (e.g. ferns)	Conifers	Angiosperms (flowering plants)
4b(H)-eudesmane [1]	X	X	-	X X	X X X
Norabietane [2]	X	X	X	X X X	X X
Abietane [2]	-	-	-	X X X	X
Beyerane [3]	-	-	X	X X	X
Phyllocladane [3]	-	-	-	X X	-
Kaurane [3]	X X	X X	X X	X X X	X X X
Retene [4]	-	-	-	X X X	X X
Cadalene [5]	X X	X X	XX	X X X	X X
Simonellite [4]	-	-	-	X X X	X X
Totarane [2]	X	-	-	X X X	X X
Sempervirane [2]	-	-	-	X X X	X X
Tetrahydroretene [4]	-	-	-	X X X	X X
2-methyl-retene [4]	-	-	-	X X X	X X
Bisnorsimonellite [4]	-	-	-	X X X	X X

a. Palaeobotanical classification used in this work (Crane et al, 1995; Kenrick and Crane, 1997; Bowe et al., 2000; Schneider et al., 2004; Martone et al., 2009; Also in Fig. 1.1)

b. Compilation information from the following review articles:

1. Asakawa, 2004.

2. Pinto et al. (1995); Otto et al. (1997); Otto and Wilde (2001); Cox et al. (2007).

3. Noble (1986); Sheng et al. (1992); Disnar and Harouna (1994); among others.

4. van Aarssen et al. (2000); Bastow et al. (2001); Hautevelle et al. (2006); among others.

5. Chopra and Kumra (1988); van Aarssen et al. (2000); among others.

1.3. Scope and significance of the present study

Several aliphatic and aromatic biomarkers have been shown to provide more specific information on the evolution of major plant groups (e.g. Noble, 1986; Otto et al., 1997; Moldowan et al., 1994; van Aarssen et al. 2000, among others). As demonstrated by Hautevelle et al. (2006), vascular plant biomarkers can be considered as a chemostratigraphic tool in order to trace palaeoflora changes on emerged land. Biomarker research can therefore play an important role in helping understanding early land plant evolution.

Although the first microfossil evidence of embryophytes occupying land is from the Middle Ordovician (Strother et al., 1996) and the earliest occurrence of vascular plants is Middle Silurian (Edwards and Wellman, 2001), few land-plant biomarker studies have been successfully carried out in sediments or extracts from Palaeozoic plants older than Middle Devonian so far (e.g. Sheng et al., 1992; Ewbank et al., 1996). Consequently, it appears that there is a gap between the first accepted evidence of land plant (Middle Ordovician) and the earliest reported terrestrial biomarker (Middle Devonian).

Due to the large gap between molecular and fossil record of plants, the first aim of this work is to extend the biomarker record of land plants in the Palaeozoic, in particular, for the Late Ordovician to Early Carboniferous. The second objective is to relate the identified biomarkers to specific kinds of Palaeozoic plants. To this end, biomarker analyses are compared with available palynological data. The last objective is to use the biomarker record in the perspective of early land plant evolution during the Palaeozoic.

1.4. Description of investigated samples

A total of 31 Paleozoic samples from the Late Ordovician (Hirnantian) to the Early Carboniferous (Visean) were investigated. Most of them are core samples containing terrestrial and marine organic matter. Some coal samples are also considered in this work. One (1) sample is assigned to Late Ordovician period, twenty-one (21) are extended along to Silurian interval, five (5) to the Early Devonian and finally four (4) belong to the Early Carboniferous. These studied samples come from two locations: The Ghadamis basin, southern Tunisia (27 samples) and The Midland Valley of Scotland (4 samples).

1.4.1. Ghadamis Basin

The Ghadamis basin is associated to a large intracratonic synform on the North African Platform (Gondwana), and covers larges areas in northwestern Libya, southern Tunisia and easternmost Algeria (Fig. 1.13). It contains a thick sequence of Paleozoic strata which are only weakly affected by tectonic metamorphism, overlain by Mesozoic and Cenozoic sediments (Underdown and Redfern, 2008). Based on palaeogeographic reconstruction, North Africa was located at the margin of Gondwana in high southern palaeolatitudes (Fig. 1.14).

The Palaeozoic sequences of the Ghadamis Basin are well known as hosting some of the largest Palaeozoic-sourced oil field in North Africa (El-Arnauti, 1988). Stratigraphic palynology has constituted the primary tool for the dating and correlation of the Palaeozoic strata in the subsurface of the basin (Deunff and Massa, 1975; Molyneux, 1988; among others), and the extremely rich and well preserved palynological content of these sediments are also well suited for detailed taxonomic and evolutionary studies of marine microphytoplankton and miospores (Vecoli, 2000; Vecoli and Le Hérissé, 2004, Vecoli et al., 2009; Spina and Vecoli, 2009).

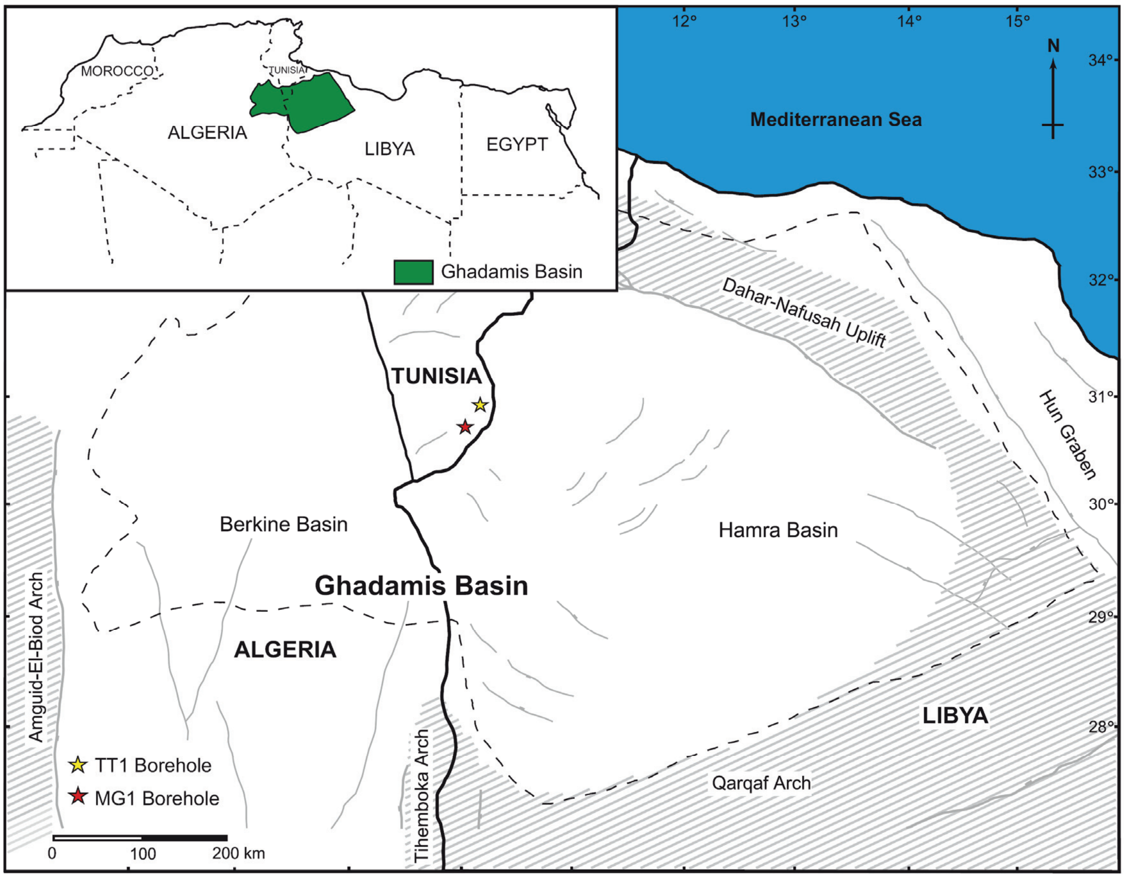


Figure 1.13: Geographic location of the Ghadamis Basin and boreholes TT1 and MG1 (modified from Underdown and Redfern, 2008)

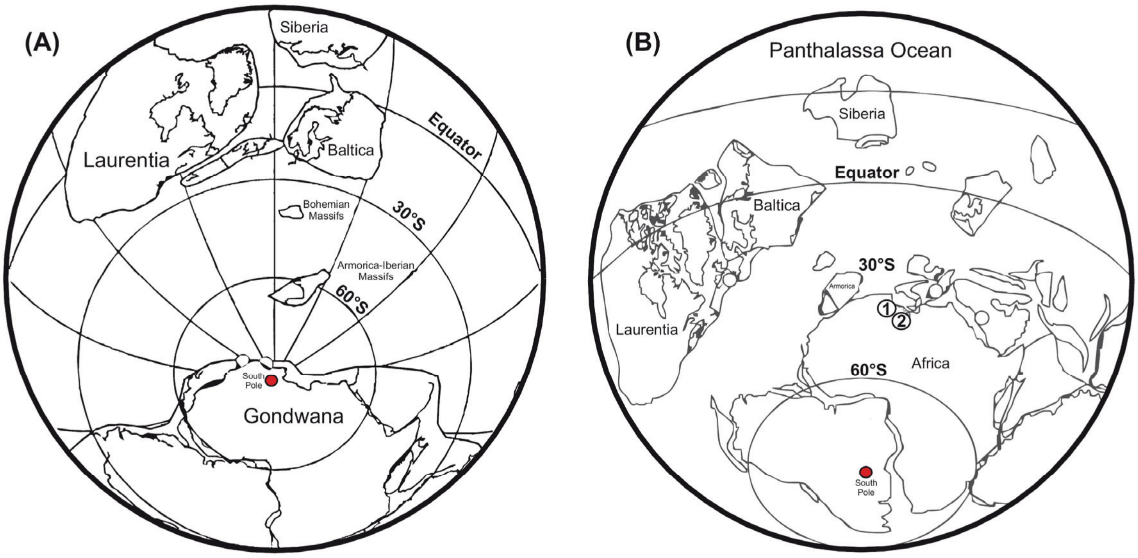


Figure 1.14: **(A)** Late Ordovician – Early Silurian palaeogeographic reconstruction (modified from Torsvik et al., 1996; Lüning et al., 2000); **(B)** Middle Silurian – Early Devonian palaeogeography (modified from Cocks and Torsvik, 2002; Spina and Vecoli, 2009). **(1)** Tunisia; **(2)** Libya

Twenty-seven samples come from two subsurface sections in southern Tunisia, North Africa (Fig. 1.13). Thirteen core samples were collected from an Ordovician – Silurian clastic sequence (borehole TT1, N 32° 18' 23"; E 10° 59' 40"). Fourteen samples were selected from a Siluro-Devonian succession penetrated by borehole MG1 (N 34° 72' 40"; E 8° 68' 00"). Both boreholes are located near to Tunisia – Libya borderline (Fig. 1.13). Table 1.2 refers to the geological setting of investigated samples from Ghadamis Basin. In addition, the photographic plates for borehole TT1 and MG1 are shown in appendices A.2 and A.3, respectively.

1.4.1.1. Borehole TT1

The TT1 borehole stratigraphy was described in detail by Massa (1988) and Vecoli et al. (2009). Samples from borehole TT1 cover the uppermost Ordovician to upper Silurian interval, occurring between -1313.6 m and -1239.0 m core depth (Fig. 1.15).

The lower part of the studied section (Fig. 1.15) consists of the latest Ordovician alternations of immature sandstones, siltstones and dark grey shales (Djeffara Formation). One sample (TT1-1313.6) was available from this interval (Fig. 1.15; Table 1.2).

An early to late Silurian silty-shaley succession formally named the Argiles Principales Formation takes place above Djeffara Formation. From the very base of the Argiles Principales Formation, one dark siltstone and two fine-grained sandstones of Rhuddanian (early Llandovery) age were collected for analysis: TT1-1300.8; TT1-1299.4 and TT1-1296.7; respectively (Fig. 1.15; Table 1.2).

Above the lower section of the Argiles Principales Formation, organic-rich black shales and shaley marls occur in between -1291.1 m and -1280.0 m core depth (Fig. 1.15; Table 1.2). These sediments represent the "hot shale" interval in the core section: samples TT1-1291.1; TT1-1286.9; TT1-1282.0 and TT1-1280.0 (Vecoli et al., 2009).

Finally, the interval between -1273.0 m and -1239.0 m consist of a succession of dark grey shale (sample TT1-1273.0) interbedded with dark grey silty shales (samples TT1-1267.0; TT1-1247.0; TT1-1243.0; TT1-1239.0). The uppermost dark grey silty-shaly interval ranging between -1247.0 m and -1239.0 m (three samples: TT1-1247.0; TT1-1243.0; TT1-1239.0; Fig. 1.15; Table 1.2) is late Ludlow (Ludfordian) in age (Vecoli et al., 2009).

Table 1.2: Geological setting of investigated samples from Ghadamis Basin

Samples	Systemes	Series	Stage	Formation	Lithology
MG1-2519.3	Devonian	Early	Lochkovian	Ouan Kasa	Grey sandstone
MG1-2635.0	Devonian	Early	Lochkovian	Ouan Kasa	Grey sandstone
MG1-2870.0	Devonian	Early	Lochkovian	Tadrart	Dark grey sandstone
MG1-2890.0	Devonian	Early	Lochkovian	Tadrart	Dark grey sandstone
MG1-2892.0	Devonian	Early	Lochkovian	Tadrart	Dark grey sandstone
MG1-2942.0	Silurian	Pridoli	Ludfordian	Acacus	Grey and green shale
MG1-3037.6	Silurian	Pridoli	Ludfordian	Acacus	Dark grey shale
MG1-3039.0	Silurian	Pridoli	Ludfordian	Acacus	Dark grey shale
MG1-3052.5	Silurian	Pridoli	Ludfordian	Acacus	Dark grey sandstone
MG1-3097.0	Silurian	Ludlow	Ludfordian	Acacus	Grey and green shale
MG1-3183.0	Silurian	Ludlow	Ludfordian	Acacus	Dark grey sandstone
MG1-3230.6	Silurian	Ludlow	Ludfordian	Acacus	Grey and green shale
TT1-1239.0	Silurian	Ludlow	Ludfordian	Argiles Principales	Dark grey silty shale
TT1-1243.0	Silurian	Ludlow	Ludfordian	Argiles Principales	Dark grey silty shale
TT1-1247.0	Silurian	Ludlow	Ludfordian	Argiles Principales	Dark grey silty shale
MG1-3710.3	Silurian	Wenlock	Homerian	Tanezzuft	Dark grey sandstone
MG1-3711.0	Silurian	Wenlock	Homerian	Tanezzuft	Grey sandstone
TT1-1267.0	Silurian	Wenlock	Homerian	Argiles Principales	Dark grey silty shale
TT1-1273.0	Silurian	Wenlock	Homerian	Argiles Principales	Dark grey shale
TT1-1280.0	Silurian	Wenlock	Sheinwoodian	Argiles Principales	Dark grey shale
TT1-1282.0	Silurian	Wenlock	Sheinwoodian	Argiles Principales	Dark grey shale
TT1-1286.9	Silurian	Wenlock	Sheinwoodian	Argiles Principales	Dark grey shale
TT1-1291.1	Silurian	Llandovery	Aeronian	Argiles Principales	Black shale
TT1-1296.7	Silurian	Llandovery	Rhuddanian	Argiles Principales	Grey sandstone
TT1-1299.4	Silurian	Llandovery	Rhuddanian	Argiles Principales	Fine-grained sandstone
TT1-1300.8	Silurian	Llandovery	Rhuddanian	Argiles Principales	Dark siltstone
TT1-1313.6	Ordovician	Late	Hirnantian	Djeffara	Laminated sandstone

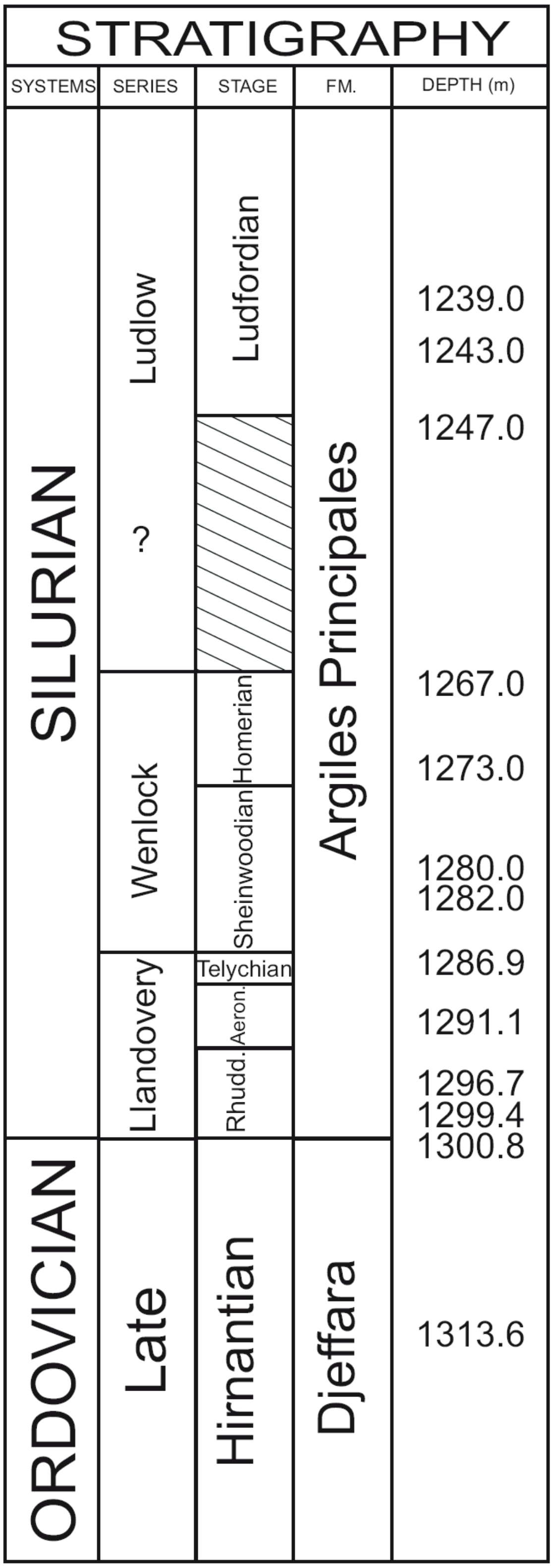


Figure 1.15: Stratigraphy and sampling levels in borehole TT1 (modified from Vecoli et al., 2009). Indicated depths correspond to the analysed samples

1.4.1.2. Borehole MG1

The investigated MG1 core section ranges between -3711.0 m and -2519.3 m core depth allows a relatively complete coverage of middle Silurian to lowermost Devonian interval (Fig. 1.16). The MG1 borehole (Fig. 1.16; Table 1.2) shows the vertical succession of Tanezzuft, Acacus, Tadrart and The Ouan Kasa Formations (Underdown and Redfern, 2008; Spina and Vecoli, 2009).

The early Silurian in MG1 section is characterized by a transgressive deposition of thick, laterally continuous marine mudstones and graptolite-rich micaceous shales, sometimes ferruginous with interbedded sandstone beds (Tanezzuft Formation, Fig. 1.16). Two dark and grey fine-grained sandstones of Homerian (Wenlock) age were collected from this formation: MG1-3711.0 and MG1-3710.3 (Fig. 1.16; Table 1.2).

The regression of the Silurian sea resulted in the deposition of late Silurian marine sandstones and mudstones of the Acacus Formation (Underdown and Redfern, 2008; Spina and Vecoli, 2009). Seven core samples from the Acacus Formation were available for analysis (Table 1.2). They are characterized by a succession of grey and green shales (samples MG1-3230.6; MG1-3097.0; MG1-3039.0; MG1-3037.6 and MG1-2942.0) interbedded with dark grey fine-to coarse-grained sandstones (samples MG1-3183.0 and MG1-3052.5).

The Caledonian unconformity separates the Silurian deposits from the overlying continental sandstones and mudstones of the Early Devonian Tadrart Formation. Three dark grey continental fine-grained sandstones of Lochkovian (Early Devonian) age were selected for analysis: MG1-2892.0; MG1-2890.0 and MG1-2870.0 (Fig. 1.16; Table 1.2).

Finally, the section ends with shallow-marine sandstones and mudstones of the Ouan Kasa Formation (Underdown and Redfern, 2008). From this interval, two grey sandstones of Lochkovian (Early Devonian) age were also chosen for analysis: MG1-2635.0 and MG1-2519.3 (Fig. 1.16; Table 1.2).

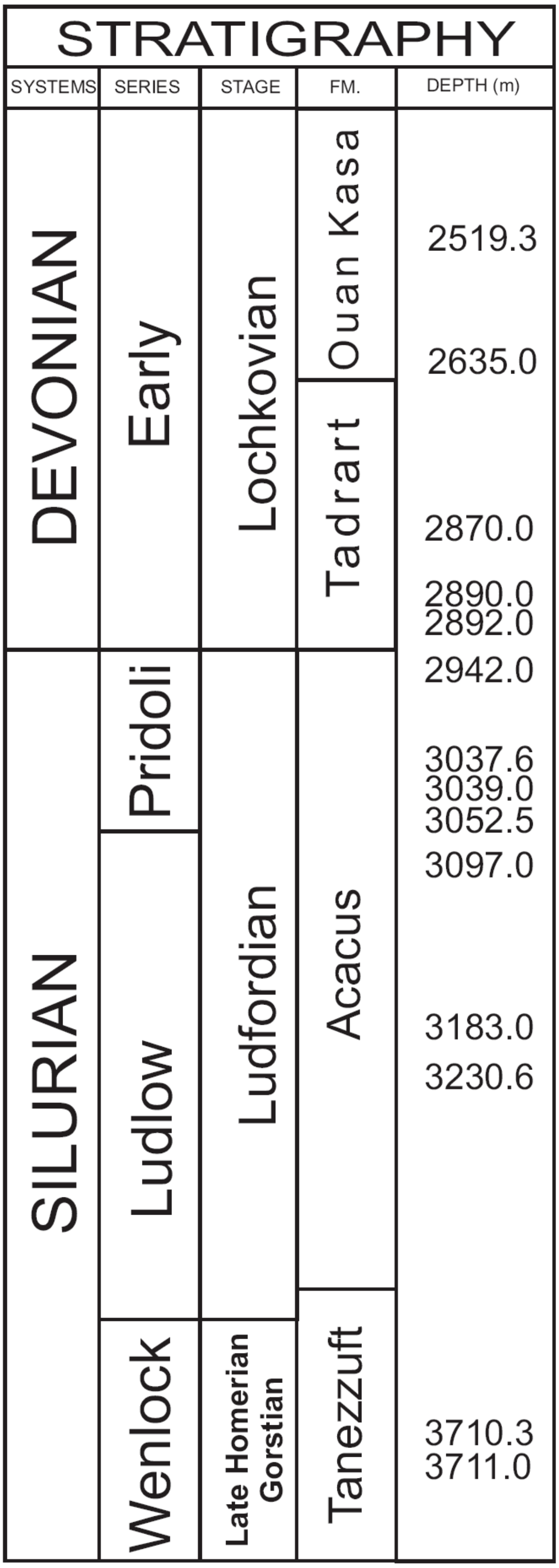


Figure 1.16: Stratigraphy and sampling levels in borehole MG1 (modified from Spina and Vecoli, 2009). Indicated depths correspond to the analysed samples

1.4.2. The Midland Valley of Scotland

The Midland Valley of Scotland is, in structural terms, a rift valley bounded by Highland Boundary and Southern Uplands Faults on the north and the south, respectively (Fig. 1.17; Murchison and Raymond, 1989; Underhill et al., 2008). This sedimentary basin evolved in response to crustal extension and especially contains Devonian to Carboniferous sediments and some igneous rocks (Murchison and Raymond, 1989; George, 1992; Underhill et al., 2008). During the Carboniferous (Early Mississippian – Visean), Scotland was located just at north of the equator in a position akin to central Africa (Fig. 1.18).

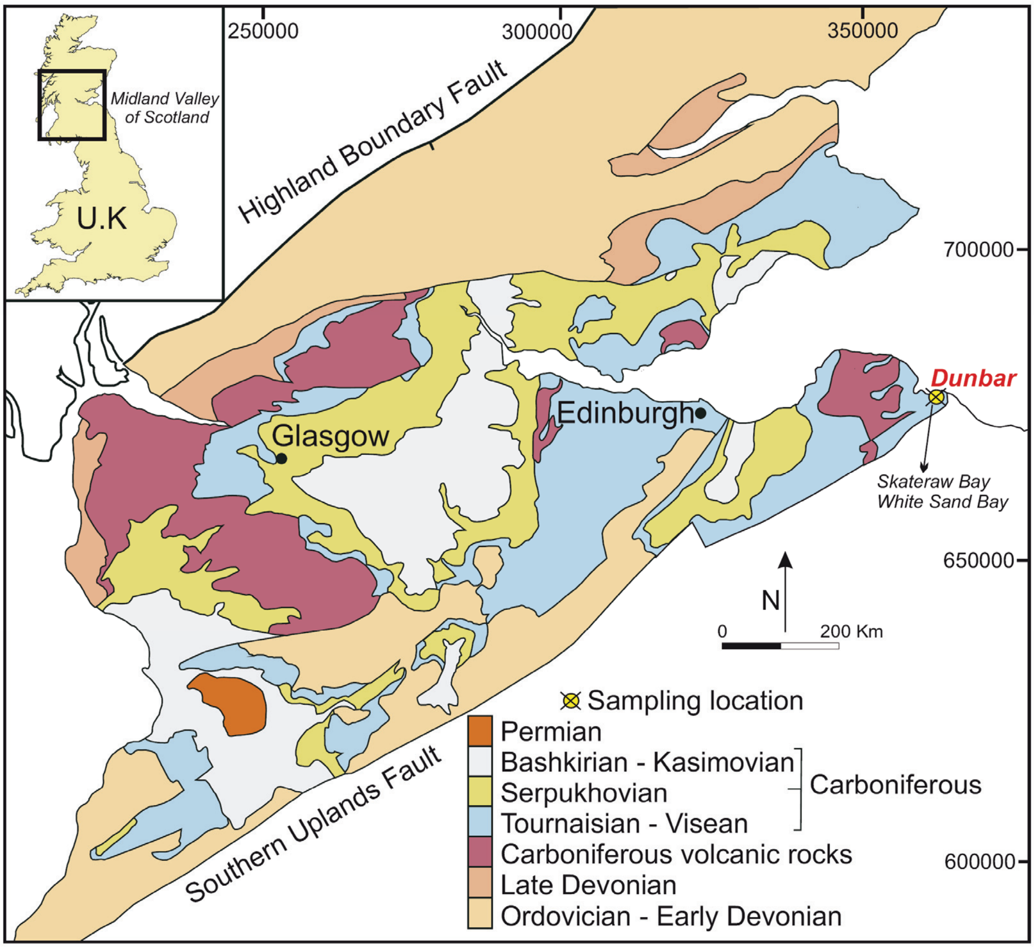


Figure 1.17: Simplified geologic map showing the outcrop sample positions at Dunbar (White Sand and Skateraw Bays) in the Midland Valley of Scotland (modified from Spinner and Clayton, 1973; Underhill et al., 2008)

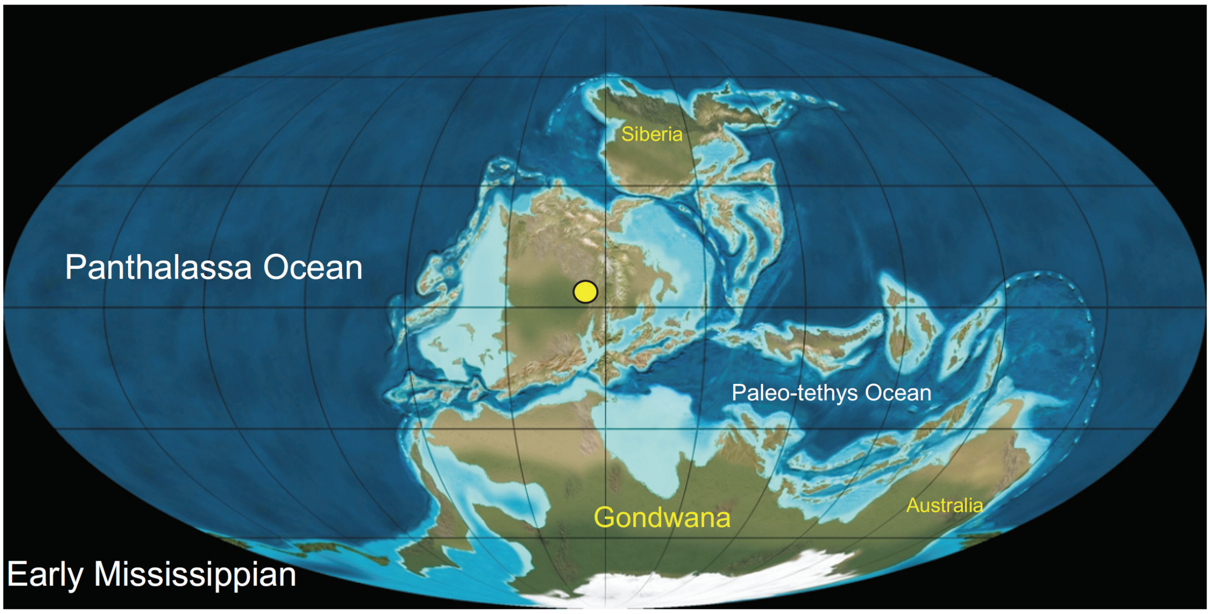


Figure 1.18: Early Carboniferous (Early Mississippian – Visean) palaeogeographic reconstruction showing the position of study area (yellow circle) (copyright figure to Ron Blakey, available online at http://jan.ucc.nau.edu/~rcb7/globaltext2.html)

The Carboniferous rocks in the Midland Valley of Scotland are only well exposed along coastlines (Murchison and Raymond, 1989). Coal samples were collected from two outcrop sections located at Dunbar, East Lothian, on the east coast of southern Scotland, some 40 km east of Edinburgh (Fig. 1.17). These sections show a group of alternating Lower Carboniferous (Visean) limestones, shales, sandstones and coals (Fig. 1.19; Spinner, 1969; Spinner and Clayton, 1973). Table 1.3 shows to the geological setting of investigated samples from The Midland Valley of Scotland. The photographic plates for Scottish coals are given in appendix A.4.

Table 1.3: Geological setting of investigated samples from The Midland Valley of Scotland

Samples	Systemes	Series	Stage	Formation	Lithology
SKT-E	Carboniferous	Mississippian	Visean	Lower Limestone	Brown coal
SKT-D	Carboniferous	Mississippian	Visean	Lower Limestone	Brown coal
WS-2	Carboniferous	Mississippian	Visean	Lower Limestone	Brown coal
WS-3	Carboniferous	Mississippian	Visean	Lower Limestone	Brown coal

SKT coal samples were collected around the bay containing Skateraw Harbour, approximately 6.4 km south-east of Dunbar, whereas WS coals were taken from rock successions exposed in White Sand Bay (Fig. 1.17). Both localities are clearly indicated on the geological map permanently exhibited just above the shoreline.

In stratigraphic order, the lower seam (WS) is located immediately above the Middle Longcraig Limestone (Fig. 1.19); the upper one occurs stratigraphically below the Chapel Point Limestone (Fig. 1.19).

WS samples are equivalent to the Longcraig coal seam described by Spinner (1969). In contrast, SKT coals are more comparable to the sample horizon SC2 detailed by Spinner and Clayton (1973).

In order to investigate the possible vertical stratigraphic variations, two samples were obtained from each coal interval (Fig. 1.19). Accordingly, samples WS-2 and WS-3 are separated by approximately 30 cm while SKT-E and SKT-D samples by 15 cm, respectively.

A fluvio-deltaic depositional environment has been assigned to these Carboniferous coals which contain mainly land-plant derived organic matter (George, 1992). After deposition, these shallow-water deltaic deposits were mainly influenced by burial history and extensive volcanic, sill and dyke activities (Murchison and Raymond, 1989).

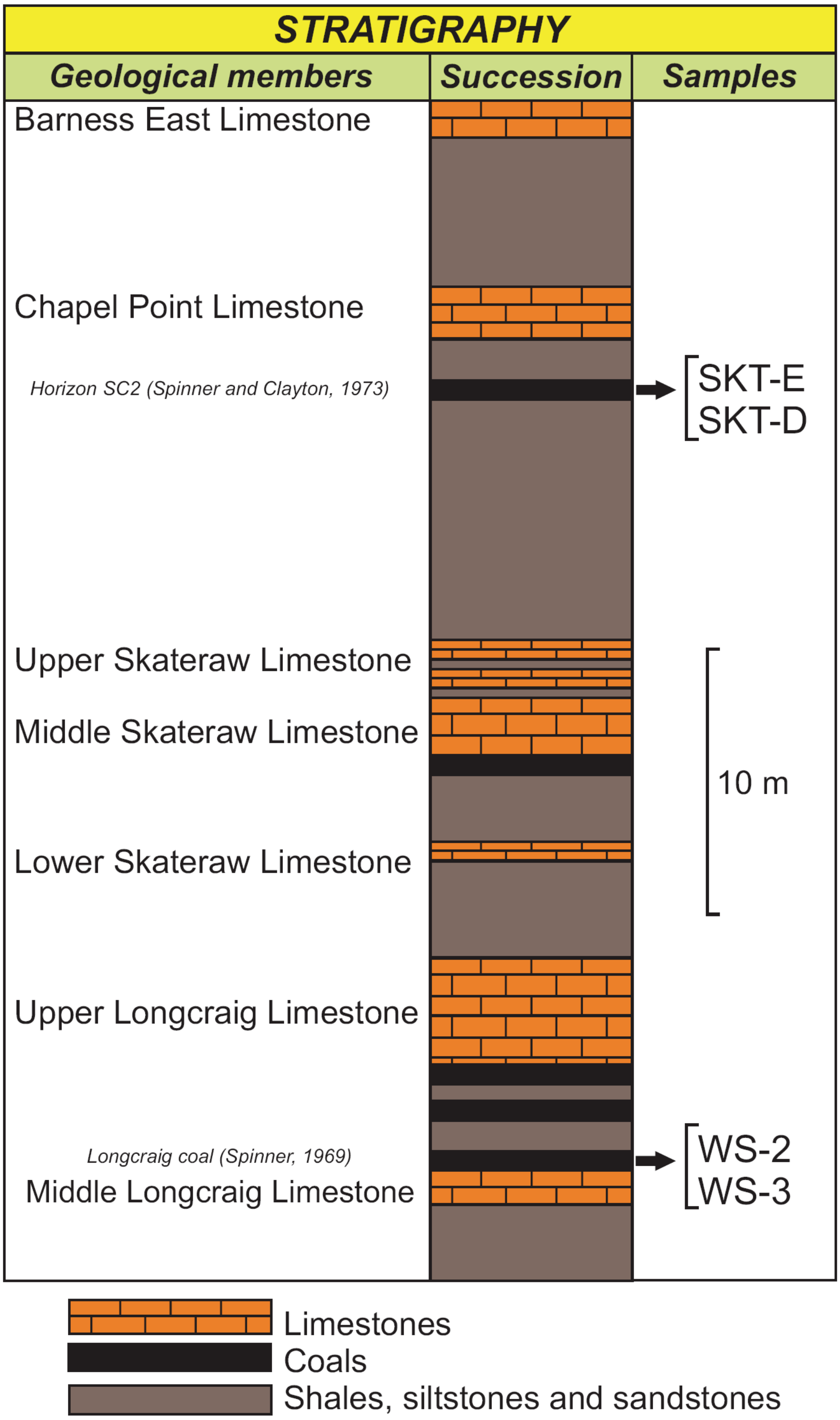


Figure 1.19: The outcrop geological succession showing alternating Carboniferous limestones, shales, sandstones and coals (modified from Spinner and Clayton, 1973)

1.4.3. Palynology

Palynology was not part of this thesis, however, previous studies were considered to correlate the detected biomarkers in samples with their palynomorph content. Accordingly, palynomorph assemblages were previously described by Spinner (1969); Spinner and Clayton (1973); Vecoli et al. (2009) and Spina and Vecoli (2009). Table 1.4 summarizes the palynomorph content of investigated samples.

Table 1.4: Palynomorph assemblages in the investigated samples

Samples	Age	Palynomorphs
SKT-E	Carboniferous	Miospore and megaspore assemblages[(a)]
SKT-D	Carboniferous	Miospore and megaspore assemblages[(a)]
WS-2	Carboniferous	Miospore and megaspore assemblages[(a)]
WS-3	Carboniferous	Miospore and megaspore assemblages[(a)]
MG1-2519.3	Devonian	**N. R**
MG1-2635.0	Devonian	**N. R**
MG1-2870.0	Devonian	Trilete spores and cryptospores[(b)]
MG1-2890.0	Devonian	Trilete spores, cryptospores and acritarchs[(b)]
MG1-2892.0	Devonian	Trilete spores, cryptospores and acritarchs[(b)]
MG1-2942.0	Silurian	Trilete spores, cryptospores and acritarchs[(b)]
MG1-3037.6	Silurian	Trilete spores, acritarchs, cryptospores and algae[(b)]
MG1-3039.0	Silurian	Trilete spores and cryptospores[(b)]
MG1-3052.5	Silurian	Trilete spores and cryptospores[(b)]
MG1-3097.0	Silurian	Algae, trilete spores, cryptospores and acritarchs[(b)]
MG1-3183.0	Silurian	Algae, trilete spores, acritarchs, chitinozoans and cryptospores[(b)]
MG1-3230.6	Silurian	Cryptospores, acritarchs, algae, trilete spores and chitinozoans[(b)]
TT1-1239.0	Silurian	Cryptospores, trilete spores, acritarchs and chitinozoans[(c)]
TT1-1243.0	Silurian	Cryptospores, trilete spores, acritarchs and chitinozoans[(c)]
TT1-1247.0	Silurian	Cryptospores, trilete spores, acritarchs and chitinozoans[(c)]
MG1-3710.3	Silurian	Cryptospores, trilete spores and acritarchs[(b)]
MG1-3711.0	Silurian	Cryptospores, trilete spores and acritarchs[(b)]
TT1-1267.0	Silurian	Acritarchs, prasinophytes, chitinozoans and miospores[(c)]
TT1-1273.0	Silurian	Acritarchs, prasinophytes and chitinozoans[(c)]
TT1-1280.0	Silurian	Acritarchs, prasinophytes and chitinozoans[(c)]
TT1-1282.0	Silurian	Acritarchs, prasinophytes and chitinozoans[(c)]
TT1-1286.9	Silurian	Acritarchs, prasinophytes and chitinozoans[(c)]
TT1-1291.1	Silurian	Acritarchs, prasinophytes and chitinozoans[(c)]
TT1-1296.7	Silurian	Prasinophytes, acritarchs and chitinozoans[(c)]
TT1-1299.4	Silurian	Prasinophytes, acritarchs and chitinozoans[(c)]
TT1-1300.8	Silurian	Acritarchs, chitinozoans and rare cryptospores[(c)]
TT1-1313.6	Ordovician	Acritarchs, chitinozoans and rare cryptospores[(c)]

Note: **(a)** Spinner (1969); Spinner and Clayton (1973)
(b) Spina and Vecoli (2009)
(c) Vecoli et al. (2009)
(N.R) – not reported.

The palynomorph content in Hirnantian (Late Ordovician) sample from Djeffara Formation (TT1-1313.6) is dominated by abundant acritarchs, chitinozoans and prasinophytes (Table 1.4). However, cryptospores also occur in very low abundance (Vecoli et al., 2009).

An increase in the abundance of *prasinophyceae*–derived microfossils (*Tasmanites*, *Leiosphaeridia* and *Cymatiosphaera*) occurs at the base of the Argiles Principales Formation (Lower – Middle Silurian), starting from sample TT1-1300.8 to sample TT1-1267.0 (Table 1.4; Vecoli et al., 2009). Acritarchs are also relatively abundant, suggesting conditions of normal marine platform environment throughout this interval (Vecoli et al., 2009).

The upper Silurian TT1 interval between samples at 1247.0 m and 1239.0 m (Argiles Principales Formation) is marked by the abundant occurrence of land-derived organic debris and terrestrial palynomorphs (Table 1.4; Vecoli et al., 2009).

The Silurian (Wenlock) core samples from Tanezzuft Formation (MG1-3711.0 and MG1-3710.3) are characterized by abundant and well preserved cryptospores and trilete spores which are less abundant than cryptospores (Table 1.4; Spina and Vecoli, 2009).

The Silurian (Ludlow – Pridoli) core samples from Acacus Formation (MG1-3230.6; MG1-3183.0; MG1-3097.0; MG1-3052.0; MG1-3039.0; MG1-3037.0 and MG1-2942.0) show a notable diversity of trilete spores, occurring in greater abundance than cryptospores (Table 1.4; Spina and Vecoli, 2009).

In general, trilete spores from these Silurian sediments are dominated by *Ambitisporites avitus*, *Retusotriletes warringtoni*, *Synosisporites verracatus*, among others (Vecoli et al., 2009; Spina and Vecoli, 2009).

Finally, the core samples from Tadrart Formation (MG1-2892.0 and MG1-2890.0) are characterized by the abundance of the trilete spore *Streelispora newportensis* (Spina and Vecoli, 2009).

Palynomorphs content of both TT1 and MG1 cores therefore demonstrates a marine contribution to the organic matter with a marked abundant of terrestrial palynomorphs from upper Silurian to lower Devonian sediments (Vecoli et al., 2009; Spina and Vecoli, 2009).

The Scottish outcrop successions were previously studied for palynological investigations by Spinner (1969) and Spinner and Clayton (1973). WS coal interval is characterized by abundant and well preserved megaspore such as: *Zonalesporites fusinatus*, *Lagenicula subpilosa* and *Setosisporites* (Spinner, 1969). Miospores are less abundant in this lower interval and are dominated by the following taxa: *Lycospora pusilla*, *Calamospora*, *Densosporites* (Spinner, 1969).

All megaspore specimens recognized in WS horizon (e.g. *Lagenicula subpilosa*, *Setosisporites* and *Zonalesporites fusinatus*) range through the SKT coal interval but the genus *Zonalesporites* is less abundant (Spinner and Clayton, 1973). This upper interval also shows a notable diversity of miospores represented by *Lycospora pusilla*, *Calamospora*, *Densosporites* and *Cingulizonates* (Spinner and Clayton, 1973).

1.5. Analytical Methods

1.5.1. Experimental procedures

1.5.1.1. Rock-Eval analysis

The Rock-Eval analyses for borehole TT1 were made by Vecoli et al. (2009). For MG1 core samples, Rock-Eval pyrolysis was performed on 100 mg of ground rock with a Turbo model RE6 pyrolyzer (Vinci Technologies) at the Institut Français du Pétrole (IFP) using the conventional temperature program described in Lafargue et al. (1998): the pyrolysis oven temperature was programmed with 25°/min from 300°C (held 3 min) to 650°C (held 3 min).

For Scottish samples, Rock-Eval pyrolysis was performed on 100mg of ground rock with an Oil Show Analyser device at the University of Paris 6, France; using the conventional temperature program described in Espitalié et al. (1986). The pyrolysis oven temperature was programmed with 25°/min from 300°C (held 3 min) to 650°C (held 3 min). However, due to the poor estimation of the total organic carbon (TOC) content of coals by Rock-Eval analysis (Espitalié et al, 1986), their TOC content was additionally determined on 100 mg of powdered decarbonated sample using a LECO carbon analyser at the same university (Paris 6, France) The hydrogen index (HI) was calculated using the Rock-Eval S_2 and the LECO TOC values.

1.5.1.2. Biomarker analyses

For biomarker analyses, rock fragments were extracted with dichloromethane (DCM) during 24 h in the refrigerator, in order to remove possible contamination on the sample surface. After this first extraction, the rock fragments were crushed to enable extraction of the lipids preserved inside the rock.

Approximately 30 g of pulverised samples were extracted with a mixture of methanol (MeOH) and dichloromethane (DCM) (1/2, v/v) for 24 h with extensive stirring. This second extract was dried by means of roto-evaporation and partly re-solubilized in cyclohexane.

The cyclohexane-soluble fraction (maltenes) was further separated by column chromatography. The apolar fraction was recovered from the maltenes by elution with cyclohexane on an activated silica column. Subsequent elution with a mixture of cyclohexane - DCM (2/1, v/v) recovered the aromatic fraction after which the polar fraction was recovered by elution with a mixture DCM – MeOH (2/1, v/v). The elemental sulphur present in some TT1 extracts was removed using activated copper.

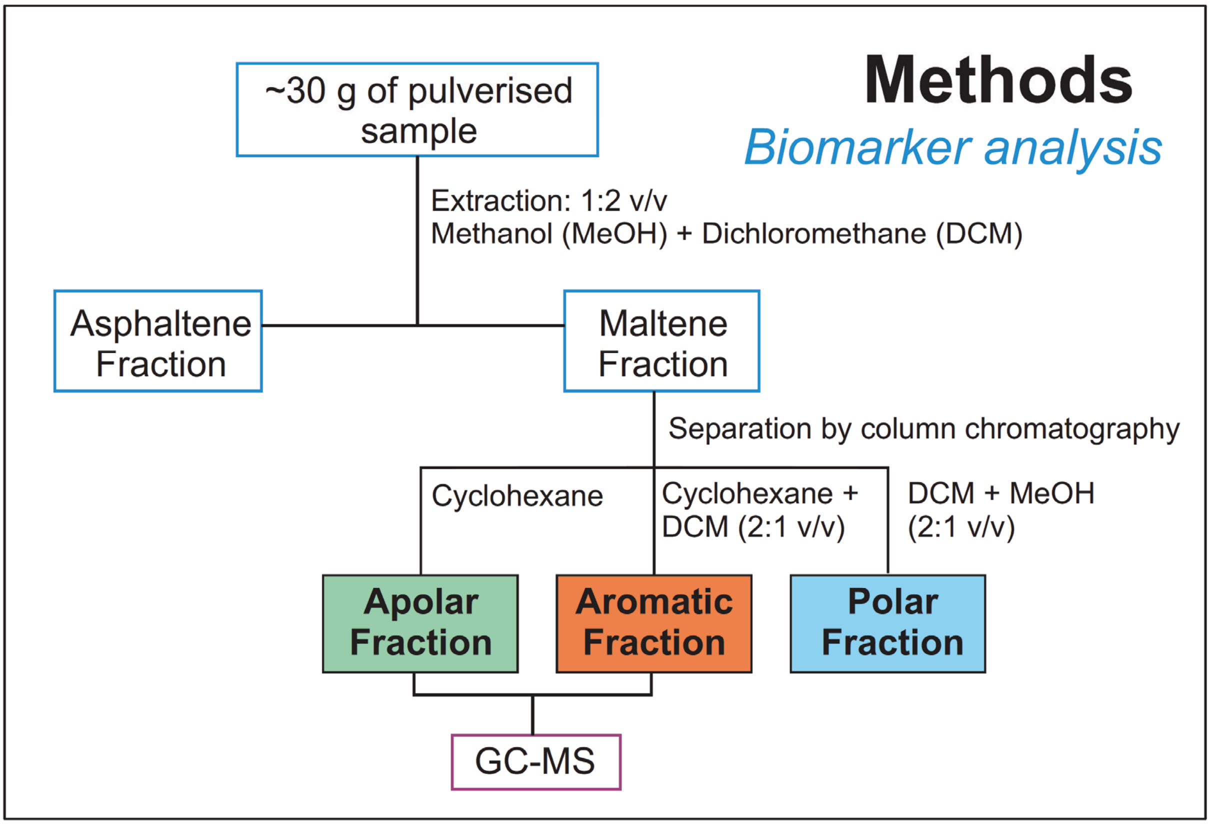


Figure 1.20: Experimental procedures applied for biomarker analyses in this work

1.5.1.3. Gas chromatography – mass spectrometry (GC-MS)

As shown in Figure 1.20, the aliphatic and aromatic fractions were analysed by gas chromatography – mass spectrometry (GC-MS) at the University of Bremen, using a Trace GC 2000 gas chromatograph and a DB5ht column (30 m length, 0.25 mm internal diameter, 0.1 µm film thickness).

The oven temperature was programmed as follows 100 °C for 1 minute, 100 °C – 310°C at a rate of 4 °C/min followed by an isothermal period of 16.5 min at 310 °C. Helium was used as carrier gas. The mass spectrometer was operated in the EI mode at electron energy of 70 eV. Full scan mass spectra were recorded over the mass range of 50 - 700Da at a scan rate of 1000 amu/s and an inter scan time of 0.67 s resulting in a scan cycle time of 0.5 s. The organic compounds were identified by comparison of their mass spectra and retention times with available published data.

1.6. References

Achari, R.G., Shaw, G., Holleyhead, R., 1973. Identification of ionene and other carotenoid degradation products from the pyrolysis of sporopollenins derived from some pollen exines, a spore coal and the Green River shale. Chemical Geology 12, 229 – 234.

Alexander, R., Kagi, R. I, Noble, R., Volkman, J., 1984. Identification of some bicyclic alkanes in petroleum. Organic Geochemistry 6, 63 – 70.

Alexander, G., Hazai, I., Grimalt, J., Albaigés, J., 1987. Occurrence and transformation of phyllocladanes in brown coals from Nograd Basin, Hungary. Geochimica et Cosmochimica Acta 51, 2065 – 2073.

Algeo, T.J., Scheckler, S.E., Maynard, J.B., 2001. Effects of the middle to late Devonian spread of vascular plants on weathering regimes, marine biotas, and global climate. In: Gensel , P., Edwards, D. (eds.): Plants invade the land. Columbia University Press, New York, 213 – 236.

Aplin, R. T., Cambie, R. C., Rutledge, P. S., 1963. The taxonomic distribution of some diterpene hydrocarbons. Phytochemistry 2, 205 – 214.

Armstroff, A., Wilkes, H., Schwarzbauer, J., Littke, R., Horsfield, B., 2006. Aromatic hydrocarbon biomarkers in terrestrial organic matter of Devonian to Permian age. Palaeogeography, Palaeoclimatology, Palaeoecology, 240, 253 – 274.

Asakawa, Y., 1982. Chemical constituents of the Hepaticeae. In: Herz, W., H., Grisebach, Kirby, G.W. (eds.): Progress in the Chemistry of Organic Natural Products 42, 15 – 126.

Asakawa, Y., 2001. Recent advances in phytochemistry of bryophytes-acetogenins, terpenoids and bis(bibenzyl)s from selected Japanese, Taiwanese, New Zealand, Argentinean and European liverworts. Phytochemistry 56, 297 – 312.

Asakawa, Y., 2004. Chemosystematics of the Hepaticae. Phytochemistry 65, 623 – 669.

Bastow, T.P., Alexander, R., Sosrowidjojo, I. B., Kagi, R.I, 1998. Pentamethylnaphthalenes and related compounds in sedimentary organic matter. Organic Geochemistry 28, 585 – 595.

Bastow, T.P., Singh, R., van Aarssen, B.K.G., Alexander, R., Kagi, R.I., 2001. 2-methylretene in sedimentary material: a new higher plant biomarker. Organic Geochemistry 32: 1211 – 1217.

Berner, R.A., 2001. The effect of the rise of land plants on atmospheric CO2 during the Paleozoic. In: Gensel and Edwards (eds.): Plants invade the land. Columbia University Press, New York, 173 – 178.

Blumer, M., 1976. Polycyclic aromatic compounds in nature. Scientifc America 234, 35 – 45.

Bordoloi, M., Shukla, V.S., Nath, S.C., Sharma, R.P., 1989. Naturally occurring cadinenes. Phytochemistry 28, 2007 – 2037.

Bowe, L.M., Coat, G., dePamphilis, C.W., 2000. Phylogeny of seed plants based on all three genomic compartments: Extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. Proceedings of the National Academy of Sciences USA 97, 4092 – 4097.

Caldicott, A. B. and Eglinton, G., 1973. Surface waxes. In Phytochemistry, III Inorganic Elements and Special Groups of Chemicals. Van Nostrand-Reinhold, New York, ed. L. P. Miller, 162 – 194.

Chaloner, W.G., 1967. Spores and land plant evolution. Review of Palaeobotany and Palynology 1, 23 – 93.

Chaw, S.-M., Parkinson, C. L., Cheng, Y., Vincent, T. M., Palmer, J. D., 2000. Seed plant phylogeny inferred from all three plant genomes: Monophyly of extant gymnosperms and origin of gnetales from conifers. Proceedings of the National Academy of Sciences 97, 4086 – 4091.

Cheng, Z.D., 1988. Retene and pimanthrene from continental source rocks in China. In: Yen, T.F., Moldowan, J.M. (eds.), Geochemical Biomarkers. Harwood Academic Press, New York, 203 – 220.

Crane, P. R., E. M. Friis, and K.R. Pedersen., 1995. The origin and early diversification of angiosperms. Nature 374, 27 – 33.

Chopra, R.N., Kumra, K.P., 1988. Biology of Bryophytes. John Wiley & Sons, New York. 350 p.

Cocks, L.R.M., Torsvik, T.H., 2002. Earth geography from 500 to 400 million years ago: a faunal and palaeomagnetic review. Journal of the Geological Society of London 159, 631 – 644.

Collinson M.E., van Bergen P.F., Scott A.C. and de Leeuw J.W., 1994. The oil-generating potential of plants from coal and coal-bearing strata through time: a review with new evidence from Carboniferous plants. In: Coal and Coal-bearing Strata as Oil-prone Source Rocks? (eds, Scott A.C. and Fleet A.J.). Geological Society Special Publication 77, 31 – 70.

Cox, R.E., Yamamoto, S., Otto, A., Simoneit, B.R.T., 2007. Oxygenated di- and tricyclic diterpenoids of southern hemisphere conifers. Biochemical Systematics and Ecology 35, 342 – 362.

Cross, A. T., Phillips, T. L., 1990. Coal-forming through time in North America. International Journal of Coal Geology 16, 1 – 46.

del Río, J. C., Garcia-Molla, J., González-Vila, F. J., Martin, F. 1994. Composition and origin of the aliphatic extractable hydrocarbons in the Puertollano (Spain) oil shale. Organic Geochemistry, 21, 897 – 909.

Deunff, J., Massa, D., 1975. Palynologie et stratigraphie du Cambro-Ordovicien (Libye nord-occidentale). Comptes Rendus de l'Académie des Sciences Paris. Série D 281, 21 – 24.

Disnar, J.R., Harouna, M., 1994. Biological origin of tetra-cyclic diterpanes, *n*-alkanes and other biomarkers found in Lower Carboniferous Gondwana coals (Niger). Organic Geochemistry 21, 143 – 152.

Dutkiewicz, A., George, S. C., Mossman, D. J., Ridley, J., Volk, H., 2007. Oil and its biomarkers associated with the Palaeoproterozoic Oklo natural fission reactors, Gabon. Chemical Geology, 244, 130 – 154.

Dzou, L. I. P., Noble, R. A., Senflte, J. T., 1995. Maturation effects on absolute biomarker concentrations in a suite of coals and associated vitrinite concentrates. Organic Geochemistry 23, 681 – 697.

Edwards, D., Richardson, J.B., 1996. Review of in situ spores in early land plants. In: Jansonius, J., McGregor, D.C. (Eds.), Palynology: Principles and Applications. American Association of Stratigraphic Palynologists Foundation, Vol. 2, 391 – 408.

Edwards, D., Wellman, C. H., 2001. Embryophytes on land: The Ordovician to Lochkovian (Lower Devonian) record. In: Gensel, P. G. and Edwards, D. (eds). Plants invade the land: Evolutionary and environmental perspectives. Columbia University Press, New York. 3 – 28.

Eglinton, G., Calvin, M., 1967. Chemical fossils. Scientific American 216, 32 – 43.

Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. Science 156, 1322 – 1335.

El-Arnauti, A., Owens, B, Thusu, B. (eds.), 1988. Subsurface palynostratigraphy of northeast Libya. Garyounis University Publishing, Benghazi, Libya, 45 – 59.

Ellis, L., Singh, R., Alexander, R., Kagi, R.I., 1996. Formation of isohexyl alkylaromatic hydrocarbons from aromatization-rearrangement of terpenoids in the sedimentary environment: A new class of biomarker. Geochimica and Cosmochimica Acta 60, 4747 – 4763.

Espitalié, J., Deroo, G., Marquis, F., 1986. La pyrolyse Rock-Eval et ses applications. Revue de l'Institut Français du Pétrole 41, 73 – 89.

Ewbank, G., Edwards, D., Abbott, G., 1996. Chemical characterization of Lower Devonian vascular plants. Organic Geochemistry 25, 461 – 473.

Fabianska, M. J., Bzowska, G., Matuszewska, A., Racka, A., Skret, U., 2003. Gas chromatography-mass spectrometry in geochemical investigation of organic matter of the Grodziec Beds (Upper Carboniferous), Upper Silesian Coal Basin, Poland. Geochemistry 63, 63 – 91.

Fanning, U., Richardson, J.B., Edwards, D., 1991. A review of in situ spores in Silurian land plants. In: Blackmore, S., Barnes, S.H. (Eds.), Pollen and Spores: Patterns of Diversification. Systematics Association Special 44. Clarendon Press, Oxford, 25 – 47.

Fleck, S., Michels, R., Izart, A., Elie, M., Landais, P., 2001. Palaeoenvironmental assessment of Westphalian fluvio–lacustrine deposits of Lorraine (France) using a combination of organic geochemistry and sedimentology. International Journal of Coal Geology 48, 65 – 88.

Fleck, S., Michels, R., Ferry, S., Malartre, F., Elion, P., Landais, P., 2002. Organic geochemistry in a sequence stratigraphic framework. The siliciclastic shelf environment of Cretaceous series, SE France. Organic Geochemistry 33, 1533 – 1557.

Gensel, P.G., 1992. Phylogenetic relationships of the *zosterophylls* and *lycopsids*: evidence from morphology, paleoecology and cladistic methods of inference. Annals of the Missouri Botanical Garden 79, 450 – 473.

Gensel, P. G., Andrews, H. N., 1987. The evolution of early land plants. American Scientist 75, 478 – 488.

George, S.C., 1992. Effect of igneous intrusion on the organic geochemistry of a siltstone and an oil shale horizon in the Midland Valley of Scotland. Organic Geochemistry 18, 705 – 723.

Gerrienne, P., Meyer-Berthaud, B., Fairon-Demaret, M., Streel, M., Steemans, P. 2004. Runcaria, a middle Devonian seed plant precursor. Science 306, 856 – 858.

Graham, L. E., 1985. The origin of the life cycle of land plants. American Scientist 73, 178 – 186.

Gray, J., 1985. The microfossil record of early land plants; advances in understanding of early terrestrialization, 1970-1984. *In*: Chaloner, W. G. & Lawson, J. D. (eds) Evolution and environment in the Late Silurian and Early Devonian. Philosophical Transactions of the Royal Society, London, B 309, 167 – 195.

Gray, J. 1991. *Tetrahedraletes, Nodospora* and the 'cross' tetrad: an accretion of myth. *In*: Blackmore, S. & Barnes, S. (eds) Pollen and spores: Patterns of diversification. The Systematics Association Special Volume Clarendon Press, Oxford, 44, 49-87.

Gray, J., Shear, W., 1992. Early life on land. American Scientist 80, 444 – 456.

Gray, J., 1993. Major Palaeozoic land plant evolutionary bio-events. Palaeogeography, Palaeoclimatology, Palaeoecology 104, 153 – 169.

Hart, J., 1987. A cladistic analysis of conifers: preliminary result. Journal of the Arnold Arboretum 68, 269 – 307.

Hartgers, W. A., Sinninghe Damsté, J. S., de Leeuw, J. W., Ling, Y., Dyrkacz, G., 1994. Molecular characterization of flash pyrolysates of two Carboniferous coals and their constituting maceral fractions. Energy & Fuels 8, 1055 – 1067.

Hautevelle, Y., Michels, R., Malartre, F., Trouiller, A., 2006. Vascular plant biomarkers as proxies for palaeoflora and palaeoclimatic changes at the Dogger/Malm transition of the Paris Basin (France). Organic Geochemistry 37, 610 – 625.

Huang, W.-Y., Meinschein, W.G., 1979. Sterols as ecological indicators. Geochimica et Cosmochimica Acta 43, 739 – 745.

Hueber, F.M., 1992. Thoughts on the early *lycopsids* and *zosterophylls*. Annals of the Missouri Botanical Garden 79, 474 – 499.

Jiang, C., Alexander, R., Kagi, R.I., Murray, A. P., 1998. Polycyclic aromatic hydrocarbons in ancient sediments and their relationships to palaeoclimate. Organic Geochemistry 29, 1721 – 1735.

Kenrick, P., Crane, P. R., 1997. The origin and early evolution of plants on land. Nature 389, 33 – 39.

Kerp, H., 1996. Post-variscan late palaeozoic northern hemisphere gymnosperms: the onset of the Mesozoic. Review of Palaeobotany and Palynology 90, 263 – 285.

Killops, S.D., 1991. Novel aromatic hydrocarbons of probable bacterial origin in a Jurassic lacustrine sequence. Organic Geochemistry 17, 25 – 36.

Killops, S. D., Massoud, M. S., 1992. Polycyclic aromatic hydrocarbons of pyrolytic origin in ancient sediments: evidence for Jurassic vegetation fires. Organic Geochemistry 18, 1 – 7.

Lafargue, E., Marquis, F., Pillot, D., 1998. Rock-Eval 6 Applications in hydrocarbon exploration, production and soils contamination studies. Oil & Gas Science and Technology, Revue de l'Institut Français du Pétrole 53, 421 – 437.

Laflamme, R. E., Hites, R. A., 1978. The global distribution of polycyclic aromatic hydrocarbons in recent sediments. Geochimica et Cosmochimica Acta 42, 289 – 303.

Lüning, S., Craig, J., Loydell, D.K., Storchd, P., Fitches, B., 2000. Lower Silurian 'hot shales' in North Africa and Arabia: regional distribution and depositional model. Earth-Science Reviews 49, 121 – 200.

Mackenzie, A., S., Brassel, S. C., Eglinton G., Maxwell, J. R., 1982. Chemical fossils: the geological fate of steroids. Science 217, 491 – 504.

Massa, D., 1988. Paléozoïque de Libye Occidentale. Stratigraphie et Paléogéographie. Thèse Sc. Univ. Nice, 1-2, 514 p.

Martin, W., Gierl, A., Saedler, H., 1989. Molecular evidence for pre-cretaceous angiosperm origins. Nature 339, 46 – 48.

Martone, P., Estevez, J. M., Lu, F., Ruel, K., Denny, M.W, Somerville, C., Ralph, J., 2009. Discovery of lignin in seaweed reveals convergent evolution of cell-wall architecture. Current Biology 19, 169 – 175.

Moldowan, J. M., Dahl, J., Huizinga, B. J., Fago, F. J., Hickey, L. J., Peakman, T. M., Taylor, D. W., 1994. The molecular fossil record of oleanane and its relation to angiosperms. Science 265, 768 – 771.

Molyneux, S.G., 1988. Late Ordovician acritarchs from northeast Libya. In: El-Arnauti, A., Owens, B, Thusu, B. (Eds.), Subsurface palynostratigraphy of northeast Libya. Garyounis University Publishing, Benghazi, Libya, 45 – 59.

Murchison D. G., Raymond A. C., 1989. Igneous activity and organic maturation in the Midland Valley of Scotland. International Journal of Coal Geology 14, 47 – 82.

Niklas, K.J., Banks, H.P., 1990. A reevaluation of the *Zosterophyllophytina* with comments on the origin of Lycopods. American Journal of Botany 77, 274 – 283.

Noble, R. A., 1986. A geochemical study of bicyclic alkanes and diterpenoid hydrocarbons in crude oils, sediments and coals. Thesis. The University of Western Australia. 398 p.

Noble, R. A., Alexander, R., Kagi, R.I., Knox, J., 1985. Tetracyclic diterpenoid hydrocarbons in some Australian coals, sediments and crude oils. Geochimica and Cosmochimica Acta 49, 2141 – 2147.

Noble, R. A., Alexander, R., Kagi, R.I., 1987. Configurational isomerization in sedimentary bicyclic alkanes. Organic Geochemistry 11, 151 – 156.

Otto, A., Walther, H., Püttmann, W., 1997. Sesqui- and diterpenoid biomarkers preserved in Taxodium-rich Oligocene Oxbow Lake Clays, Weisselster basin, Germany. Organic Geochemistry 26, 105–115.

Otto, A., Simoneit, B.R.D., 2001. Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz Formation, Saxony, Germany. Geochimica et Cosmochimica Acta 65, 3505 – 3527.

Otto, A., Wilde, V., 2001. Sesqui-, di- and triterpenoids as chemosystematic markers in extant conifers—A review. The Botanical Review 67, 141 – 238.

Ourisson, G., 1973. Some aspects of the distribution of diterpenes in plants. In Chemistry in Botanical Classification. (eds) G. Bendz, J. Santesson. Nobel Symposium London 25, 129 – 134.

Peters, K.E., Walters, C.W., Moldowan, J.M., 2005. The Biomarker Guide. Cambridge University Press. Cambridge -706 pp.

Pentegova, V.A., Dubovenko, Z.V., Vol'skii, L.N., Vasilyuk, S.M., Chirkova, M.A., Schmidt, E.N., 1968. Sesquiterpenoid hydrocarbons from the oleoresins of some Siberian conifer species. Siberian Chemistry 211 – 214.

Philp, R. P., 1994. Geochemical characteristics of oils derived predominantly from terrigenous source materials. In: Coal and Coal-bearing Strata as Oil-prone Source Rocks? (Eds, Scott A.C. and Fleet A.J.). Geological Society Special Publication 77, 71 – 91.

Pinto A.C., Zocher D.H.T., Rezende C.M., Gottlieb H.E., 1995. A new diterpene with a totarane skeleton from Vellozia flavicans. Natural Product Research 6, 209 – 213

Pryer, K. M., Schneider, H., Smith, A. R., Cranfill, R., Wolf, P. G., Hunt, J. S., Sipes, S. D., 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature 409, 618 – 622.

Püttmann, W., Villar, H., 1987. Occurrence and geochemical significance of 1,2,5,6-tetramethylnaphthalene. Geochimica et Cosmochimica Acta 51, 3023 – 3029.

Radke M., Willsch H., Leythaeuser D., Teichmuller M., 1982. Aromatic components of coal: relation of distribution pattern to rank. Geochimica et Cosmochimica 46, 1831 – 1848.

Radke, M., Rullkötter, J., Vriend, S. P., 1994. Distribution of naphthalenes in crude oils from the Java Sea: Source and maturation effects. Geochimica et Cosmochimica Acta 58, 3675 – 3689.

Radke, M., Hilkert, A., Rullkötter, J., 1998. Molecular stable carbon isotope compositions of alkylphenanthrenes in coals and marine shales related to source and maturity. Organic Geochemistry 28, 785 – 795.

Radke, M., Vriend, S. P., Ramanampisoa, L. R., 2000. Alkyldibenzofurans in terrestrial rocks: Influence of organic facies and maturation. Geochimica et Cosmochimica 64, 275 – 286.

Retallak, G.J., 2000. Ordovician life on land and early Palaeozoic global change. In: Gastaldo, R.A., Di Michele, W.A. (Eds.), Phanerozoic terrestrial ecosystems. Paleontological Society Papers 6, 21 – 45.

Retallak, G.J, 2008, in press. Cambrian paleosols and landscapes of South Australia. Australian Journal of Earth Sciences 55.

Schmidt, M., Schneider-Poetsch, H. A. W., 2002. The evolution of gymnosperms redrawn by phytochrome genes: The gnetatae appear at the base of the gymnosperms. Journal of Molecular Evolution 54, 715 – 724.

Schneider, H., Schuettpelz, E., Pryer, K.M., Cranfill, R., Magallon, S., Lupia, R., 2004. Ferns diversified in the shadow of angiosperms. Nature 428, 553 – 557.

Schulze, T., Michaelis, W., 1990. Structure and origin of terpenoid hydrocarbons in some German coals. Organic Geochemistry 16, 1051 – 1058.

Scott, A., 1974. The earliest conifer. Nature 25, 707 – 708.

Sheng, G., Simoneit, B. R. T., Leif, R. N., Chen, X., Fu, J., 1992. Tetracyclic terpanes enriched in Devonian cuticle humic coals. Fuel, 71, 523 – 532.

Simoneit, B.R.T., 1977. Diterpenoid compounds and other lipids in deep-sea sediments and their geochemical significance. Geochimica et Cosmochimica Acta 41, 463 – 476.

Simoneit, B.R.T., 1978. The organic chemistry of marine sediments. In: Riley J.P., Chester R. (Eds.), Chemical Oceanography, Vol. 7, 2nd Edition. Academic Press, New York, pp. 233 – 311.

Simoneit, B.R.T., 1986. Cyclic terpenoids of the geosphere. In: Johns, R.B. (Ed.), Biological Markers in the Sedimentary Record. Elsevier, Amsterdam, 41 – 99.

Simoneit, B.R.T., Grimalt, J.O., Wang, T.G., Cox, R.E., Hatcher, P.G., Nissenbaum, A., 1986. Cyclic terpenoids of contemporary resinous plant detritus and of fossil woods, ambers and coals. Organic Geochemistry 10, 877 – 889.

Spina, A., Vecoli, M., 2009. Palynostratigraphy and vegetational changes in the Siluro-Devonian of the Ghadamis Basin, North Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 282, 1 – 18.

Spinner, E., 1969. Megaspore assemblages from Visean deposits at Dunbar, East Lothian, Scotland. Palaeontology 12, 441 – 458.

Spinner, E., Clayton, G., 1973. Visean spore assemblages from Skateraw, East Lothian, Scotland. Pollen et Spores XV, 139 – 165.

Steemans, P., Le Hérissé, A., Bozdogan, N., 1996. Ordovician and Silurian cryptospores and miospores from Southeastern Turkey. Review of Palaeobotany and Palynology 93, 35 – 76.

Steemans, P. 1999. Paléodiversification des spores et des cryptospores del'Ordovician au Dévonien Inférieur. Geobios 32, 341 – 352.

Steemans, P., 2000. Miospore evolution from the Ordovician to the Silurian. Review of Palaeobotany and Palynology 113, 189 – 196.

Steemans, P., Le Hérissé, A., Melvin, J., Miller, M.A., Paris, F., Verniers, J., Wellman, C., 2009. Origin and radiation of the earliest vascular land plans. Science 324.

Stewart, W.N., Rothwell, G.W., 1993. Paleobotany and the evolution of plants. Cambridge University Press, London. 521 p.

Strachan, M.G., Alexander, R., Kagi, R.I., 1988. Trimethylnaphthalenes in crude oils and sediments: effects of source and maturity. Geochimica et Cosmochimica Acta 52, 1255 – 1264.

Strother, P.K., Al-Hajri, S., Traverse, A., 1996. New evidence for land plants from the lower Middle Ordovician of Saudi Arabia. Geology 24, 55 – 58.

Thomas, B. R., 1969. Organic Geochemistry. Methods and results, chapter Kauri resins-modern and fossil. Springer Verlag, Berlin, 599 – 618.

Tissot, B., Welte, D.M., 1984. Petroleum Formation and Occurrence. Springer-Verlag, Berlin, 699 p.

Torsvik, T.H., Smethurst, M.A., Meert, J.G., der Voo, R.V., McKerrow, W.S., Brasier, M.D., Sturt, B.A., Walderhaug, H.J., 1996. Continental break-up and collision in the Neoproterozoic and Palaeozoic — a tale of Baltica and Laurentia. Earth-Science Reviews 40, 229 – 258.

Toyota, M., Asakawa, Y., 1993. Bibenzyl and sesquiterpenoids from the liverwort *Jubula japonica*. Phytochemistry 34, 1135 – 1137.

Tuo, J., Philp, R. P., 2005. Saturated and aromatic diterpenoids and triterpenoids in Eocene coals and mudstones from China. Applied Geochemistry 20, 367 – 381.

Underdown, R., Redfern, J., 2008. Petroleum generation and migration in the Ghadames Basin, North Africa: A two-dimensional basin-modeling study. AAPG Bulletin 92, 53 – 76.

Underhill, J. R., Monaghan, A. A., Browne, M. A. E., 2008. Controls on structural styles, basin development and petroleum prospectivity in the Midland Valley of Scotland. Marine and Petroleum Geology 25, 1000 – 1022.

van Aarssen, B.K.G., Cox, H.C., Hoogendoorn, P., de Leeuw, J.W., 1990. A cadinene biopolymer in fossil and extant dammar resins as a source for cadinanes and bicadinanes in crude oils from South East Asia. Geochimica et Cosmochimica Acta 54, 3021 – 3031.

van Aarssen, B.K.G., Alexander, R., Kagi, R.I, 2000. Higher plant biomarkers reflect palaeovegetation changes during Jurassic times. Geochimica and Cosmochimica Acta 64, 1417 – 1424.

van Aarssen, B.K.G., Bastow, T.P., Alexander, R., Kagi, R.I, 1999. Distributions of methylated naphthalenes in crude oils: indicators of maturity, biodegradation and mixing. Organic Geochemistry 30, 1213 – 1227.

Vecoli, M., 2000. Palaeoenvironmental interpretation of microphytoplankton diversity trends in the Cambrian–Ordovician of the northern Sahara Platform. Palaeogeography, Palaeoclimatology, Palaeoecology 160, 329 – 346.

Vecoli, M., Le Hérissé, A., 2004. Biostratigraphy taxonomic diversity, and patterns of morphological evolution of Ordovician acritarchs (organic-walled microphytoplankton) from the northern Gondwana margin in relation to palaeoclimatic and palaeogeographic changes. Earth-Science Reviews 67, 267 – 311.

Vecoli, M., Riboulleau, A., Versteegh, G., 2009. Palynology, organic geochemistry and carbon isotope analysis of a latest Ordovician through Silurian clastic succession from borehole T.T.1, Ghadamis Basin, southern Tunisia, North Africa: Palaeoenvironmental interpretation. Palaeogeography, Palaeoclimatology, Palaeoecology 273, 378 – 394.

Volkman, J., 1986. A review of sterol markers for marine and terrigenous organic matter. Organic Geochemistry 9, 83 – 99.

Wang, T.G., Simoneit, B.R.T., 1990. Organic geochemistry and coal petrology of Tertiary brown coal in the Zhoujing mine, Baise Basin, South China. Fuel 69, 12 – 20.

Wellman, C. H. and Gray, J., 2000. The microfossil record of early land plants. Philosophical Transactions of the Royal Society London B 355, 717 – 732.

Wellman, C.H., Osterloff, P.L., Mohiuddin, U., 2003. Fragments of the earliest land plants. Nature 425, 282 – 285.

Wen, Z., Ruiyong, W., Radke, M., Qingyu, W., Guoying, S., Zhili, L., 2000. Retene in pyrolysates of algal and bacterial organic matter. Organic Geochemistry 31, 757 – 762.

Zhang, L., Huang, D., Liao, Z., 1999. High concentration retene and methylretene in Silurian carbonate of Michigan Basin. Chinese Science Bulletin 44, 2083 – 2086.

Zhu, W.-Q., Kenrick P., 1999. A *Zosterophyllum*-like plant from the Lower Devonian of Yunnan Province, China. Review of Palaeobotany and Palynology 105, 111 – 118.

Chapter 2

Occurrence of retene in upper Silurian – lower Devonian sediments from North Africa: Origin and implications

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2.1. Abstract

The biomarkers retene, cadalene, tetrahydroretene and isohexylalkylnapthalene have been identified in the extracts of Silurian – Devonian clastic sediments from southern Tunisia. The occurrence of retene is unexpected as it is generally considered to be a diagenetic product of the conifer constituent abietic acid; however, conifers evolved only after the Devonian. The retene shows a strong correlation with the abundant terrestrial signal from bryophyte-derived cryptospores in the palynofacies, suggesting that it derives from a terrestrial source. Accordingly, we propose that either abietic acid synthesis evolved prior to the appearance of conifers or retene is a diagenetic product of precursors other than abietic acid, which were synthesized by the earliest land plants. Without excluding the first possibility, it appears likely that retene derives here from the diagenesis of compounds with a kaurane-type skeleton, which were abundantly produced by the early Palaeozoic bryophytes.

Keywords: retene; Silurian; Devonian; land plant biomarkers; palynofacies

2.2. Introduction

Retene is an aromatic hydrocarbon which has generally been considered as a conifer biomarker (van Aarssen et al., 2000). Partial degradation of abietic acid, a major constituent of conifer resins, leads to the formation of typical aromatic abietanes such as retene and simonellite. In fact, all abietane class bioditerpenoids may be degraded to produce retene (Otto and Simoneit, 2001; Hautevelle et al., 2006).

Phytoplankton has also been considered a source of retene, most particularly in sediments of pre-Devonian age (Zhang et al., 1999). Retene also appeared among the pyrolysis products of phytoplankton material (Wen et al., 2000). These results indicate that coniferous resins are not the only possible source of retene in sediments. In sediments with contributions from both terrestrial plants and algal matter its origin therefore remains subject of discussion (Wen et al., 2000).

We performed molecular biomarker analysis on a Silurian-Devonian clastic sequence (borehole MG1, southern Tunisia); known to contain abundant and well-preserved algal and terrestrial plant remains such as acritarchs, miospores and phytodebris (Spina and Vecoli, 2009). Comparison of concomitant changes between biomarkers and palynomorph concentrations allowed consideration of the origin of retene in these sediments, i.e. algal vs terrestrial plant sources, and of possible implications for land plant evolution.

2.3. Geological setting

The samples were collected from a Siluro-Devonian succession penetrated by borehole MG1 (34°72’40”N; 8°68’00”E), near the Tunisia – Libya border (Ghadamis Basin, southern Tunisia; Fig. 2.1).

The early Silurian is characterized by a transgressive deposition of thick, laterally continuous marine mudstones and graptolite-rich micaceous shales, sometimes ferruginous with interbedded sandstone beds (Tanezzuft Formation, Fig. 2.2). The regression of the Silurian sea resulted in the deposition of late Silurian marine sandstones and mudstones of the Acacus Formation (Underdown and Redfern, 2008; Spina and Vecoli, 2009). The Caledonian unconformity separates the Silurian deposits from the overlying continental sandstones and mudstones of the Early Devonian Tadrart Formation. Finally, the section ends with shallow-marine sandstones and mudstones of the Ouan Kasa Formation (Underdown and Redfern, 2008).

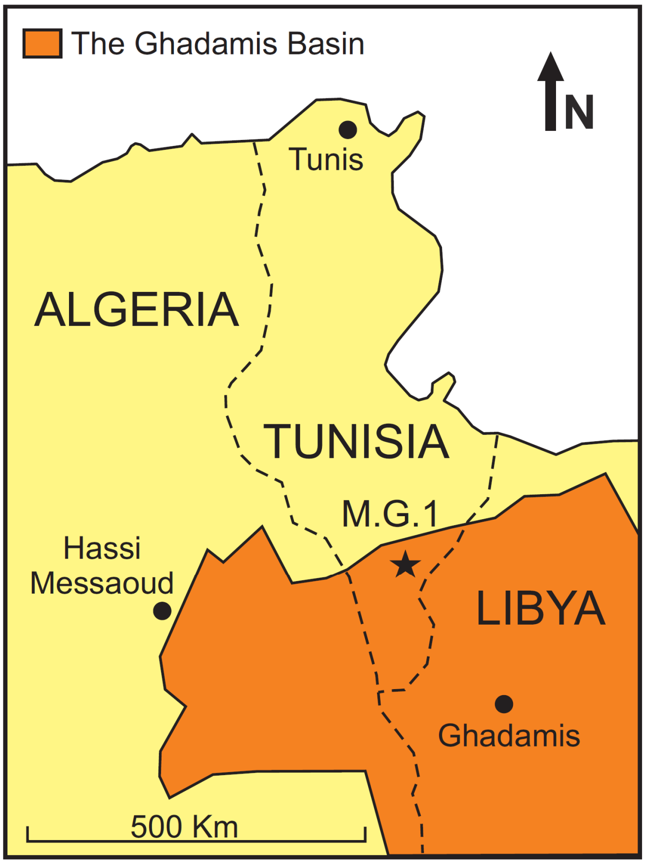

Figure 2.1: Location of borehole MG1

2.4. Analytical methods

2.4.1. Experimental procedures

All 11 samples (Fig. 2.2) were studied using Rock-Eval pyrolysis and biomarker analysis. Rock-Eval pyrolysis was performed on 100 mg ground rock with a Turbo model RE6 pyrolyzer (Vinci Technologies) at the Institut Français du Pétrole (IFP) using the conventional temperature programme described by Lafargue et al. (1998). The pyrolysis oven temperature was: 300°C (3 min) to 650°C (held 3 min) at 25 °C/min. For biomarker analyses, rock fragments were extracted with dichloromethane (DCM) during 24 h in the refrigerator, in order to remove possible contamination on the sample surface. After this first extraction, the rock fragments were crushed to enable extraction of the lipids preserved inside the rock. Approximately 30 g pulverised samples were extracted with a mixture of methanol (MeOH) and dichloromethane (DCM) (1/2, v/v) for 24 h with extensive stirring. This second extract was dried by means of roto-evaporation and partly redissolved in cyclohexane. The cyclohexane-soluble fraction (maltenes) was further separated using column chromatography.

The apolar fraction was recovered from the maltenes by elution with cyclohexane on an activated silica column. Subsequent elution with a mixture of cyclohexane - DCM (2/1, v/v) recovered the aromatic fraction after which the polar fraction was recovered by elution with a mixture DCM – MeOH (2/1, v/v).

2.4.2. Gas chromatography – mass spectrometry (GC-MS)

The aliphatic and aromatic fractions were analysed by using GC-MS with a Trace GC 2000 gas chromatograph and a DB5ht column (30 m length, 0.25 mm i.d., 0.1 µm film thickness). The oven temperature was programmed as follows: 100 °C (1 min), to 310°C (held 16.5 min) at 4 °C/min. Helium was used as carrier gas. Compounds were identified by comparison of mass spectra and retention times with available published data.

2.5. Results

The total organic carbon (TOC) values obtained from Rock-Eval analysis range between 0.1% and 2.6%. The hydrogen index (HI) values range between 15 and 148 mg HC/g TOC, indicating that the section contains Type II-III kerogen and suggesting a mixture of organic matter (OM) of terrestrial and marine origin. The average Tmax value of 437 °C indicates that the sediments are at the beginning of the oil window. From their low TOC and HI values, they show poor to fair source rock potential.

The apolar fraction (not shown) is dominated by a series of *n*-alkanes ranging from C_{14} to C_{35}. Series of C_{29} to C_{34} αβ-hopanes and C_{19} to C_{23} cheilantanes were observed in all the samples while steranes and diterpanes were only detected in very low abundance. In addition, a C_{20} tricyclic diterpane present in low abundance was tentatively assigned for sample MG1-2890.0. It has a molecular ion at m/z 274 ($C_{20}H_{34}$) and from its retention time could correspond to *ent*-16β(H)-kaurane; however, its low intensity did not allow us to unambiguously confirm its presence.

The m/z 133 + 134 + 147 + 169 + 183 + 197 mass chromatograms from the aromatic fractions show the presence of a pseudo-homologous series of 1-alkyl-2,3,6-trimetylbenzenes from C_{13} to C_{20}, and of C_{15} to C_{17} isohexylalkylbenzenes (Fig. 2.2). The relative abundance of both aryl-isoprenoids and isohexylalkylbenzenes generally decreases upsection despite some fluctuations. Series of C_{15} to C_{20} isohexylalkylnaphthalenes also are visible on the m/z 133 + 134 + 147 + 169 + 183 + 197 mass chromatograms (Fig. 2.2).

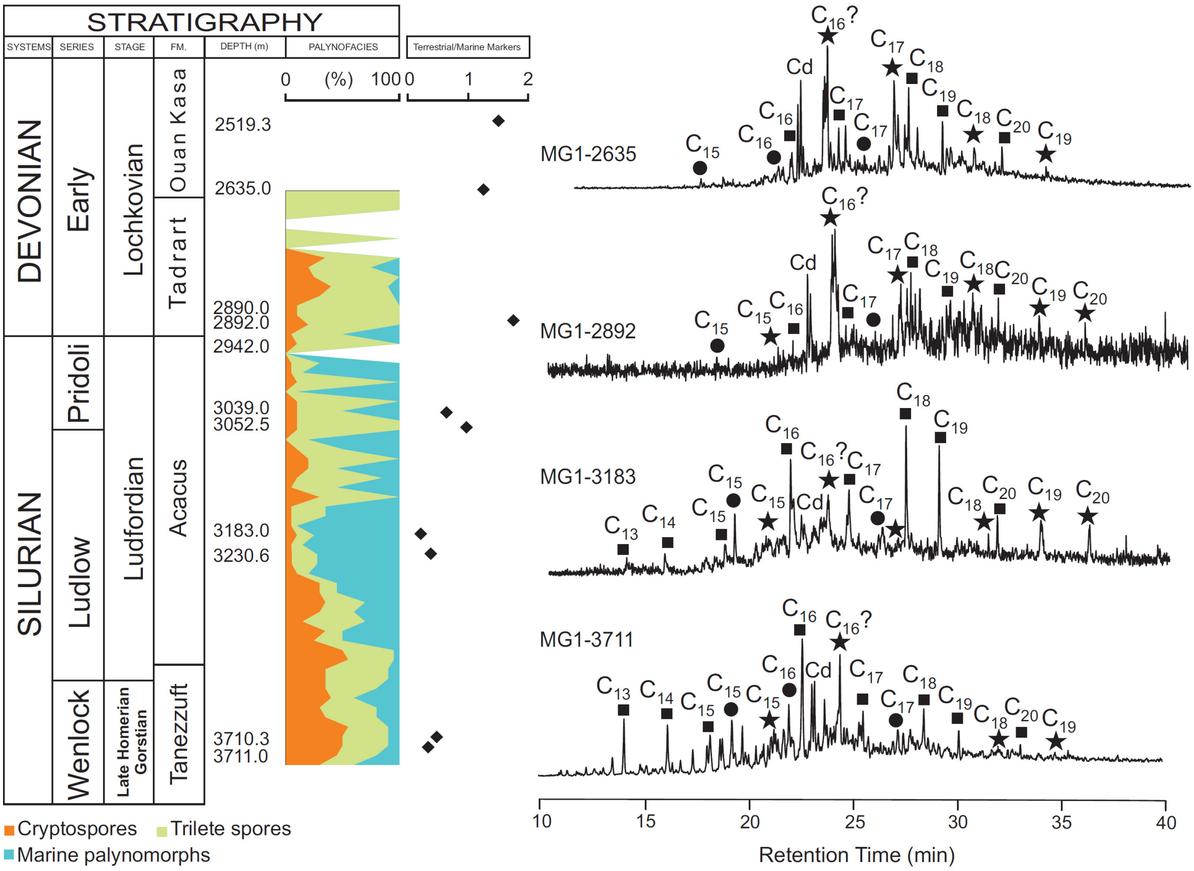

Figure 2.2: m/z 133 + 134 + 147 + 169 + 183 + 197 chromatograms from aromatic fractions of selected samples from borehole MG1, showing the distribution of aryl isoprenoids, isohexylalkylbenzenes and isohexylalkylnaphthalenes. Stratigraphy and palynological content from Spina and Vecoli (2009). Indicated depths correspond to analysed samples. ■ aryl isoprenoids; **Cd** cadalene; ● isohexylalkylbenzenes; ★ isohexylalkylnaphthalenes. The terrestrial/marine ratio is based on peak heights of (cadalene + isohexylalkylnaphthalenes) / (aryl isoprenoids + isohexylalkylbenzenes) in the chromatograms

The m/z 183 + 219 + 223 chromatograms from the aromatic fractions revealed the presence of C_{19} isohexylalkylnaphthalene, cadalene, retene and tetrahydroretene (Fig. 2.3). While the presence of cadalene and retene could be clearly established in the samples of the Acacus, Tadrart and Ouan Kasa Formations (Fig. 2.3), their presence in the samples of the lower part of the section (Tanezzuft Formation) is more doubtful, because of their low abundance. Simonellite was present in very low amounts in two samples (not shown in Fig. 2.3).

The relative abundance of (cadalene + isohexylalkylnaphthalenes) versus (aryl isoprenoids + isohexylalkylbenzenes) in the aromatic fraction generally increases upsection (Fig. 2.2). This increase takes place in parallel with a transition from marine upper Silurian to more continent-influenced lower Devonian sediments. The trend also parallels the increasing proportion of continental palynomorphs (Fig. 2.2). Note that the Tanezzuft Formation contains high proportions of fine-grained marine amorphous OM. As a result of sieving of the palynological residue (Spina and Vecoli, 2009), the proportion of terrestrial palynomorphs vs. marine OM is overestimated in this part of the interval (Fig. 2.2).

2.6. Discussion

Both the bulk geochemical features (Rock-Eval) and the biomarker content of the aliphatic and aromatic fractions of the samples indicate a mixed marine and terrestrial origin for the OM in core MG1. These conclusions are consistent with the palynological content showing a mixture of terrestrial and marine OM (Spina and Vecoli, 2009).

Short chain *n*-alkanes, cheilantanes and hopanes, present in the apolar fraction, are typical biomarkers of an algal/bacterial origin for the OM (Peters et al., 2005). In the apolar fraction, no biomarkers of terrestrial origin could be clearly identified. Long chain > C_{20} *n*-alkanes can be present in significant proportion, but they do not show the typical odd carbon number predominance generally observed in higher plant waxes (Eglinton and Hamilton, 1967; Peters et al., 2005). Higher plant diterpanes, monitored using m/z 123 and 109 mass fragmentograms (Noble et al., 1985), were hardly detected in the aliphatic fraction. Only kaurane and norabietane were tentatively identified in two samples (MG1-2890.0, MG1-3183.0), in very low amounts. It therefore appears that the apolar fractions do not bring to light the contribution of land plants since the terrestrial plant terpenoids have been mainly converted to their aromatic counterparts. This feature might be related to a combination of factors which favoured the aromatisation these compounds: thermal maturity (Alexander et al., 1987a) and the abundant presence of clay minerals in the sediment (Haberer et al., 2006). Clays are known to catalyse molecular rearrangements, e.g. in the case of steroids (Sieskind et al., 1979) and apparently could also favour aromatisation of terrestrial plant terpenoids (Haberer et al., 2006).

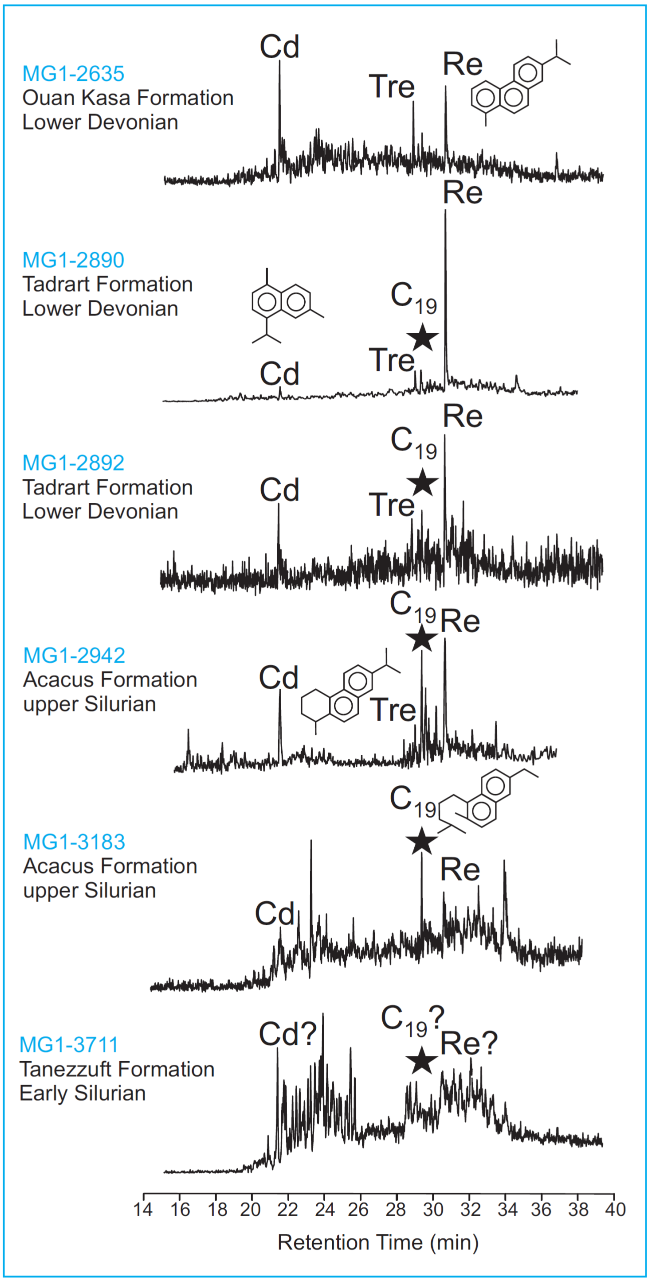

Figure 2.3: m/z 183 + 219 + 223 chromatograms from aromatic fractions of selected samples from borehole MG1, showing the presence of cadalene, retene, tetrahydroretene and C_{19} isohexylalkylnaphthalene. Indicated depths correspond to the analysed samples. **Re** retene; **Tre** tetrahydroretene; **Cd** cadalene; ★ isohexylalkylnapthalenes

Aryl isoprenoids are generally considered to derive from green sulphur bacteria (Summons and Powell, 1987; Requejo et al., 1992; Hartgers et al., 1994). However, Sinninghe Damsté et al. (1988) and Koopmans et al. (1996) demonstrated that they could also derive from β-carotene. Consistently, the presence of aryl isoprenoids in our Tunisian samples is definitively associated with a marine source. These compounds are relatively more abundant in the samples of the lower section and, more particularly, in samples containing abundant marine palynomorphs (e.g. sample MG1-3183.0; Fig. 2.2).

In addition, land plant biomarkers also are recovered in the aromatic fraction. Cadalene is a well recognised biomarker for terrestrial plants (van Aarssen et al., 2000; Hautevelle et al., 2006). It derives from cadinenes and cadinols synthesized by most land plants (Simonsen and Barton, 1952; Bordoloi et al., 1989) and in particular bryophytes (Chopra and Kumra, 1988; Asakawa, 2004). Bryophytes are among the earliest plants that developed on land and their presence on emerged lands around the Ghadamis Basin during the deposition of the studied sediments is attested by way of the abundant bryophyte-derived cryptospores in the palynological spectrum of core MG1 (Spina and Vecoli, 2009; Fig. 2.2).

Isohexyl aromatic compounds (isohexylalkybenzenes and isohexylalkylnaphthalenes) were shown to be formed by ring A opening of terpenoids possessing an A-B ring system (Ellis et al., 1996). According to Ellis et al. (1996), isohexylalkylbenzenes can be generated in low amounts from the aromatisation and rearrangement of algal or bacterial terpenoids. However, according to these authors, both series of isohexyl aromatic compounds originate mainly from the aromatisation and rearrangement of terrestrial plant terpenoids. In particular, diterpenoids with a labdane, pimarane and kaurane skeleton, synthetised by bryophytes (Alexander et al. 1987b) appear among the precursors of isohexyl aromatic compounds (Ellis et al., 1996).

The presence of retene has generally been considered to result from diagenesis of the conifer biomarker abietic acid (van Aarssen et al., 2000; Hautevelle et al., 2006), and therefore to indicate terrestrial input. Conifers had not yet, however, evolved during the Silurian – Devonian period. Algal sources of retene have also been suggested by others (Zhang et al., 1999; Wen et al., 2000) and might be consistent with the presence of algal organic matter in the study material. However, retene shows a general correlation with the other land plant biomarkers such as cadalene, tetrahydroretene and isohexylalkylnaphthalenes, as well as with the abundance of trilete spores and cryptospores in the palynofacies (Fig. 2.2). From these observations, it appears more likely that retene in core MG1 has a terrestrial origin.

In order to explain the terrestrial origin of retene in these upper Silurian – lower Devonian samples, it must be assumed that either abietic acid synthesis evolved prior to the conifers, or that different kinds of terrestrial plant biomolecules can produce retene through diagenesis. It is possible that the Silurian land plants were already able to synthesize abietic acid. However, this implies that this character would have been lost later in most of more evolved plants (P. Strother, personal communication).

In the most primitive terrestrial plants currently present on land, i.e. the bryophytes, abietic acid has not been detected (Chopra and Kumra, 1988; Asakawa, 2004), but rearranged abietanes have been observed in some liverworts (Liu and Wu, 1997; Wu and Jong, 2001). Such compounds could therefore be precursors of retene, though they were only detected in low amounts in bryophytes (Liu and Wu, 1997; Wu and Jong, 2001). Alexander et al. (1987a) and Ellis et al. (1996), among others, demonstrated that retene can be produced by maturation of the terrestrial plant diterpenoids phyllocladanes and kauranes. Although the presence of compounds possessing a phyllocladane skeleton in bryophytes has not been reported (Alexander et al., 1987b; Chopra and Kumra, 1988; Asakawa, 2004), compounds with a kaurane skeleton are widespread among bryophytes, and in particular liverworts (Chopra and Kumra, 1988; Asakawa, 2004).

Retene could alternatively derive from *ent*-kaurene, a biosynthetic intermediate to gibberellin hormones, which are widespread among plants, in particular flowering plants, and fungi (Graebe et al., 1965; MacMillan, 2001). However, if kaurene is present in numerous plants, its accumulation possibilities are very low because it may be rapidly converted to gibberellin hormones.

Abietanoid precursors of retene could also derive from abiogenic cyclisation and rearrangement of bicyclic terpenoids (Hall and Oehlschlager, 1972), which are widespread among plant oils and resins (Anderson and Crelling, 1996). However, bicyclic terpenoids are highly volatile compounds and similarly have low preservation degree (Anderson and Crelling, 1996). Moreover, though possible, their conversions to abietanoids is of low efficiency (a few % yield) (Hall and Oehlschlager, 1972). The fact that retene is not systematically present in petroleums derived from terrestrial plants supports the hypothesis that it does not derive from rearrangement of these widespread compounds.

Based on these observations and, consistent with the presence of bryophyte-derived cryptospores in the palynofacies (Spina and Vecoli, 2009), the occurrence of retene in our samples may be related to the maturation of kaurane-type compounds associated with the very primitive flora of bryophytes, and in particular liverworts, which covered emerged lands around the Ghadamis Basin during Silurian – Devonian times.

2.7. Conclusions

Several land plant biomarkers have been identified in Silurian – Devonian sediments from Tunisia. The presence of retene in these sediments suggests that this biomarker may not be associated exclusively with gymnosperms, which only evolved during the Late Devonian. Based on the observed correlation between retene abundance, sedimentary facies and/or presence of other terrestrial plant biomarkers and palynomorphs, we propose that retene in our samples probably derives from a land plant source, either via degradation of abietic acid, in which case abietic acid synthesis must have evolved prior to the emergence of the conifers, or more likely, via degradation of kaurane-type compounds synthesized by the primitive flora of bryophytes.

2.8. Acknowledgements

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2.9. References

Alexander, G., Hazai, I., Grimalt, J., Albaigés, J., 1987a. Occurrence and transformation of phyllocladanes in brown coals from Nograd Basin, Hungary. Geochimica et Cosmochimica Acta 51, 2065 – 2073.

Alexander, R., Noble, R.A., Kagi, R.I., 1987b. Fossil resin biomarkers and their application in oil to source rock correlation, Gippsland basin, Australia. Australian Petroleum Exploration Association Journal 27, 63 – 72.

Anderson, K.B., Crelling, J.C., 1996. Amber, resinite and fossil resins. ACS symposium series 617, 296p.

Asakawa, Y., 2004. Chemosystematics of the Hepaticae. Phytochemistry 65, 623 – 669.

Bordoloi, M., Shukla, V.S., Nath, S.C., Sharma, R.P., 1989. Naturally occurring cadinenes. Phytochemistry 28, 2007-2037.

Chopra, R.N., Kumra, K.P., 1988. Biology of Bryophytes. John Wiley & Sons, New York, 350p.

Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. Science 156, 1322 – 1335.

Ellis, L., Singh, R., Alexander, R., Kagi, R., 1996. Formation of isohexyl alkylaromatic hydrocarbons from aromatization-rearrangement of terpenoids in the sedimentary environment: A new class of biomarker. Geochimica et Cosmochimica Acta 60, 4747 – 4763.

Graebe, J.E., Dennis, D.T., Upper, C.D., West, C.A., 1965. Biosynthesis of Gibberellins. Journal of Biological Chemistry 240, 1847 – 1854.

Haberer, R.M., Mangelsdorf, K., Wilkes, H., Horsfield, B., 2006. Occurrence and palaeoenvironmental significance of aromatic hydrocarbon biomarkers in Oligocene sediments from the Mallik 5L-38 Gas Hydrate Production Research Well (Canada). Organic Geochemistry 37, 519 – 538.

Hall, S. F., Oehlschlager, A. C., 1972. Cationic rearrangements and cyclizations of diterpenoid olefins. Tetrahedron 28, 3155 – 3173.

Hartgers, .A., Sinninghe Damsté, J.S., Requejo, A.G., Allan, J., Hayes, J.M., Ling, Y., Xie, T.M., Primack, J., de Leeuw, J.W., 1994. A molecular and carbon isotopic study towards the origin and diagenetic fate of diaromatic carotenoids. Organic Geochemistry 22, 703 – 725.

Hautevelle, Y., Michels, R., Malartre, F., Trouiller, A., 2006. Vascular plant biomarkers as proxies for palaeoflora and palaeoclimatic changes at the Dogger/Malm transition of the Paris Basin (France). Organic Geochemistry 37, 610 – 625.

Koopmans, M.P., Köster, J., Van Kaam-Peters, H.M.E., Kenig, F., Schouten, S., Hartgers, W.A., de Leeuw, J.W., Sinninghe Damsté, J.S., 1996. Diagenetic and catagenetic products of isorenieratene: Molecular indicators for photic zone anoxia Geochimica et Cosmochimica Acta 60, 4467 – 4496.

Lafargue, E., Marquis, F., Pillot, D., 1998. Rock-Eval 6 Applications in hydrocarbon exploration, production and soils contamination studies. Oil & Gas Science and Technology, Revue de l'Institut Français du Pétrole 53, 421 – 437.

Liu, H.-J., Wu, C.-L., 1997. A rearranged abietane-type diterpenoid from the liverwort *Makinoa crispata*. Phytochemistry 44, 1523-1525.

MacMillan, J., 2001. Occurrence of Gibberellins in vascular plants, fungi, and bacteria. Journal of Plant Growth Regulation 20, 387-442.

Noble, R. A., Alexander, R., Ian Kagi, R., Knox, J., 1985. Tetracyclic diterpenoid hydrocarbons in some Australian coals, sediments and crude oils. Geochimica and Cosmochimica Acta 49, 2141 – 2147.

Otto, A., Simoneit, B.R.D., 2001. Chemosystematics and diagenesis of terpenoids in fossil conifers species and sediment from the Eocene Zeitz Formation, Saxony, Germany. Geochemica et Cosmochimica Acta 65, 3505-3527.

Peters, K.E., Walters, C.W., Moldowan, J.M., 2005. The Biomarker Guide. Cambridge University Press.Cambridge. 706 pp.

Requejo, A., Allan, J., Creaney S., Gray, N., Cole K., 1992. Aryl isoprenoids and diaromatic carotenoids in Paleozoic source rocks and oils from the Western Canada and Williston Basins. Organic Geochemistry 19, 245 – 264.

Sieskind, O., Joly, G., Albrecht, P., 1979. Simulation of the geochemical transformations of sterols; superacid effect of clay minerals. Geochimica et Cosmochimica Acta 43, 1675–1680.

Simonsen, J.L., Barton, D.H.R., 1952. The Terpenes: The sesquiterpenes, diterpenes and their derivatives. Cambridge University Press, New York, 580p.

Sinninghe Damsté, J.S., Kock-van, D.A.C., de Leeuw, J.W., 1988. Identification of isoprenoid alkylbenzenes in sediments and crude oils. Geochimica et Cosmochimica Acta 52, 2671 – 2677.

Spina, A., Vecoli, M., 2009. Palynostratigraphy and vegetational changes in the Siluro-Devonian of the Ghadamis Basin, North Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 282, 1-18.

Summons, R. E., Powell, T. G., 1987. Identification of aryl isoprenoids in source rocks and crude oils: Biologicalmarkers for the green sulphur bacteria. Geochimica et Cosmochimica Acta 51, 557-566.

Underdown, R., Redfern, J., 2008. Petroleum generation and migration in the Ghadames Basin, North Africa: A two-dimensional basin-modeling study. AAPG Bulletin 92, 53-76.

van Aarssen, B., Alexander, R., Kagi, R., 2000. Higher plant biomarkers reflect palaeovegetation changes during Jurassic times. Geochimica et Cosmochimica Acta 64, 1417 – 1424.

Wen, Z., Ruiyong, W., Radke, M., Qingyu, W., Guoying, S., Zhili, L., 2000. Retene in pyrolysates of algal and bacterial organic matter. Organic Geochemistry 31, 757 – 762.

Wu, C.L., Jong, J.R., 2001. A cyclic peroxide of clerodenoic acid from the Taiwanese liverwort *Schistochila acuminata*. Journal of Asian natural products research 3, 241 – 246.

Zhang, L., Huang, D., Liao, Z., 1999. High concentration retene and methylretene in Silurian carbonate of Michigan Basin. Chinese Science Bulletin 44, 2083 – 2086.

Chapter 3

Aliphatic and aromatic biomarkers from Gondwanan sediments of Late Ordovician to Early Devonian age: An early terrestrialization approach

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3.1. Abstract

Twenty-one core samples of Late Ordovician to Early Devonian age from sections in southern Tunisia, North African Platform (Gondwana) containing marine and terrestrial organic matter with microbial input have been investigated to link the distribution of their aliphatic and aromatic hydrocarbons with their terrestrial and marine palynomorph content (e.g. acritarchs, prasinophytes, chitinozoans, cryptospores and trilete spores). Long chain *n*-alkanes which could derive from land plants and the terrestrial diterpenoid norabietane were mainly identified in the aliphatic fractions obtained from middle Silurian – lower Devonian clastic sediments. In the aromatic fractions, alkylphenanthrenes, perylene and combustion-derived polycyclic aromatic hydrocarbons have been detected. In addition, several land-plant-derived aromatic biomarkers such as retene, cadalene, simonellite, tetrahydroretene and C_{19} isohexylalkylnaphthalene have been observed. While these terrestrial biomarkers could be clearly recognized in the middle Silurian – lower Devonian samples, their presence in the upper Ordovician – lower Silurian sediments is more doubtful, due to their much lower abundances. The identified land plant biomarkers in our samples show a fairly good correlation with the occurrence and abundance of cryptospores and trilete spores, derived from bryophytes and tracheophytes, which covered the emerged lands around the Ghadamis Basin during the Silurian and Devonian. The early tracheophytes (e.g. *Cooksonia*, lycophytes and zosterophylls) are therefore suggested as a new terrestrial source for most of the detected saturated and aromatic biomarkers in sediments of Middle Silurian to Early Devonian age.

Keywords: terrestrialization, Ghadamis basin; Ordovician, Silurian; Devonian; land plant biomarkers; crytospores; trilete spores, tracheophytes; *Cooksonia*

3.2. Introduction

The Ordovician to Devonian time interval is a key period for the development of life on emerged lands: the so-called terrestrialization process, which includes the origin and early evolution of land plants and their progressive spread on the continents. Traditionally, the terrestrialization process is studied by means of the analysis of pollen, spores and plant macrofossils. While microfossil evidence supports a Middle Ordovician age for the origin of land plants, based on the earliest occurrence (Darriwilian) of bryophytic-derived cryptospore assemblages in the subsurface of Saudi Arabia (Strother et al., 1996) and, more controversially, from a section in Bohemia (Vavrdovà, 1990), the earliest micro and macrofossils ascribed to vascular plants are of Middle Silurian age (Edwards and Wellman, 2001). An alternative route to obtain information on the evolution of early land plants is analysis of the organic molecules that have been preserved in the sediments. Although several biomarker reports on the lipids of sediments or extracts from Palaeozoic plants older than Middle Devonian have been published (e.g. Sheng et al., 1992; Ewbank et al., 1996), few of these have paid special attention to relating the obtained biomarkers with the early Palaeozoic palynomorphs to further elucidate the terrestralization process and development of land plants.

In this context, the scope of this study was to test, first if land plant biomarkers in Ordovician – Devonian sediments could be identified and, in this case, if they could be related to specific taxonomic groups based on the comparison with the microfossil contents of the sediments. For this, we performed molecular biomarker analyses on two clastic sedimentary sequences from the subsurface of the Ghadamis Basin in southern Tunisia spanning a stratigraphic interval from Late Ordovician to Early Devonian (boreholes TT1 and MG1). The choice of these two sections was based on their abundant and well-preserved palynological contents, which include amorphous and structured organic debris, marine microphytoplankton, and miospores represented by both cryptospores and trilete spores (Vecoli et al., 2009; Spina and Vecoli, 2009). In the study area and in the material available to us, lower and middle Ordovician sediments are mainly marine and their palynological content does not provide any evidence of input from terrestrial sources. For this reason the present biomarker study has been conducted on sediments containing some evidence of terrestrial organic matter input, in the upper Ordovician through lowermost Devonian stratigraphic interval. This allows for a direct comparison of palynomorphs and lipid biomarkers.

3.3. Geological setting and previous studies

The Ghadamis basin is associated to a large intracratonic synform on the North African Platform, and covers large areas in northwestern Libya, southern Tunisia and easternmost Algeria (Fig. 3.1). It contains a thick sequence of Palaeozoic strata which are only weakly affected by tectonic metamorphism, overlain by Mesozoic and Cenozoic sediments (Underdown and Redfern, 2008). The Palaeozoic sequences of the Ghadamis Basin are well known as hosting some of the largest Palaeozoic-sourced oil field in North Africa (El-Arnauti, 1988). Stratigraphic palynology has constituted the primary tool for the dating and correlation of the Palaeozoic strata in the subsurface of the basin (Deunff and Massa, 1975; Molyneux, 1988; among others), and the extremely rich and well preserved palynological content of these sediments are also well suited for detailed taxonomic and evolutionary studies of marine microphytoplankton and miospores (Vecoli, 2000; Vecoli and Le Hérissé, 2004, Vecoli et al., 2009; Spina and Vecoli, 2009).

Samples from borehole TT1 cover the uppermost Ordovician to upper Silurian interval, occurring between -1313.6 m and -1239.0 m core depth (Fig. 3.2). The investigated MG1 core section ranges between -3711.0 m and -2890.0 m core depth and allows a relatively complete coverage of middle Silurian to lowermost Devonian interval (Fig. 3.2).

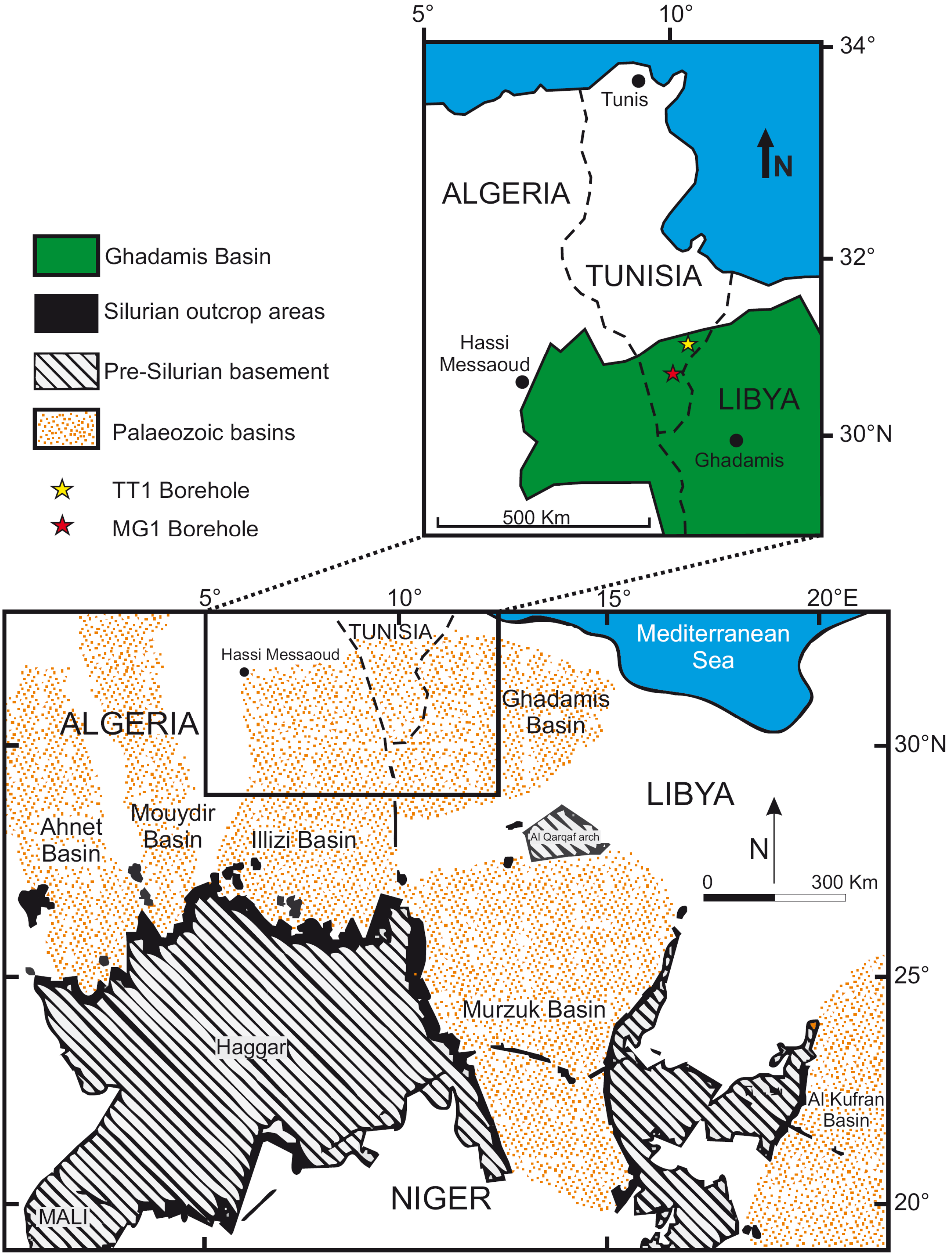

Figure 3.1: Location and simplified geological map of boreholes TT1 and MG1

The TT1 borehole stratigraphy was described in detail by Massa (1988) and Vecoli et al. (2009). The lower part of this core section (Fig. 3.2) consists of the latest Ordovician alternations of immature sandstones, siltstones and dark grey shales (Djeffara Formation). An early to late Silurian silty-shaley succession formally named the Argiles Principales Formation takes place above this last formation. Organic-rich black shales and shaley marls occur in the lower part of the Argiles Principales Formation. These sediments represent the "hot shale" interval in the core section (Vecoli et al., 2009).

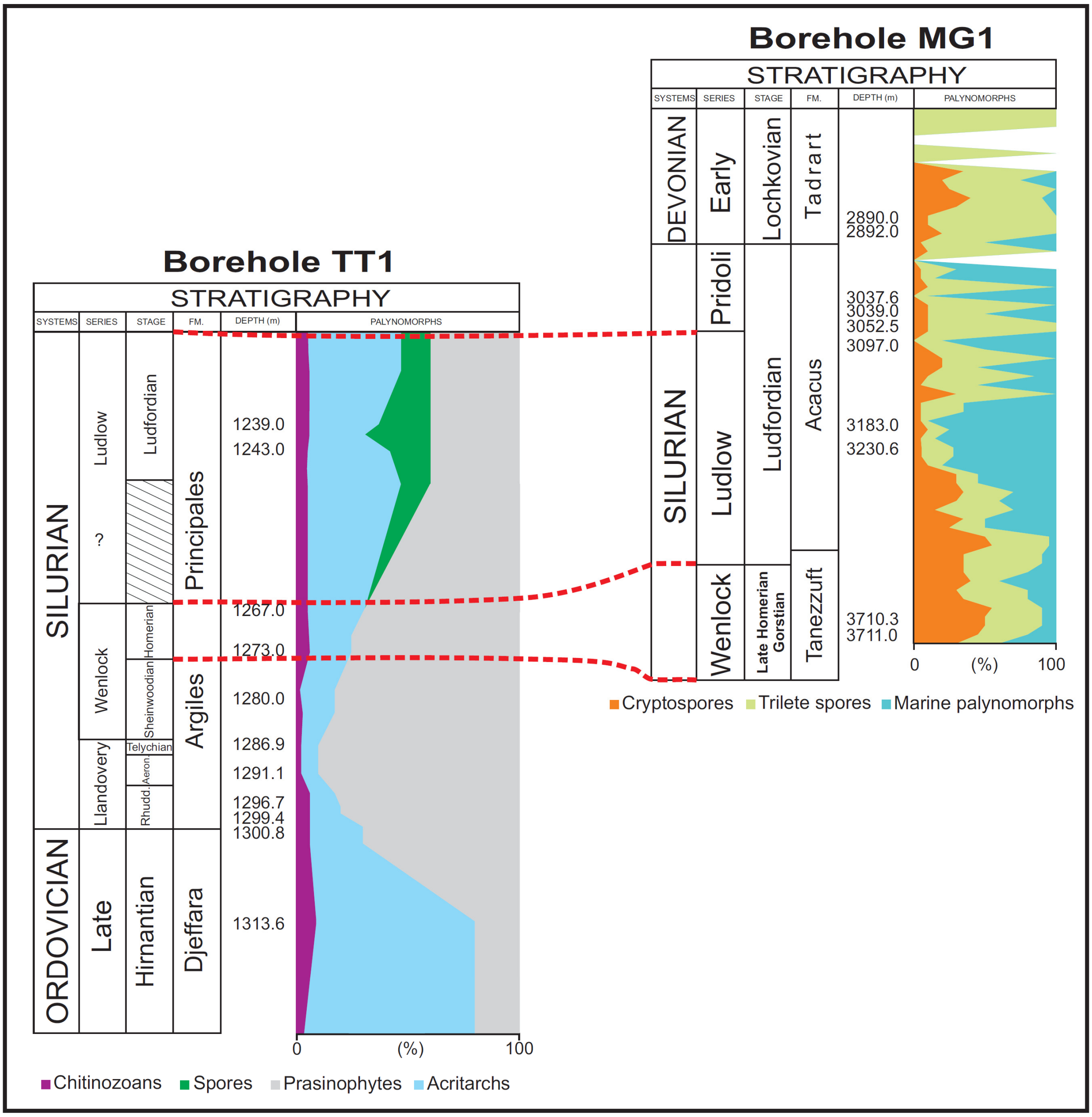

Figure 3.2: Stratigraphy and palynological content of the TT1 and MG1 boreholes (modified from Vecoli et al., 2009 and Spina and Vecoli, 2009). Indicated depths correspond to the analysed samples

The MG1 borehole (Fig. 3.2) shows the vertical succession of the middle Silurian marine mudstones, shales and sandstones (Tanezzuft Formation), the overlying late Silurian marine sandstones and mudstones of the Acacus Formation and finally, the Early Devonian continental sandstones and mudstones (Tadrart Formation) (Underdown and Redfern, 2008; Spina and Vecoli, 2009).

Previous studies on palynofacies and the organic carbon isotope composition of terminal Ordovician through Silurian sediments of TT1 samples were carried out by Vecoli et al. (2009). In addition, Spina and Vecoli (2009) provided a detailed description of miospores assemblages from borehole MG1 contributing to the palaeoclimatic and palaeofloristic evolution across the middle Silurian to Early Devonian times.

3.4. Analytical methods

3.4.1. Experimental procedures

All 21 core samples were studied by Rock-Eval pyrolysis and biomarker analysis. The Rock-Eval analyses for borehole TT1 were made by Vecoli et al. (2009). For the rest of the samples, Rock-Eval pyrolysis was performed on 100mg of ground rock with a Turbo model RE6 pyrolyzer (Vinci Technologies) at the Institut Français du Pétrole (IFP) using the conventional temperature program described in Lafargue et al. (1998): the pyrolysis oven temperature was programmed with 25 °C/min from 300°C (held 3 min) to 650°C (held 3 min). For biomarker analyses, rock fragments were extracted with dichloromethane (DCM) during 24 h in the refrigerator, in order to remove possible contamination on the sample surface. After this first extraction, the rock fragments were crushed to enable extraction of the lipids preserved inside the rock. Approximately 30 g of pulverised samples were extracted with a mixture of methanol (MeOH) and dichloromethane (DCM) (1/2, v/v) for 24 h with extensive stirring. This second extract was dried by means of roto-evaporation and partly re-solubilized in cyclohexane. The cyclohexane-soluble fraction (maltenes) was further separated by column chromatography.

The aliphatic fraction was recovered from the maltenes by elution with cyclohexane on an activated silica column. Subsequent elution with a mixture of cyclohexane - DCM (2/1, v/v) recovered the aromatic fraction after which the polar fraction was recovered by elution with a mixture DCM – MeOH (2/1, v/v).

3.4.2. Gas chromatography – mass spectrometry (GC-MS)

The aliphatic and aromatic fractions were analysed by gas chromatography – mass spectrometry (GC-MS) using a Trace GC 2000 gas chromatograph and a DB5ht column (30 m length, 0.25 mm internal diameter, 0.1 µm film thickness). The oven temperature was programmed as follows 100 °C for 1 minute, 100 °C – 310°C at a rate of 4 °C/min followed by an isothermal period of 16.5 min at 310 °C. Helium was used as carrier gas. The mass spectrometer was operated in the EI mode at electron energy of 70 eV. Samples were analysed in full scan (m/z 50 – 700; scan rate 1000 amu/s; scan speed 1.49/s, scan time 0.67 s) The organic compounds were identified by comparison of their mass spectra and retention times with available published data.

3.5. Results

3.5.1. Rock-Eval

The total organic carbon (TOC) and Rock-Eval parameters from MG1 core samples are displayed in Table 3.1. The vertical variations of TOC content, the hydrogen index (HI) and the source rock potential parameter (S_2) show a relatively similar trend throughout borehole MG1 (Fig. 3.3). The TOC values range between 0.11 and 0.42 % whereas the HI and Tmax values vary between 67 to 160 mg HC/g TOC and 434 to 439 °C, respectively (Figs. 3.3 and 3.4; Table 3.1).

The MG1 core samples have an average Tmax of 436 °C. The typical HI-Tmax diagram (Fig. 3.4; Espitalié et al., 1986) reflects that most of the investigated samples plot in the type II – III kerogen part. The Rock-Eval analyses for borehole TT1 showed an average Tmax of 437 °C and most of the TT1 core samples also plotted in the type II – III organic matter sector (Vecoli et al., 2009).

Table 3.1: TOC and Rock-Eval parameters from MG1 borehole

	Parameters				
Samples	TOC (%)	Tmax (°C)	HI (mgHC/gTOC)	S_1 (mg/g)	S_2 (mg/g)
MG1-2890.0	0.11	434	109	0.01	0.12
MG1-2892.0	0.15	435	67	0.01	0.10
MG1-3037.6	0.25	435	160	0.14	0.40
MG1-3039.0	0.22	437	145	0.01	0.32
MG1-3052.5	0.20	437	70	0.01	0.14
MG1-3097.0	0.23	436	126	0.01	0.29
MG1-3183.0	0.31	438	148	0.03	0.46
MG1-3230.3	0.17	435	112	0.02	0.19
MG1-3710.3	0.42	439	124	0.10	0.52
MG1-3711.0	0.35	438	97	0.07	0.34

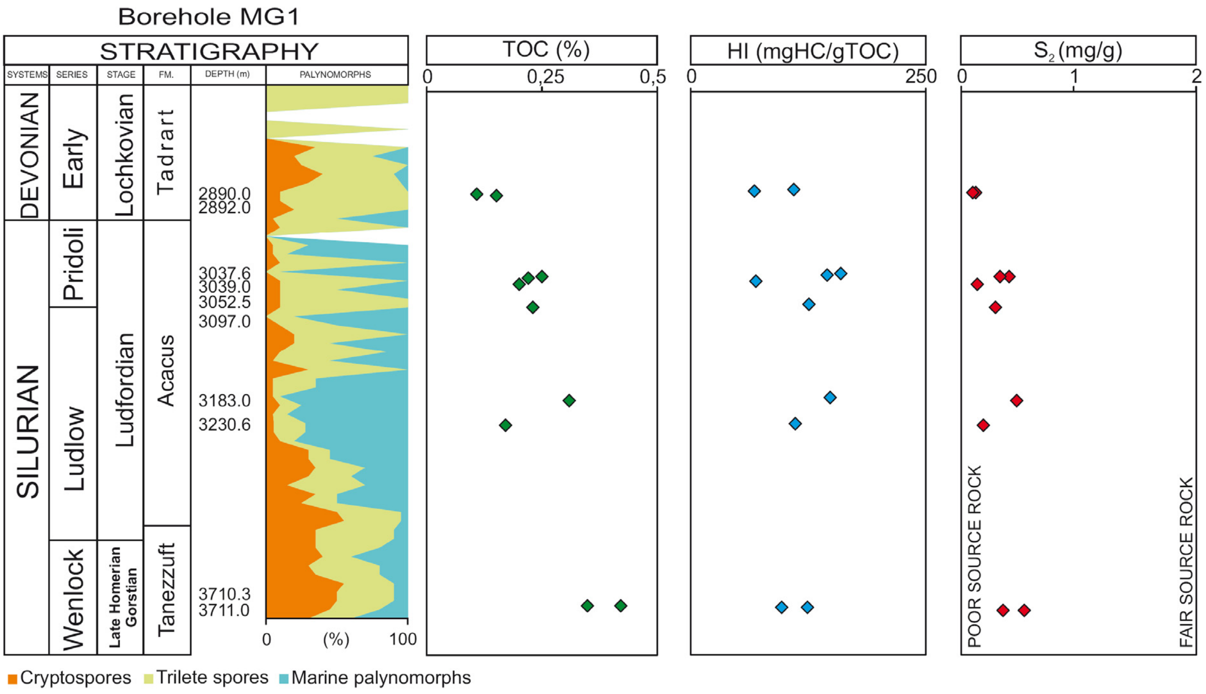

Figure 3.3: Stratigraphic variations of the TOC content and the Rock-Eval parameters S_2 and HI in core MG1. Stratigraphy and palynological content from Spina and Vecoli (2009). Indicated depths correspond to the analysed samples

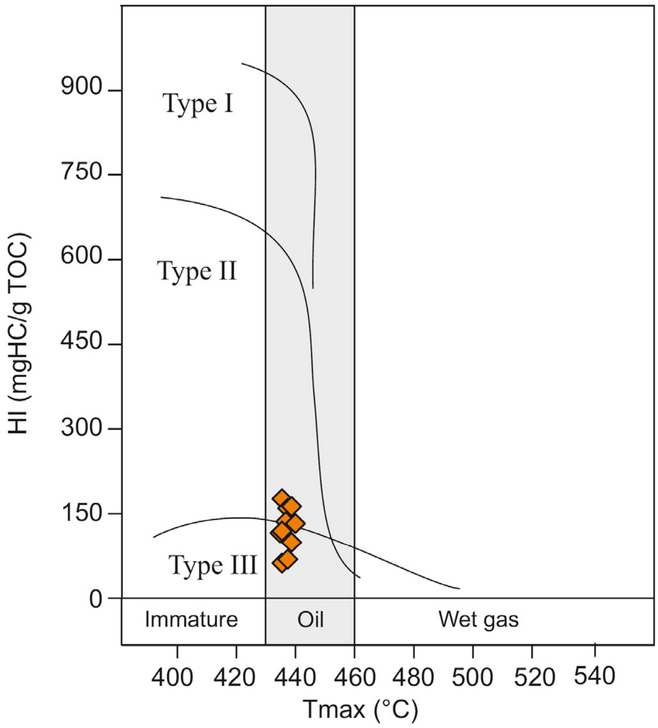

Figure 3.4: Plot of HI vs Tmax values for core MG1 (diagram from Espitalié et al., 1986)

3.5.2. Aliphatic fractions

The aliphatic fractions obtained from upper Ordovician to lower Devonian core samples are generally dominated by C_{14} to C_{35} *n*-alkanes (Fig. 3.5). Based on the distribution of *n*-alkanes, two major groups of samples can be recognized:

Group 1 comprises the investigated TT1 interval (-1313.6 m and -1267.0 m core depth) conformed by upper Ordovician to lower Silurian sediments. This group is characterized by a marked predominance of short chain *n*-alkanes with a maximum at C_{16} or C_{17}. However, Hirnantian sample of Djeffara Formation (TT1-1313.6) also contains minor amounts of long-chain *n*-alkanes (> C_{20} *n*-alkanes; Fig. 3.5).

Group 2 includes the remaining (middle Silurian to lower Devonian samples), up to MG1-2890. This group reveals a marked presence of long chain *n*-alkanes with no odd-over-even predominance (> C_{22} *n*-alkanes; Fig. 3.5), though short chain C_{17} – C_{18} *n*-alkanes are also present in significant proportion. Devonian sample MG1-2890 is the only one that has abundant heavy *n*-alkanes with *n*-C_{27} as the dominant compound in the aliphatic fraction (Figs. 3.5 and 3.6B).

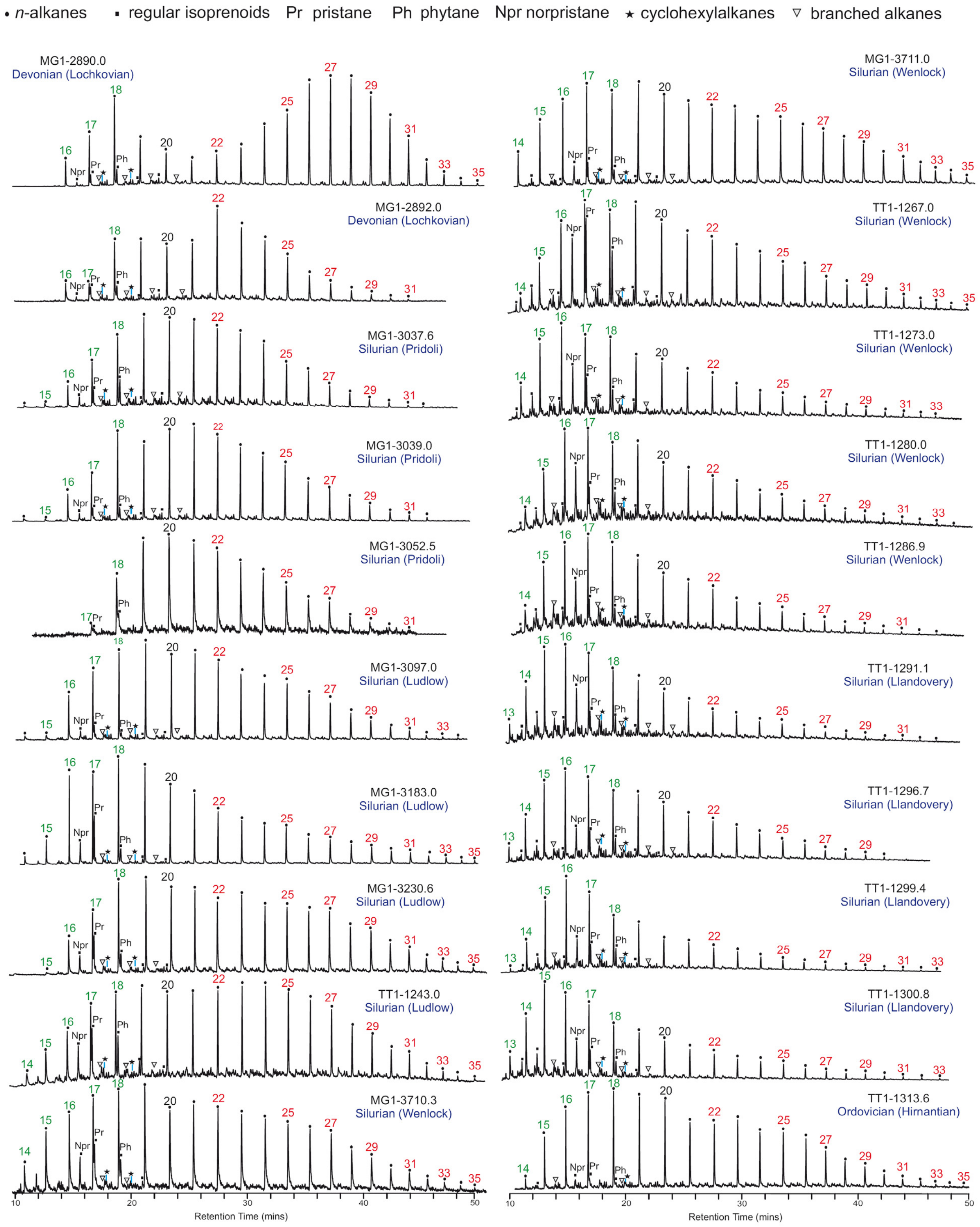

Figure 3.5: Mass chromatograms m/z 57 of aliphatic fractions from upper Ordovician to lower Devonian core samples, showing the distribution of *n*-alkanes, regular isoprenoids, cyclohexylalkanes and branched alkanes. Numbers above symbols indicate carbon number. Samples are displayed in stratigraphic order from bottom right to top left

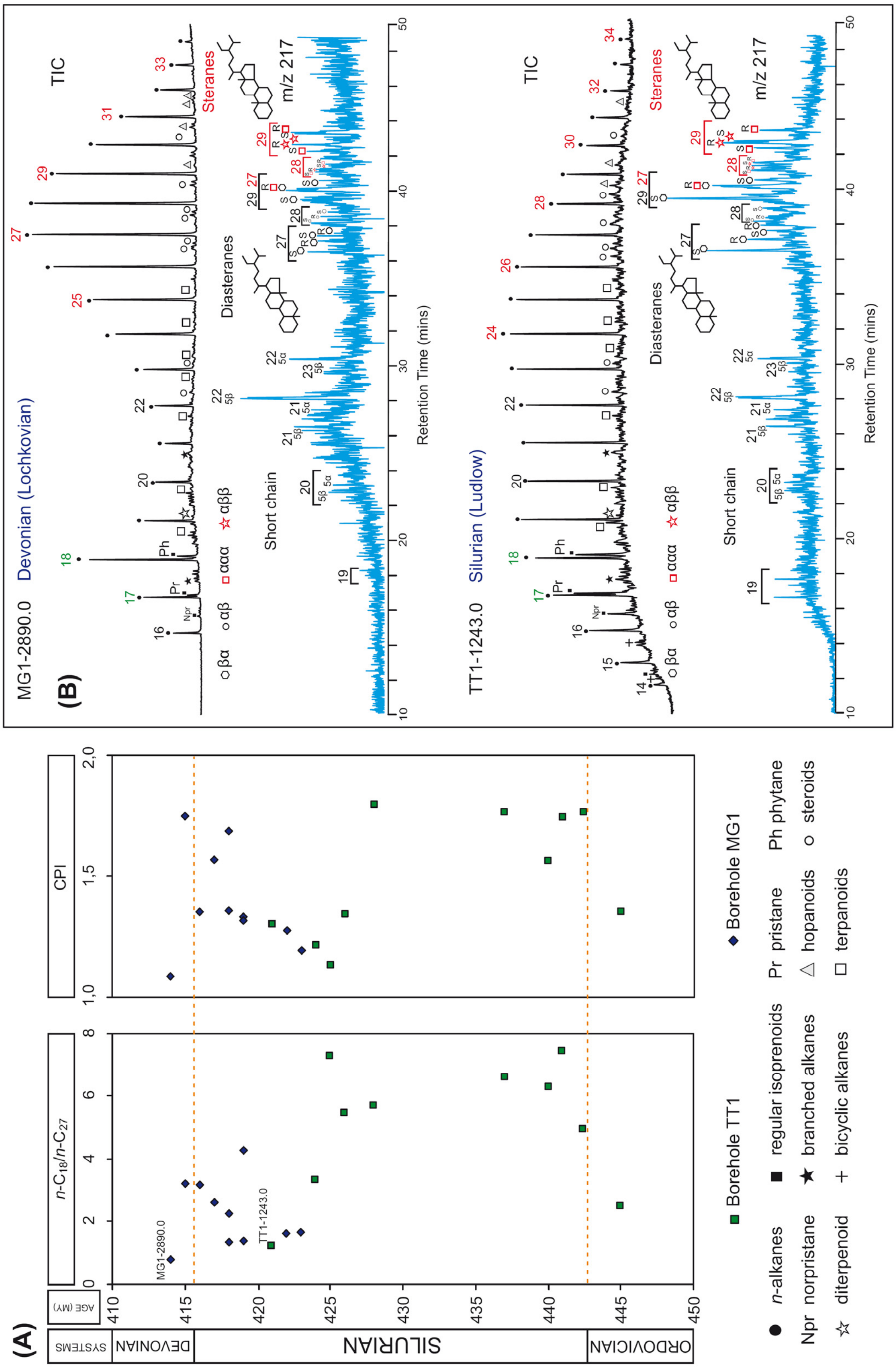

Figure 3.6: (A) Stratigraphic variations of n-C_{18}/n-C_{27} ratio and the carbon preference index in studied core samples. The n-C_{18}/n-C_{27} ratio was calculated using peak height of n-octadecane (n-C_{18}) and n-heptacosane (n-C_{27}) on m/z 57 chromatogram. **(B)** Total ion current (TIC) and m/z 217 mass chromatograms of aliphatic fraction from extracts of two selected samples. Numbers above symbols denote the carbon number. The molecular structures represent the standard diasterane and sterane skeletons

Romero-Sarmiento, M.-F., et al. (In preparation)

The stratigraphic variations of *n*-C_{18}/*n*-C_{27} ratio and the carbon preference index (CPI) for studied core samples are shown in figure 3.6A. CPI ranges between 1.09 and 2.00 (Table 3.2). The *n*-C_{18}/*n*-C_{27} ratio is high for the lower Silurian (***Group 1***) and low for the middle Silurian to lower Devonian (***Group 2***) with some exceptions (Fig. 3.6; Table 3.2).

Series of C_{14} to C_{21} regular isoprenoids dominated by norpristane (C_{18}), pristane (C_{19}) and phytane (C_{20}) were detected in all samples especially in lower Silurian sediments (Fig. 3.5). The pristane/phytane (Pr/Ph) ratio ranges from 0.75 to 7.25 (Table 3.2). The pristane/C_{17} *n*-alkane (Pr/*n*-C_{17}) ratio is > 1 except for TT1-1313.6, TT1-1286.9, MG1-3711.0 and MG1-2890. The phytane/C_{18} *n*-alkane (Ph/*n*-C_{18}) ratio is < 1 (Table 3.2; Fig. 3.7). Cyclohexylalkanes and branched alkanes without odd or even chain length predominance were also detected in low abundance (Fig. 3.5). Steranes and diterpanes were only observed in very low amounts. These latter compounds together with short-chain steroids dominated by C_{22} have only been clearly identified in four samples: TT1-1313.6; TT1-1267.0; TT1-1243.0 and MG1-2890.0 (e.g. Fig. 3.6).

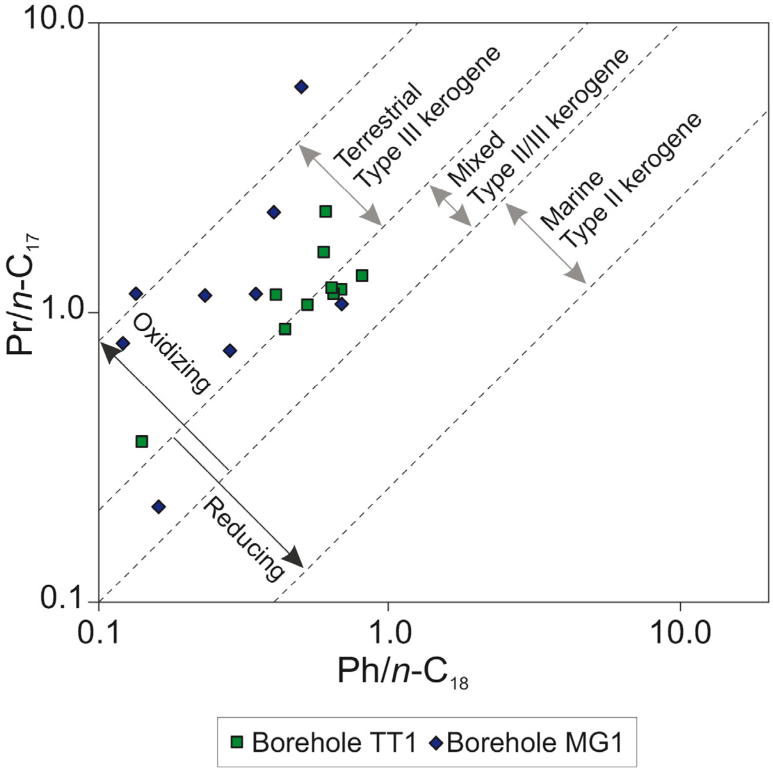

Figure 3.7: Plot of Pr/*n*-C_{17} vs. Ph/*n*-C_{18} for TT1 and MG1 core samples (diagram from Hunt, 1995)

Table 3.2: Biomarker ratios from the aliphatic fractions of the investigated core samples

	Parameters				
Samples	Pr/Ph$^{(1)}$	Pr/C$_{17}$$^{(2)}$	Ph/C$_{18}$$^{(3)}$	n-C$_{18}$/n-C$_{27}$$^{(4)}$	CPI$^{(5)}$
MG1-2890.0	0.75	0.21	0.16	0.78	1.09
MG1-2892.0	2.25	6.00	0.50	3.20	1.75
MG1-3037.6	1.08	1.08	0.68	3.17	1.35
MG1-3039.0	1.56	1.17	0.35	2.60	1.57
MG1-3052.5	N.D.	N.D.	N.D.	1.33	1.69
MG1-3097.0	4.67	0.78	0.12	2.27	1.36
MG1-3183.0	7.25	1.16	0.13	4.29	1.33
MG1-3230.3	3.10	2.21	0.40	1.39	1.32
TT1-1239.0	N.D.	N.D.	N.D.	10.00	2.00
TT1-1243.3	1.38	1.06	0.52	1.25	1.31
MG1-3710.3	3.83	1.15	0.23	1.63	1.28
MG1-3711.0	2.86	0.74	0.28	1.67	1.20
TT1-1267.0	2.82	1.15	0.41	3.38	1.22
TT1-1273.0	2.62	1.62	0.59	7.33	1.14
TT1-1280.0	2.07	1.21	0.64	5.50	1.35
TT1-1286.9	2.10	0.88	0.43	5.75	1.80
TT1-1291.1	2.08	1.17	0.65	6.67	1.77
TT1-1296.7	2.00	1.18	0.68	6.33	1.57
TT1-1299.4	2.33	1.33	0.80	7.50	1.75
TT1-1300.8	3.22	2.23	0.60	5.00	1.77
TT1-1313.6	2.75	0.35	0.14	2.55	1.36

N.D. ; not determined.

(1) Pr/Ph = Pristane/Phytane; m/z = 183 (Didyk et al., 1978).

(2) Pr/n-C$_{17}$= Pristane/n-heptadecane; m/z = 183 (Didyk et al., 1978).

(3) Pr/*n*-C_{18}= Pristane/*n*-octadecane; m/z = 183 (Didyk et al., 1978).

(4) *n*-C_{18}/*n*-C_{27}= *n*-octadecane/*n*-heptacosane; m/z = 57 (Didyk et al., 1978).

(5) CPI= Carbon Preference Index = $\{[(n\text{-}C_{25}+n\text{-}C_{27}+n\text{-}C_{29}+n\text{-}C_{31}+n\text{-}C_{33})/(n\text{-}C_{26}+n\text{-}C_{28}+n\text{-}C_{30}+n\text{-}C_{32}+n\text{-}C_{34})]+[(n\text{-}C_{25}+n\text{-}C_{27}+n\text{-}C_{29}+n\text{-}C_{31}+n\text{-}C_{33})/(n\text{-}C_{26}+n\text{-}C_{28}+n\text{-}C_{30}+n\text{-}C_{32}+n\text{-}C_{34})]\}/2$; m/z = 57 (Bray and Evans, 1961).

Hopanes, moretanes, tricyclic terpanes, bicyclic alkanes and a diterpanoid were also recognized in the aliphatic fractions (Fig. 3.6). While tricyclic terpanes were clearly detected in all the samples (Fig. 3.8), hopanes and moretanes were mostly identified in middle Silurian to lower Devonian sediments (***Group 2***; Fig. 3.8). However, hopanes and moretanes were also recognized in the Ordovician sample (TT1-1313.6).

The αβ-hopanes are dominated by 17α(H),21β(H)-hopanes (22R and 22S epimers) from C_{27} to C_{33}, with C_{29} or C_{30} representatives dominating (Fig. 3.8). The βα-moretanes vary between C_{27} to C_{32}, with a maximum at C_{30} moretanes (Fig. 3.8). The tricyclic terpanes are represented by a series of C_{19} to C_{26} cheilantanes in all the samples (Fig. 3.8).

The aliphatic extracts from some Silurian to Devonian core samples also exhibit the occurrence of ten bicyclic alkanes ranging from C_{15} to C_{16} carbon atoms and a tricyclic diterpenoid, visible on the expanded m/z 109 + 123 + 193 mass chromatograms (Fig. 3.9). Peak assignments for these detected aliphatic compounds are summarized in Table 3.3.

Bicyclic alkanes containing the drimane and homodrimane skeletons were mainly identified in Silurian sediments (Fig. 3.9; Noble, 1986; Noble et al., 1987), whereas the tricyclic diterpenoid norabietane was recognized in middle Silurian to Devonian samples (Fig. 3.9; Philp, 1985).

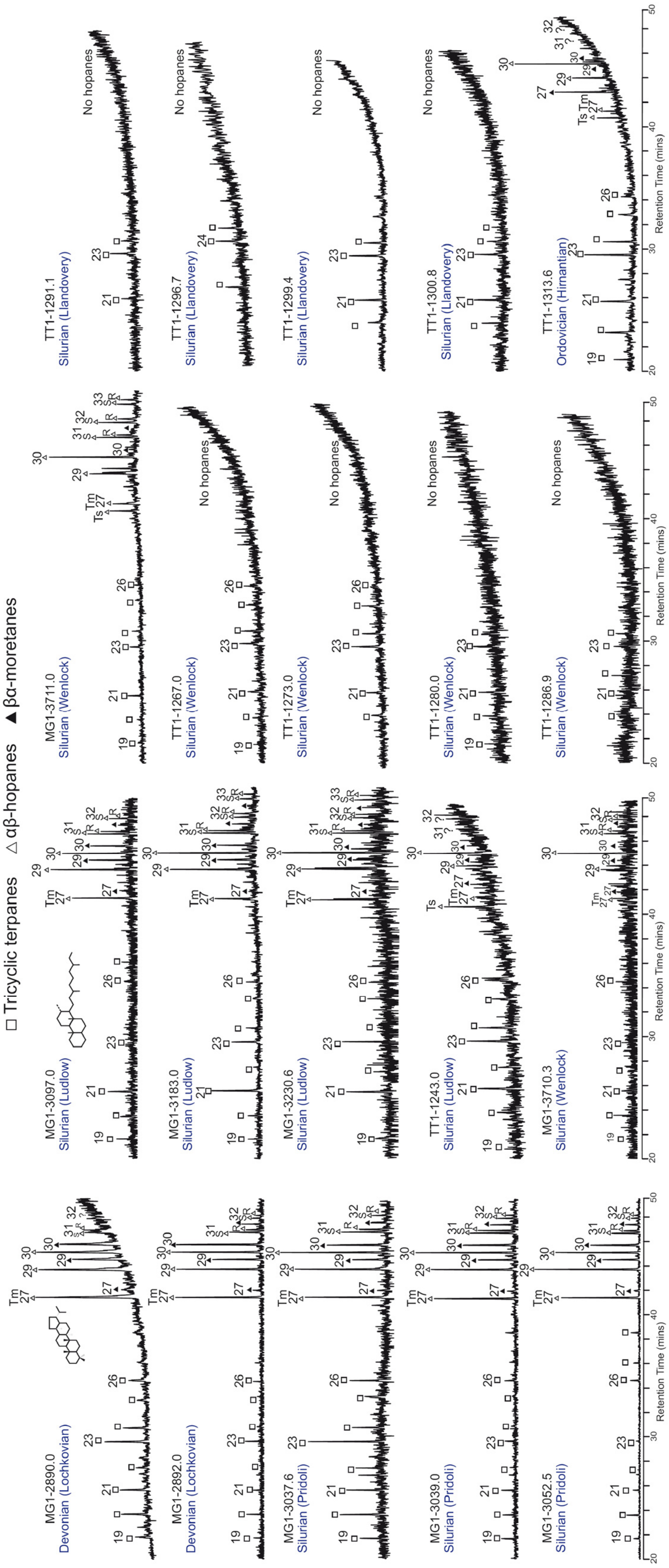

Figure 3.8: Mass chromatograms m/z 191 of aliphatic fractions from Ordovician to Devonian core samples, showing the distribution of hopanes, moretanes and tricyclic terpanes. Numbers above symbols indicate carbon number. The molecular structures represent the standard hopane and tricyclic terpane skeletons

Romero-Sarmiento, M.-F., et al. (In preparation)

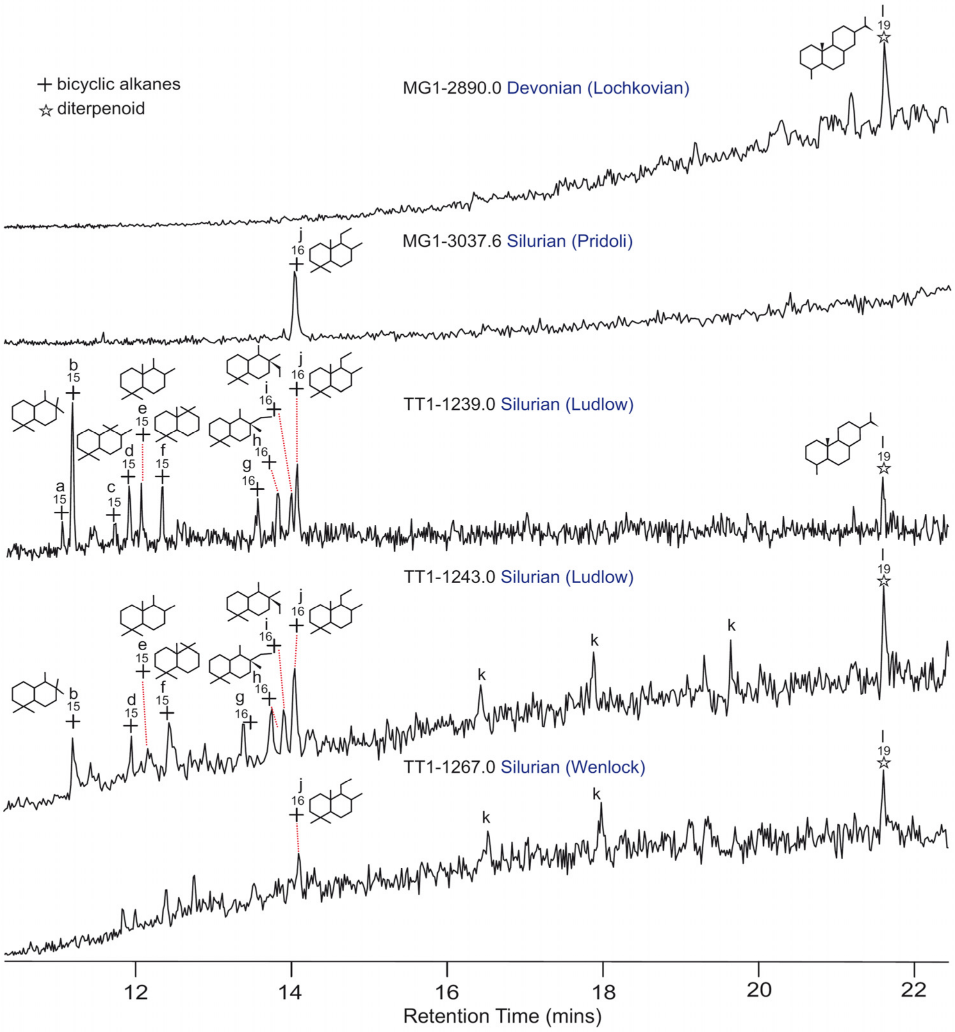

Figure 3.9: Partial m/z 109 + 123 + 193 mass fragmentograms of aliphatic fractions from extracts of selected Silurian to Devonian samples. Peak assignments are given in Table 3.3. Numbers above symbols denote carbon number

Table 3.3: Bicyclic alkanes and diterpenoid identified in Silurian – Devonian core samples from Ghadamis Basin, North Africa

Peak	m/z	MW	Compound	References
a	193	208	C_{15} bicyclic alkane? $^{+}$	-
b	193	208	C_{15} bicyclic alkane (Compound A)	Noble, 1986
c	109	208	C_{15} bicyclic alkane? $^{+}$	-
d	193	208	C_{15} bicyclic alkane (Compound B)	Noble, 1986
e	123	208	8β(H)-drimane	Noble et al., 1987
f	123	208	C_{15} bicyclic alkane (Compound D)	Noble, 1986
g	179	222	C_{16} bicyclic alkane? $^{+}$	-
h	109	222	C_{16} bicyclic alkane (Compound F)	Noble, 1986
i	109	222	C_{16} bicyclic alkane (Compound G)	Noble, 1986
j	123	222	8β(H)-homodrimane	Noble et al., 1987
k	179	-	Unknown compounds? $^{+}$	-
l	109	262	Norabietane	Philp, 1985

($^{+}$) Mass spectra are shown in Appendix A.6

3.5.3. Aromatic fractions

Forty-six aromatic hydrocarbons were detected by their characteristic mass fragments, using the partial m/z 178 + 183 + 184 + 192 + 198 + 202 + 206 + 219 + 220 + 223 + 228 + 237 + 252 mass chromatograms of the aromatic fractions from TT1-1243.0 (Silurian) and MG1-2890.0 (Devonian) core samples (Fig. 3.10; Table 3.4). These two representative core samples were selected considering their abundant terrestrial palynomorphs content (e.g. miospores; Fig. 3.2) and their biomarker record (e.g. long chain *n*-alkanes; Figs. 3.5 and 3.6). However, Table 3.5 summarizes the most predominant aromatic compounds recovered in all studied samples.

Phenanthrene (P), methylphenanthrene (MP), ethylphenanthrene (EP), dimethylphenanthrene (DMP) and trimethylphenanthrene (TMP) isomers represent the most abundant components in all aromatic fractions (Table 3.5; e.g. Fig. 3.10). Accordingly, methylphenanthrenes indices (MPI 1, MPR and MPI 2) could be calculated for most of the samples (Table 3.6). In general, the MPI 1 and MPI 2 ratios are < 1 (MPI 1 = 0.53 – 0.89; MPI 2 = 0.51 – 1.00), whereas MPR ratios are > 1 except for MG1-3052.5 and MG1-3039.0 (Table 3.6). While C_{19} isohexylalkylnaphthalene was detected in middle Silurian to lower Devonian sediments (Table 3.5), tetramethylnaphthalene (TeMN) isomers were found in most samples in low amounts (e.g. Fig. 3.10; Tables 3.4 and 3.5). Dibenzothiophene (DBT) and methyldibenzothiophene (MDBT) homologues were also detected (Fig. 3.10; Table 3.4). The relative abundances of 1,2,5,6- and 1,2,3,5-TeMN are higher than those of 1,3,6,7-TeMN for most of the samples (e.g. samples TT1-1243.0 and MG1-2890.0; Fig. 3.10). The 4-MDBT is the predominant methyldibenzothiophene isomer in sample MG1-2890.0 (Fig. 3.10), but also in upper Ordovician to middle Silurian samples (e.g. TT1-1300.8; TT1-1280.0; TT1-1267.0; among others; not shown in Fig. 3.10).

The polycyclic aromatic hydrocarbons (PAHs) retene, cadalene, simonellite, tetrahydroretene, pyrene, fluoranthene and benzo[a]anthracene were clearly recognized in the middle Silurian to lower Devonian sediments from the uppermost part of the Argiles Principales and Tadrart Formations (Table 3.5; e.g. Fig. 3.10). Retene is the most abundant aromatic compound in the partial mass chromatogram of the lower Devonian sample MG1-

2890.0 (Fig. 3.10). In addition, the PAH perylene has been identified in most of these middle Silurian to lower Devonian samples (Table 3.5; e.g. Fig. 3.10).

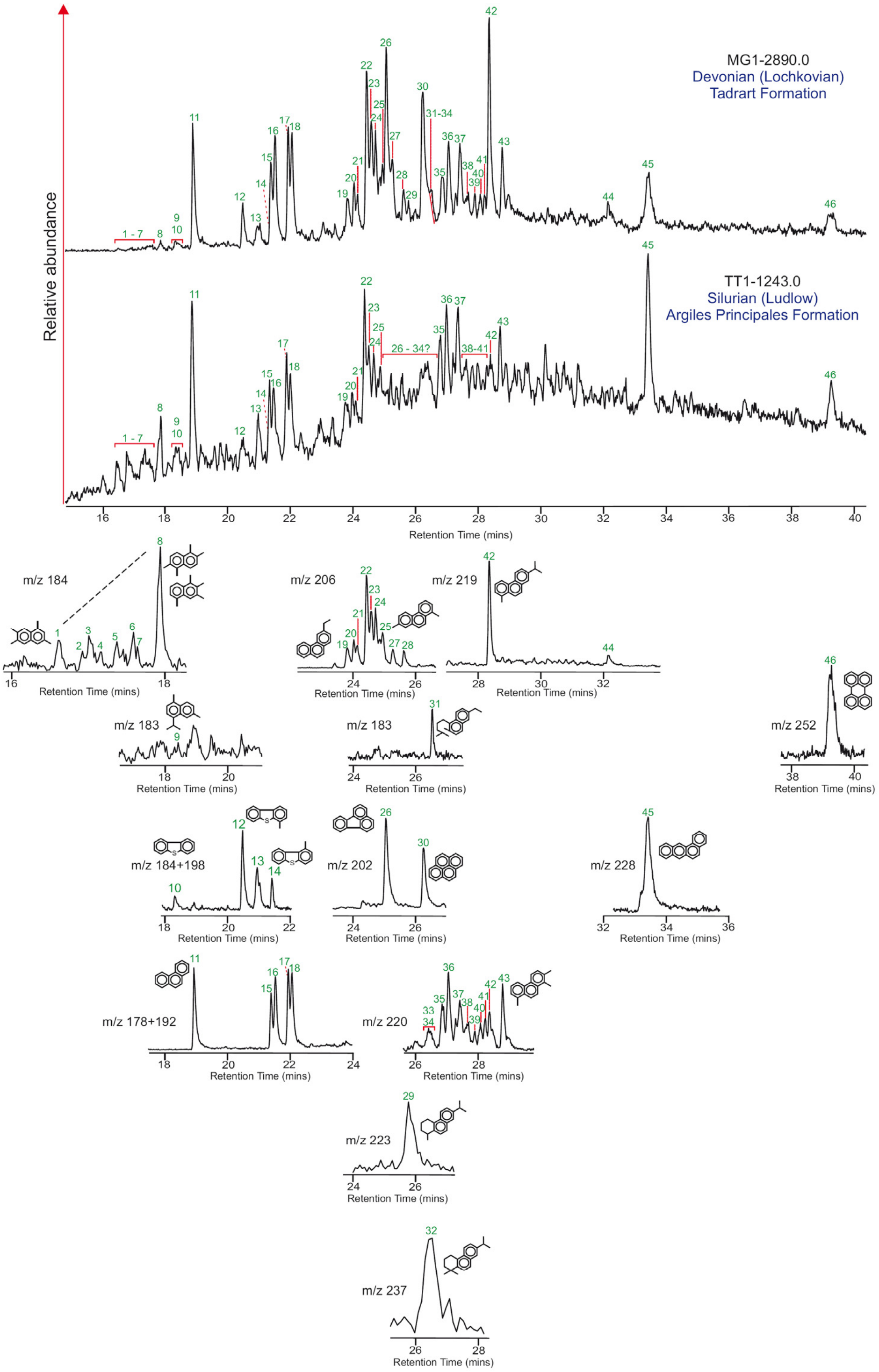

Figure 3.10: Partial m/z 178 + 183 + 184 + 192 + 198 + 202 + 206 + 219 + 220 + 223 + 228 + 237 + 252 mass chromatograms of aromatic fraction from extracts of two selected samples: TT1-1243.0 (Silurian) and MG1-2890.0 (Devonian). Peak assignments are given in Table 3.4

Table 3.4: Aromatic hydrocarbons identified in Silurian – Devonian core samples from Ghadamis Basin, North Africa

Peak	m/z	Compound
1	184	1,3,6,7-TeMN
2	184	1,2,4,6- + 1,2,4,7- + 1,4,6,7-TeMN
3	184	1,2,5,7-TeMN
4	184	2,3,6,7-TeMN
5	184	1,2,6,7-TeMN
6	184	1,2,3,7-TeMN
7	184	1,2,3,6-TeMN
8	184	1,2,5,6- + 1,2,3,5-TeMN
9	183	Cadalene
10	184+198	Dibenzothiophene
11	178+192	Phenanthrene
12	184+198	4-MDBT
13	184+198	2+3-MDBT
14	184+198	1-MDBT
15	178+192	3-MP
16	178+192	2-MP
17	178+192	9-MP
18	178+192	1-MP
19	206	3-EP
20	206	2-EP + 9-EP + 3,6-DMP
21	206	1-EP
22	206	2,6- + 2,7-DMP
23	206	1,3- + 2,10- + 3,9- + 3,10-DMP
24	206	1,6- + 2,9-DMP
25	206	1,7-DMP
26	202	Fluoranthene
27	206	2,3- + 1,9-DMP
28	206	1,8-DMP
29	223	Tetrahydroretene
30	202	Pyrene
31	183	C_{19} Isohexylalkylnaphthalene
32	237	Simonellite
33	220	1,3,6- + 1,3,10- + 2,6,10-TMP
34	220	1,3,7- + 2,6,9- + 2,7,9-TMP
35	220	1,3,9- + 2,3,6-TMP
36	220	1,6,9- + 1,7,9- + 2,3,7-TMP
37	220	1,3,8-TMP
38	220	2,3,10-TMP
39	220	1,6,7-TMP
40	220	1,2,6-TMP
41	220	1,2,7-TMP
42	219	Retene
43	220	1,2,8-TMP
44	219, 234	C_4-Phenanthrene
45	228	Benzo[a]anthracene
46	252	Perylene

Abbreviations: TeMN – tetramethylnaphthalene, MDBT – methyldibenzothiophene, MP – methylphenanthrene, EP – ethylphenanthrene, DMP – dimethylphenanthrene, TMP – trimethylphenanthrene.

Table 3.5: Relative abundance of aromatic compounds identified in all the samples

Samples	Age	Cd	Re	Tre	Sim	C_{19}	Pery	Pyre	Fluo	Benz	TeMNs	APs
MG1-2890.0	Devonian	+++	+++	+++	++	++	++	+++	+++	++	++	+++
MG1-2892.0	Devonian	+++	+++	+++	++	++	+	++	++	+	++	+
MG1-3037.6	Silurian	++	++	++	++	++	+	++	++	+	++	+
MG1-3039.0	Silurian	++	++	++	++	++	+	++	++	+	++	++
MG1-3052.5	Silurian	++	++	++	++	++	+	++	++	++	++	++
MG1-3097.0	Silurian	++	++	++	++	++	+	++	++	++	++	+
MG1-3183.0	Silurian	++	++	++	++	++	+	+	+	+	++	+
MG1-3230.6	Silurian	+	+	+	+	+	-	+	+	+	++	+
TT1-1239.0	Silurian	+	+	+	+	+	+	+	+	+	+ +	++
TT1-1243.0	Silurian	+	+	+	+	+	++	+	+	+++	++	+++
MG1-3710.3	Silurian	+	+	+	+	+	+	-	-	+	+ +	+ +
MG1-3711.0	Silurian	+	+	+	+	+	+	-	-	++	++	+++
TT1-1267.0	Silurian	-	-	-	-	-	+	-	-	+ +	+ +	+++
TT1-1273.0	Silurian	-	-	-	-	-	+	-	-	++	++	+++
TT1-1280.0	Silurian	-	-	-	-	-	+	-	-	++	++	+++
TT1-1286.9	Silurian	-	-	-	-	-	-	-	-	++	++	+++
TT1-1291.1	Silurian	-	-	-	-	-	-	-	-	-	-	-
TT1-1296.7	Silurian	-	-	-	-	-	-	-	-	++	++	+++
TT1-1299.4	Silurian	-	-	-	-	-	-	-	-	++	++	+++
TT1-1300.8	Silurian	-	-	-	-	-	-	-	-	++	++	+++
TT1-1313.6	Ordovician	-	-	-	-	-	-	-	-	-	-	-

Legend: +++ abundant; ++ frequent; + poor to faire; - rare to poor

Abbreviations: Cd – cadalene; **Re** – retene; **Tre** – tetrahydroretene; **Sim** – simonellite; **C_{19}** – C_{19} isohexylakylnaphthalene; **Pery** – perylene; **Pyre** – pyrene; **Fluo** – fluoranthene; **Benz** – benzo[a]anthracene; **TeMNs** – tetramethylnaphthalenes; **APs** – alkylphenanthrenes (This last compound family includes phenanthrene and its C_1, C_2 and C_3 isomers)

Table 3.6: Methylphenanthrene (MP) indices from aromatic fractions of the investigated core samples

	Parameters		
Samples	MPI 1$^{(a)}$	MPR$^{(b)}$	MPI 2$^{(c)}$
MG1-2890.0	0.83	1.40	1.00
MG1-2892.0	N.D.	N.D.	N.D.
MG1-3037.6	N.D.	N.D.	N.D.
MG1-3039.0	0.89	0.94	0.87
MG1-3052.5	0.53	0.85	0.65
MG1-3097.0	N.D.	N.D.	N.D.
MG1-3183.0	N.D.	N.D.	N.D.
MG1-3230.3	N.D.	N.D.	N.D.
TT1-1239.0	N.D.	N.D.	N.D.
TT1-1243.3	0.68	1.33	0.71
MG1-3710.3	N.D.	N.D.	N.D.
MG1-3711.0	0.54	1.14	0.51
TT1-1267.0	0.80	1.71	0.84
TT1-1273.0	0.67	1.57	0.73
TT1-1280.0	0.70	1.43	0.73
TT1-1286.9	0.68	1.50	0.78
TT1-1291.1	N.D.	N.D.	N.D.
TT1-1296.7	0.71	1.63	0.76
TT1-1299.4	0.63	1.67	0.70
TT1-1300.8	0.70	2.20	0.80
TT1-1313.6	N.D.	N.D.	N.D.

N.D. ; not determined.

(a) MPI 1 = {1.5([2-MP]+[3-MP])}/{[P]+[1-MP]+[9-MP]}; m/z=178+192 (Radke et al., 1986).

(b) MPR = [2-MP]/[1-MP]; m/z=178+192 (Radke et al., 1986).

(c) MPI 2 = {3[2-MP]}/{[P]+[1-MP]+[9-MP]}; m/z=178+192 (Radke et al., 1986).

3.6. Discussion

3.6.1. Organic matter maturity

The average Tmax from upper Ordovician to lower Devonian sediments (437°C for TT1 core; Vecoli et al., 2009 and 436°C for MG1 core; Table 3.1) indicates that the organic matter has just reached the beginning of the oil window. The relatively low thermal maturity of these sediments is further supported by: (1) a high carbon preference index (CPI > 1; Table 3.2; Bray and Evans, 1961); (2) the distribution patterns of TeMN isomers with 1,2,5,6- and 1,2,3,5-TeMN dominating such as in sample MG1-2890.0 (Fig. 3.10; van Aarssen et al., 1999); (3) the alkylphenanthrenes show the predominance of 9-MP over the 1-MP (Fig. 10; Table 3.5; Radke et al., 1982); (4) a certain contribution of $\beta\alpha$-moretanes (Fig. 3.8; Seifert and Moldowan, 1980) and finally, (5) the colour of palynomorphs, ranging from pale yellow to light brown, and corresponding to a TAI (Thermal Alteration Index; Staplin, 1982) between 2.0 and 2.5, also equivalent to a AAI (Acritarch Alteration Index; Williams et al., 1998) of ca. 2. In contrast, Ordovician and three Silurian samples reveal the presence of 18α-22,29,30-trisnorneohopane (Ts) which has been widely considered as a thermal maturity marker in sediments (Fig. 3.8; Seifert and Moldowan, 1978; 1986). However, in general, the low thermal history allows for a minor maturity effect on the distribution of aliphatic and aromatic biomarkers.

3.6.2. Organic matter origin

The HI values of the MG1 core samples (67 – 160 mg HC/g TOC; Table 3.1) demonstrate that the organic matter (OM) in middle Silurian to lower Devonian sediments is mainly type II – III kerogen, suggesting a mixture of terrestrial and marine OM (Fig. 3.4). From their low TOC (0.11 – 0.42%) and S_2 values, the studied samples from borehole MG1 show poor to fair source rock potential (Fig. 3.3; Table 3.1). Most of the TT1 samples have also of type II – III OM, similarly indicating a mixture of organic matter of terrestrial and marine origin in this upper Ordovician to upper Silurian section (Vecoli et al., 2009). The type of organic matter is also corroborated by the cross plot of Pr/n-C_{17} vs. Ph/n-C_{18} (Fig. 3.7; Hunt, 1995). Within this diagram, most of the TT1 core samples plot in mixed marine and terrestrial type II – III organic matter whereas most of the MG1 sediments plot in the terrestrial type III OM. In addition, the biomarker content of the aliphatic and aromatic fractions from Tunisian samples also suggests a mixed marine and terrestrial origin for the organic matter of Late Ordovician to Early Devonian age (both cores TT1 and MG1). This interpretation is also consistent with the palynological content showing an abundance of algal (acritarchs, prasinophytes, chitinozoans) and terrestrial palynomorphs (cryptospores, trilete spores) in these core samples (Fig. 3.2; Vecoli et al., 2009; Spina and Vecoli, 2009).

3.6.2.1. Marine contribution to the organic matter

An increase in the abundance of *prasinophyceae–*derived microfossils (*Tasmanites*, *Leiosphaeridia* and *Cymatiosphaera*) occurs at the base of the Argiles Principales Formation, starting from sample TT1-1300.8 to sample TT1-1267.0 (Fig. 3.2; ***Group 1***; Vecoli et al., 2009). Acritarchs are also relatively abundant, suggesting conditions of normal marine platform environment throughout this interval (Vecoli et al., 2009). Chitinozoans, acritarchs, scolecodonts and tasmanites have been also reported in middle Silurian to lower Devonian sediments (Fig. 3.2; ***Group 2***; Spina and Vecoli, 2009). Palynomorphs content of both TT1 and MG1 cores therefore demonstrates a marine contribution to the organic matter (Vecoli et al., 2009; Spina and Vecoli, 2009).

The *n*-alkanes occur widely in crude oils and sediments. These compounds and their alkanol and carboxylic-acid precursors generally occur as fats, oils and waxes in aquatic organisms and land plants (Noble, 1986). Their distribution in sediments and crude oils often provides information relating to the source of the sedimentary organic matter (Noble, 1986). The short chain *n*-alkanes in the aliphatic fractions from upper Ordovician to lower Devonian sediments may indicate an algal/bacterial contribution to the organic matter (Peters et al., 2005 and references therein; Fig. 3.5) and these lipids are present in considerable amounts in samples with a particularly high marine palynomorph contribution (Figs. 3.5 and 3.2). The high concentration of tricyclic terpanes (cheilantanes) in *Tasmanite* rock extracts indicates a possible origin from these algae (Simoneit et al., 1990; Aquino Neto et al., 1992; Azevedo et al., 1992). However, Dutta et al., (2006) demonstrated that tricyclic terpanes could be also produced by *Leiosphaeridia* prasinophytes suggesting that the genus *Tasmanites* is not exclusively the only precursor for tricyclic terpanes (Dutta et al., 2006). The presence of tricyclic terpanes in all investigated core samples (Fig. 3.8) is therefore consistent with the recorded prasinophyte palynomorphs. The previously detected isohexylalkylbenzenes and aryl isoprenoids in MG1 aromatic fractions (Romero-Sarmiento et al., 2010), may also be considered to be indicative for a marine contribution since they are similarly more abundant in samples from marine settings and samples with marine palynomorphs (Vecoli et al., 2009; Spina and Vecoli, 2009; Romero-Sarmiento et al., 2010).

Dibenzothiophenes and methyldibenzothiophenes are particularly abundant in marine oils and carbonates (Radke et al., 2000). Alkyldibenzothiophenes are considered as indicators of sulphate reduction resulting in free reduced sulphur species (e.g. H_2S) reacting with the organic matter in an anoxic setting (e.g. Radke and Willsch, 1994). This process is much more likely to occur in marine settings since SO_4^{-2} is much more abundant in these environmental conditions. Although alkyldibenzothiophenes are not typical markers to organic matter sources, the occurrence of these aromatic sulphur compounds in our samples may therefore be related to a marine depositional setting (e.g. Fig. 3.10; White and Lee, 1980; Radke and Willsch, 1994; Radke et al., 2000; among others).

3.6.2.2. Terrestrial organic matter input

Most samples from Middle Silurian to Early Devonian (Fig. 3.5; ***Group 2***) show a notable contribution of long-chain *n*-alkanes which could derive from land plants. These long *n*-alkanes do not show the typical odd carbon number predominance generally observed in higher plant waxes (Eglinton and Hamilton, 1967; Peters et al., 2005 and references therein). Since the palynological information from this stratigraphic interval displays the presence of terrestrial palynomorphs, the long *n*-alkanes could derive from a terrestrial source. Tricyclic diterpenoids are widespread in the plant kingdom, and are the major constituents of conifer resins (e.g. Simoneit, 1977). The saturated diterpanoid with a norabietane skeleton (Fig. 3.9) is therefore considered as a higher plant contribution to the organic matter (Simoneit, 1977; Simoneit, 1986; Philp, 1985; Noble et al., 1985; del Rio et al, 1994), but other biological precursors than conifers are needed in order to explain its presence in our geological samples, since the oldest report of conifers is Westphalian B – Carboniferous (Scott, 1974).

Nevertheless, the low concentrations of this terrestrial diterpenoid in the Tunisian cores seem to suggest a very low land plant contribution. This interpretation, however, underestimates the terrestrial input since the terrestrial plant terpenoids have been mostly converted into their aromatic counterparts (Romero-Sarmiento et al., 2010). This aromatisation may have been catalysed by clay minerals (Haberer et al., 2006) which dominate in our samples as well as by the thermal evolution of the organic matter (Alexander et al., 1987b). Considering this aromatisation of the organic matter, we focused on the aromatic compounds to identify additional land plant biomarkers. The aromatic hydrocarbons 1,2,5,6-tetramethylnaphthalene (1,2,5,6-TeMN), C_{19} isohexylalkylnaphthalene, 1-methylphenanthrene (1-MP), pimanthrene (1,7-DMP), cadalene, retene, simonellite and tetrahydroretene (Fig. 3.10; Table 3.4) have been frequently considered as indicators of terrestrial organic matter (Radke et al., 1986; Püttmann and Villar, 1987; Ellis et al., 1996; van Aarssen et al., 2000; Hautevelle et al., 2006; among others). Their occurrences in Silurian to Devonian core samples, therefore, reflect a terrestrial contribution to the organic matter since these compounds are considered to derive from different plant types (Jiang et al., 1998).

Cadalene (e.g. Fig. 3.10; Table 3.5) is a relatively unspecific terrestrial marker because it occurs in a wide variety of vascular plants (van Aarssen et al., 2000; Hautevelle et al. 2006). This well recognised biomarker derives from cadinenes and cadinols (Simonsen and Barton, 1952; Bordoloi et al., 1989) synthesised by, for instance, bryophytes (Chopra and Kumra, 1988; Asakawa, 2004), fungi (Bordoloi et al., 1989), plant resins (van Aarssen et al., 1990) and some conifers (Bordoloi et al., 1989; Otto and Wilde, 2001).

Retene, simonellite and tetrahydroretene are thought to be derived from partial degradation of abietic acid (van Aarssen et al., 2000; Hautevelle et al., 2006). Since abietic acid represents the major constituent of some families of conifer resins, these aromatic biomarkers have been widely used as evidence for a contribution from conifers. However, retene could also be derived from the maturation of phyllocladane and/or kaurane-type compounds associated to the early Palaeozoic bryophytes (Alexander et al., 1987a; Romero-Sarmiento et al., 2010). Due to the fact that the 1-MP and 1,7-DMP generally occur in conjunction with retene in crude oils and shales, an origin from abietane-type diterpenoids is also proposed for these alkylphenanthrene compounds (Alexander et al., 1995, Radke et al., 1998).

3.6.2.3. Microbial activity

Bicyclic alkanes with drimane and homodrimane skeletons in Silurian aliphatic fractions (Fig. 3.9; Noble, 1986; Noble et al., 1987) and the occurrence of hopanoids from extracts of middle Silurian to lower Devonian sediments (Fig. 3.8; Ourisson et al., 1979) suggest a bacterial contribution to the organic matter. The Silurian to Devonian aromatic fractions also reveal the presence of 1,3,6,7-tetramethylnaphthalene (1,3,6,7-TeMN; e.g. Fig. 3.10; Table 3.4), which has been frequently associated to microbial source (van Aarssen et al., 2000). The detected C_4-phenanthrene in Silurian to Devonian core samples (Fig. 3.10; compound 44) has been previously observed by Killops (1991). The origin of this compound is not clear and its structure cannot be anticipated but it has probably a bacterial origin (Killops, 1991). It is however interesting to notice that hopanoids, which are characteristic biomarkers of oxygenic bacteria (eubacteria) are mostly present in the samples where a significant contribution from terrestrial plants is observed. These bacterial contributions could therefore mainly correspond to soil bacteria (e.g. Cooke et al., 2008 and references therein).

3.6.2.4. Other sources

Several non-specific aromatic hydrocarbons have been also detected as considerable contributors to the organic matter. For instance, 1,2,8-trimethylphenanthrene (1,2,8-TMP; Fig. 3.10) has been frequently reported in samples from the Early Cretaceous and younger (Budzinski et al., 1995). High proportions of this alkylphenanthrene compound have been recently reported also in Devonian samples by Kruge (2000) and Armstroff et al. (2006). The latter authors concluded that 1,2,8-TMP is a promising age-related biomarker in the Late Palaeozoic. Although this biomarker may derive from the degradation of certain triterpenoids or hopanoids (Budzinski et al., 1995), the origin of this compound remains unknown (Armstroff et al., 2006). 1,2,8-TMP in our Silurian and Devonian core sediments (TT1-1243.0 and MG1-2890.0, respectively; Fig. 3.10) agrees with its previously reported occurrence (Kruge, 2000; Armstroff et al., 2006).

Pyrene, fluoranthene and benzo[a]anthracene represent a well-established combustion-derived PAHs group in modern geological settings (Laflamme and Hites, 1978; Jiang et al., 1998 and references therein). Although we have no palynological evidence for ancient fires (e.g. carbonised woody materials) in our samples, the presence of these PAHs (e.g. Fig. 3.10; Table 3.5) may suggest at least that fire events took place in Silurian – Devonian interval around Ghadamis Basin, North Africa.

The occurrence of perylene in several samples (Fig. 3.10; Table 3.5) represents an exceptional report of this compound. Although perylene has been detected in our sediments from Late Silurian to Early Devonian (Table 3.5), few published studies reveal the presence of this PAH in Palaeozoic sediments and oils (Grice et al., 2007; 2009). The biological origin of perylene in sediments has never been established. However, the most likely source for this aromatic compound appears to be wood rotting fungi (Grice et al., 2009). Fungi are classified as a kingdom that is separate from plants, animals and bacteria. It is probable that the first terrestrial fungi colonized land during the Cambrian (542 – 488.3 Ma), long before plants did (Brundrett, 2002). In order to explain the presence of perylene in marine sediments, Grice et al. (2009) proposed that this PAH can be associated to marine and/or terrestrial-derived fungi, anoxia conditions and/or contamination of core material by fungi. At least, the two possible sources of perylene (e.g. fungi and anoxia) are feasible for our setting since anoxia is suggested by the sulphur compounds detected and fungi occurred prior to the terrestrialization by land plants (e.g. Brundrett, 2002). However, since perylene was not detected in samples from the "hot shale" interval in the TT1 core section, which were deposited under anoxic conditions (Vecoli et al., 2009), fungi probably are the precursors for this compound in our sediments.

3.6.3. Specific origin of terrestrial biomarkers and their relationships to palynological distributions

The distribution of terrestrial aliphatic and aromatic biomarkers in the siliciclastic sediments from the Ghadamis Basin is both controlled by the geological setting (a progradation of shallow and marginal marine deposits into more basinal areas) and the development of plants on emerged lands during the Late Ordovician – Early Devonian period. The recorded input of miospores in the investigated sedimentary sequence (Vecoli et al., 2009; Spina and Vecoli, 2009) also evidences these palaeoenvironmental and palaeobotanical conditions. It appears, in general, that the occurrence of land plant biomarkers takes place with the progressive transition from a marine setting during Late Ordovician to Early Silurian to a more continental one during the Early Devonian (Figs. 3.5, 3.9 and 3.10).

Acritarchs, prasinophytes and chitinozoans are typical palynomorphs in marine sediments of Late Ordovician to Late Silurian age, whereas cryptospores and trilete spores are produced on the continent by land plants (Traverse, 2007; e.g. Fig. 3.2). The oldest uncontroversial report of cryptospores thought to have been produced by land plants (bryophytes) is from middle Ordovician (Darriwilian) sediments in Saudi Arabia (Strother et al., 1996). Cryptospores show a rapid diversification and increase in abundance during Late Ordovician – Early Silurian times, and then a diversity and abundance decrease during middle to late Silurian times, when they are superseded by trilete spores, which dominate the terrestrial palynomorph record during Devonian times (Steemans, 2000). The first trilete spores are known to occur in latest Ordovician (Hirnantian) Turkish sediments (Steemans et al., 1996). Trilete spores are generally interpreted as being produced in sporangia of tracheophytic land plants. However, it is also known that trilete spore morphology is not a totally exclusive feature of tracheophytic plants, as rare bryophyte plants can also produce spores with trilete marks (Steemans et al., 2009). In any case, we can therefore consider, for our purposes, that the late Silurian – earliest Devonian trilete spores from our studied samples represent tracheophyte plants. The major tracheophyte taxa are generally divided into Rhyniophytina (e.g *Cooksonia*), Trimerophytina and Zosterophyllophytina (Gray, 1993).

The abundances of long chain *n*-alkanes (Fig. 3.5), tricyclic diterpenoid (Fig. 3.9) and terrestrial aromatic biomarkers (Fig. 3.10) in our investigated samples covary with the abundance of miospores during the target Silurian – Devonian interval. Although the palynomorph content in Hirnantian sample from Djeffara Formation (TT1-1313.6) is dominated by abundant acritarchs, chitinozoans and prasinophytes (Fig. 3.2), the observed long-chain *n*-alkanes in this sample (Fig. 3.5) can be eventually related to the presence of bryophyte-derived cryptospores which also occur in low abundance (Vecoli et al., 2009; not shown in Fig. 3.2).

Following the stratigraphic order, the upper Silurian TT1 interval between samples at 1243.0 m and 1239.0 m (Argiles Principales Formation) is marked by the abundant occurrence of land-derived organic debris and terrestrial palynomorphs (Fig. 3.2; Vecoli et al., 2009). The Silurian (Wenlock) core samples from Tanezzuft Formation (MG1-3711.0 and MG1-3710.3) are characterized by abundant and well preserved cryptospores and trilete spores which are less abundant than cryptospores (Fig. 3.2; Spina and Vecoli, 2009). In contrast, the Silurian (Ludlow – Pridoli) core samples from Acacus Formation (MG1-3230.6; MG1-3183.0; MG1-3097.0; MG1-3052.0; MG1-3039.0 and MG1-3037.0) show a notable diversity of trilete spores, occurring in greater abundance than cryptospores (Fig. 3.2; Spina and Vecoli, 2009). In general, trilete spores from these Silurian sediments are dominated by *Ambitisporites avitus*, *Retusotriletes warringtoni*, *Synosisporites verracatus*, among others (Vecoli et al., 2009; Spina and Vecoli, 2009). Specimens of *Ambitisporites avitus* and *Synosisporites verracatus* have been found in sporangia of *Cooksonia pertoni pertoni* and *Cooksonia pertoni synorispora* respectively (Habgood et al., 2002). In addition, it seems that the genus *Retusotriletes* can be related to lycophytes and zosterophylls which are also other families of land plant recorded in Silurian – Devonian sediments (Li et al., 2000). For instance, small herbaceous lycopsids and zosterophylls comprise almost 65% of the documented species diversity from the Posonchong Formation in China, indicating that these groups could be the major component of the lower Devonian floras of Gondwana (Zhu and Kenrick, 1999). Finally, the core samples from Tadrart Formation (MG1-2892.0 and MG1-2890.0) are characterized by the abundance of the trilete spore *Streelispora newportensis* (Spina and Vecoli, 2009) and these specimens have been recovered from one single sporangium of *Cooksonia pertoni apiculispora* (Habgood et al., 2002).

From the available data, we propose, for our studied sequence, that cryptospores-producing land plants (e.g. bryophytes) dominated the Late Ordovician to Early Silurian and these cryptospores producers can be only linked to the distribution of long chain *n*-alkanes in this geological interval (sample TT1-1313.6). In contrast, the development of trilete spore-producing land plants, in particular, tracheophytes in the Middle Silurian to Early Devonian, is characterized by the appearance of a certain diversity of saturated and aromatic terrestrial biomarkers (Figs. 3.5, 3.9 and 3.10). These compounds are therefore related to the early Palaeozoic tracheophytes *Cooksonia*, lycophytes and zosterophylls which covered emerged lands around the Ghadamis Basin during the Silurian and Devonian. It is important to notice that several biomarkers which had been previously related to conifers and abietic acid (retene, tetrahydroretene, norabietane and simonellite) appear as typical biomarkers for earliest tracheophytes.

3.7. Conclusions

The biomarker record of upper Ordovician to lower Devonian siliciclastic sediments deposited on the northern margins of the North African Platform (Gondwana) reveals a large number of biomarkers which have previously been considered to be conifer derived solely such as norabietane, simonellite, retene, tetrahydroretene and C_{19} isohexylalkylnaphthalene since the middle Silurian (both boreholes TT1 and MG1). Since conifers did not yet exist, alternative sources for these biomarkers had to be inferred. On the basis of covarving distribution patters of the lipid biomarkers and palynomorphs, the compounds are proposed to have been derived from the very primitive flora with bryophytes and early tracheophytes (e.g. *Cooksonia*, lycophytes and zosterophylls), which covered the lands around the Ghadamis Basin during the Silurian – Devonian.

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3.9. References

Alexander, G., Hazai, I., Grimalt, J., Albaigés, J., 1987a. Occurrence and transformation of phyllocladanes in brown coals from Nograd Basin, Hungary. Geochimica et Cosmochimica Acta 51, 2065 – 2073.

Alexander, R., Noble, R.A., Kagi, R.I., 1987b. Fossil resin biomarkers and their application in oil to source rock correlation, Gippsland basin, Australia. Australian Petroleum Exploration Association Journal 27, 63 – 72.

Alexander, R., Bastow, T. P., Fisher, S. J., Kagi, R.I, 1995. Geosynthesis of organic compounds: II. Methylation of phenanthrene and alkylphenanthrenes. Geochimica et Cosmochimica Acta 59, 4259 – 4266.

Asakawa, Y., 2004. Chemosystematics of the Hepaticae. Phytochemistry 65, 623 – 669.

Aquino Neto, F.R., Trigüis, J., Azevedo, D.A., Rodriques, R., Simoneit, B.R.T., 1992. Organic geochemistry of geographically unrelated Tasmanites. Organic Geochemistry 18, 791 – 803.

Armstroff, A., Wilkes, H., Schwarzbauer, J., Littke, R., Horsfield, B., 2006. Aromatic hydrocarbon biomarkers in terrestrial organic matter of Devonian to Permian age. Palaeogeography, Palaeoclimatology, Palaeoecology, 240, 253 – 274.

Azevedo, D.A., Aquino Neto, F.R., Simoneit, B.R.T. and Pinto, A.C., 1992. Novel series of tricyclic aromatic terpanes characterized in *Tasmanian* tasmanite. Organic Geochemistry 18, 9 – 16.

Bordoloi, M., Shukla, V.S., Nath, S.C., Sharma, R.P., 1989. Naturally occurring cadinenes. Phytochemistry 28, 2007 – 2037.

Bray, E.E., Evans, E.D., 1961. Distribution of *n*-paraffins as a clue to recognition of source beds. Geochimica et Cosmochimica Acta 22, 2 – 15.

Brundrett, Mark C., 2002. Coevolution of roots and mycorrhizas of land plants. New Phytologist 154, 274 – 304.

Budzinski, H., Garrigues, P., Connan, J., Devillers, J., Domine, D., Radke, M., Oudins, J.L., 1995. Alkylated phenanthrene distributions as maturity and origin indicators in crude oils and rock extracts. Geochimica et Cosmochimica Acta 59, 2043 – 2056.

Chopra, R.N., Kumra, K.P., 1988. Biology of Bryophytes. John Wiley & Sons, New York, 350 p.

Cooke, M. P., Talbot, H. M., Farrimond, P., 2008. Bacterial populations recorded in bacteriohopanepolyol distributions in soils from Northern England. Organic Geochemistry 39, 1347 – 1358.

del Río, J. C., Garcia-Molla, J., González-Vila, F. J., Martin, F. 1994. Composition and origin of the aliphatic extractable hydrocarbons in the Puertollano (Spain) oil shale. Organic Geochemistry 21, 897 – 909.

Deunff, J., Massa, D., 1975. Palynologie et stratigraphie du Cambro-Ordovicien (Libye nord-occidentale). Comptes Rendus de l'Académie des Sciences Paris. Série D 281, 21 – 24.

Didyk, B.M., Simoneit, B.R.T., Brassell, S.C., Eglinton, G., 1978. Organic geochemical indicators of palaeoenvironmental conditions of sedimentation. Nature 272, 216 – 222.

Dutta, S., Greenwood, P. F., Brocke, R., Schaefer, R. G., Mann, U., 2006. New insights into the relationship between Tasmanites and tricyclic terpenoids. Organic Geochemistry 37, 117 – 127.

Edwards, D., Wellman, C. H., 2001. Embryophytes on land: The Ordovician to Lochkovian (Lower Devonian) record. In: Gensel, P. G. and Edwards, D. (eds). Plants invade the land: Evolutionary and environmental perspectives. Columbia University Press, New York, 3 – 28.

Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. Science 156, 1322 – 1335.

El-Arnauti, A., Owens, B, Thusu, B. (Eds.), 1988. Subsurface palynostratigraphy of northeast Libya. Garyounis University Publishing, Benghazi, Libya.

Ellis, L., Singh, R., Alexander, R., Kagi, R.I, 1996. Formation of isohexyl alkylaromatic hydrocarbons from aromatization-rearrangement of terpenoids in the sedimentary environment: A new class of biomarker. Geochimica and Cosmochimica Acta 60, 4747 – 4763.

Espitalié, J., Deroo, G., Marquis, F., 1986. La pyrolyse Rock-Eval et ses applications. Revue de l'Institut Français du Pétrole 41, 73 – 89.

Ewbank, G., Edwards, D., Abbott, G., 1996. Chemical characterization of Lower Devonian vascular plants. Organic Geochemistry 25, 461 – 473.

Gray, J. 1993. Major Palaeozoic land plant evolutionary bio-events. Palaeogeography, Palaeoclimatology, Palaeoecology 104, 153 – 169.

Grice, K., Nabbefeld, B., Maslen, E., 2007. Source and significance of selected polycyclic aromatic hydrocarbons in sediments (Hovea-3 well, Perth Basin, Western Australia) spanning the Permian – Triassic boundary. Organic Geochemistry 38, 1795 – 1803.

Grice, K., Lu, H., Atahan, P., Asif, M., Hallmann, C., Greenwood, P., Maslen, E., Tulipani, S., Williford, K., Dodson, J., 2009. New insights into the origin of perylene in geological samples. Geochimica et Cosmochimica Acta 73, 6531 – 6543.

Haberer, R.M., Mangelsdorf, K., Wilkes, H., Horsfield, B., 2006. Occurrence and palaeoenvironmental significance of aromatic hydrocarbon biomarkers in Oligocene sediments from the Mallik 5L-38 Gas Hydrate Production Research Well (Canada). Organic Geochemistry 37, 519 – 538.

Habgood, K.S., Edwards, D., Axe, L., 2002. New perspectives on *Cooksonia* from the Lower Devonian of the Welsh Borderland. Botanical Journal of the Linnaean Society 139, 339 – 359.

Hautevelle, Y., Michels, R., Malartre, F., Trouiller, A., 2006. Vascular plant biomarkers as proxies for palaeoflora and palaeoclimatic changes at the Dogger/Malm transition of the Paris Basin (France). Organic Geochemistry 37, 610 – 625.

Hunt, J.M., 1995. Petroleum Geochemistry and Geology. W.H. Freeman and Co., New York.

Jiang, C., Alexander, R., Kagi, R., Murray, A. P., 1998. Polycyclic aromatic hydrocarbons in ancient sediments and their relationships to palaeoclimate. Organic Geochemistry 29, 1721 – 1735.

Killops, S.D., 1991. Novel aromatic hydrocarbons of probable bacterial origin in a Jurassic lacustrine sequence. Organic Geochemistry 17, 25 – 36.

Kruge, M.A., 2000. Determination of thermal maturity and organic matter type by principal components analysis of the distributions of polycyclic aromatic compounds. International Journal of Coal Geology 43, 27 – 51.

Lafargue, E., Marquis, F., Pillot, D., 1998. Rock-Eval 6 Applications in hydrocarbon exploration, production and soils contamination studies. Oil & Gas Science and Technology, Revue de l'Institut Français du Pétrole 53, 421 – 437.

Laflamme, R. E. and Hites, R. A., 1978. The global distribution of polycyclic aromatic hydrocarbons in recent sediments. Geochimica et Cosmochimica Acta 42, 289 – 303.

Li, C.-S., Hueber, F.M., Hotton, C., 2000. A neotype for *Drepanophycus spinaeformis* Göppert 1852. Canadian Journal of Botany 78, 889 – 902.

Massa, D., 1988. Paléozoïque de Libye Occidentale. Stratigraphie et Paléogéographie. Thèse Sc. Univ. Nice, 1-2, 514 p.

Molyneux, S.G., 1988. Late Ordovician acritarchs from northeast Libya. In: El-Arnauti, A., Owens, B, Thusu, B. (Eds.), Subsurface palynostratigraphy of northeast Libya. Garyounis University Publishing, Benghazi, Libya, pp. 45 – 59.

Noble, R. A. 1986. A geochemical study of bicyclic alkanes and diterpenoid hydrocarbons in crude oils, sediments and coals. Thesis. The University of Western Australia. 398 p.

Noble, R. A., Alexander, R., Kagi, I. R., Knox, J., 1985. Tetracyclic diterpenoid hydrocarbons in some Australian coals, sediments and crude oils. Geochimica and Cosmochimica Acta 49, 2141 – 2147.

Noble, R. A., Alexander, R., Kagi, I. R., 1987. Configurational isomerization in sedimentary bicyclic alkanes. Organic Geochemistry 11, 151 – 156.

Ourisson, G., Albrecht, P., Rohmer, M., 1979. The hopanoids. Palaeochemistry and biochemistry of a group of natural products. Pure and Applied Chemistry 51, 709 – 729.

Otto, A., Wilde, V., 2001. Sesqui-, di- and triterpenoids as chemosystematic markers in extant conifers— A review. The Botanical Review 67, 141 – 238.

Peters, K.E., Walters, C.W., Moldowan, J.M., 2005. The Biomarker Guide. Cambridge University Press. Cambridge -706 p.

Philp, R.P., 1985. Fossil Fuel Biomarkers Applications and Spectra. Elsevier, Amsterdam.

Püttmann, W., Villar, H., 1987. Occurrence and geochemical significance of 1,2,5,6-tetramethylnaphthalene. Geochimica et Cosmochimica Acta 51, 3023 – 3029.

Radke, M., Willsch, H., Leythaeuser, D., 1982. Aromatic components of coal: relation of distribution pattern to rank. Geochimica et Cosmochimica Acta 46, 1831 – 1848.

Radke, M., Willsch, H., Welte, D.H., 1986. Maturity parameters based on aromatic hydrocarbons: influence of the organic matter type. Organic Geochemistry 10, 51 – 63.

Radke, M., Willsch, H., 1994. Extractable alkyldibenzothiophenes in Posidonia shale (Toarcian) source rocks: relationship of yields to petroleum formation and expulsion. Geochimica et Cosmochimica Acta 58, 5223 – 5244.

Radke, M., Hilkert, A., Rullkötter, J., 1998. Molecular stable carbon isotope compositions of alkylphenanthrenes in coals and marine shales related to source and maturity. Organic Geochemistry 28, 785 – 795.

Radke, M., Vriend, S. P., Ramanampisoa, L. R., 2000. Alkyldibenzofurans in terrestrial rocks: Influence of organic facies and maturation. Geochimica et Cosmochimica 64, 275–286.

Romero-Sarmiento, M. F., Riboulleau, A., Vecoli, M. Versteegh., G., 2010. Occurrence of retene in upper Silurian – Lower Devonian sediments from North Africa: Origin and implications. Organic Geochemistry 41, 302 – 306.

Scott, A., 1974. The earliest conifer. Nature 25, 707 – 708.

Seifert, W.K., Moldowan, J.M., 1978. Applications of steranes, terpanes and monoaromatics to the maturation, migration and source of crude oils. Geochimica et Cosmochimica Acta 42, 77 – 95.

Seifert, W.K., Moldowan, J.M., 1980. The effect of thermal stress on source rock quality as measured by hopane stereochemistry. In: Douglas, A.G., Maxwell, J.R. (Eds.), Advances in Organic Geochemistry, 1979. Pergamon Press, 229 – 237.

Seifert, W.K., Moldowan, J.M., 1986. Use of biological markers in petroleum exploration. In: Johns, R.B. (Ed.), Biological Markers in the Sedimentary Record, Methods in Geochemistry and Geophysics, vol. 24. Elsevier, Amsterdam, 261 – 290.

Sheng, G., Simoneit, B. R. T., Leif, R. N., Chen, X. & Fu, J., 1992. Tetracyclic terpanes enriched in Devonian cuticle humic coals. Fuel, 71, 523 – 532.

Simoneit, B. R. T., 1977. Diterpenoid compounds and other lipids in deep-sea sediments and their geochemical significance. Geochimica et Cosmochimica Acta 41, 463 – 476.

Simoneit, B.R.T., 1986. Cyclic terpenoids of the geosphere. In: Johns, R.B. (Ed.), Biological Markers in the Sedimentary Record. Elsevier, Amsterdam, 41 – 99.

Simoneit, B. R. T., Leif, R. N., Aquino Neto, F. R.., Azevedo, D. A., Pinto, A. C., Albrecht, P., 1990. On the presence of tricyclic terpane hydrocarbons in Permian Tasmanite algae. Naturwissenschaften 77, 380 – 383.

Simonsen, J.L., Barton, D.H.R., 1952. The Terpenes: The sesquiterpenes, diterpenes and their derivatives. Cambridge University Press, New York, 580p.

Spina, A., Vecoli, M., 2009. Palynostratigraphy and vegetational changes in the Siluro-Devonian of the Ghadamis Basin, North Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 282, 1 – 18.

Staplin, F.L., 1982. Determination of thermal alteration index from color of exinite (pollen, spores). In: Staplin, F.L. (Ed.), How to Assess Maturation and Paleotemperatures. SEPM Short Course 7, 7 – 11.

Steemans, P., 2000. Miospore evolution from the Ordovician to the Silurian. Review of Palaeobotany and Palynology 113, 189 – 196.

Steemans, P., Le Hérissé, A., Bozdogan, N., 1996. Ordovician and Silurian cryptospores and miospores from Southeastern Turkey. Review of Palaeobotany and Palynology 93, 35 – 76.

Steemans, P., Le Hérissé, A., Melvin, J., Miller, M.A., Paris, F., Verniers, J., Wellman, C., 2009. Origin and radiation of the earliest vascular land plans. Science 324, 353.

Strother, P.K., Al-Hajri, S., Traverse, A., 1996. New evidence for land plants from the lower Middle Ordovician of Saudi Arabia. Geology 24, 55 – 58.

Tissot, B., Welte, D.M., 1984. Petroleum Formation and Occurrence. Springer-Verlag, Berlin, 699 p.

Traverse, A., 2007. Paleopalynology. Second edition. Volume 28 Springer. DOI: 10.1007/1-4020-5610-9

Underdown, R., Redfern, J., 2008. Petroleum generation and migration in the Ghadames Basin, North Africa: A two-dimensional basin-modeling study. AAPG Bulletin 92, 53 – 76.

van Aarssen, B.G.K, Cox, H., Hoogendoorn, P., de Leeuw, J., 1990. A cadinene biopolymer present in fossil and extant drammar resins as a source fro cadinanes and bicadinanes in crude oils from South East Asia. Geochimica et Cosmochimica Acta 54, 3021 – 3031.

van Aarssen, B.G.K., Bastow, T.P., Alexander, R., Kagi, R., 1999. Distributions of methylated naphthalenes in crude oils: indicators of maturity, biodegradation and mixing. Organic Geochemistry 30, 1213 – 1227.

van Aarssen, B.G.K., Alexander, R., Kagi, R., 2000. Higher plant biomarkers reflect palaeovegetation changes during Jurassic times. Geochimica and Cosmochimica Acta 64, 1417 – 1424.

Vavrdovà, M., 1990. Coenobial acritarchs and other palynomorphs from the Arenig/Llanvirn boundary Prague Basin. Vestnik Ustredniho ustavu Geologického 65, 237 – 242.

Vecoli, M., 2000. Palaeoenvironmental interpretation of microphytoplankton diversity trends in the Cambrian–Ordovician of the northern Sahara Platform. Palaeogeography, Palaeoclimatology, Palaeoecology 160, 329 – 346.

Vecoli, M., Le Hérissé, A., 2004. Biostratigraphy taxonomic diversity, and patterns of morphological evolution of Ordovician acritarchs (organic-walled microphytoplankton) from the northern Gondwana margin in relation to palaeoclimatic and palaeogeographic changes. Earth-Science Reviews 67, 267 – 311.

Vecoli, M., Riboulleau, A., Versteegh, G., 2009. Palynology, organic geochemistry and carbon isotope analysis of a latest Ordovician through Silurian clastic succession from borehole T.T.1, Ghadamis Basin, southern Tunisia, North Africa: Palaeoenvironmental interpretation. Palaeogeography, Palaeoclimatology, Palaeoecology 273, 378 – 394.

White, C. M., Lee, M. L., 1980. Identification and geochemical significance of some aromatic components of coals. Geochimica et Cosmochimica Acta 44, 1825 – 1832.

Williams, S. H., Burden, E. T., Mukhopadhyay, P. K., 1998. Thermal matutity and burial history of Palaeozoic rocks in western Newfoundland. Canadian Journal of Earth Sciences 35, 1307 – 1322.

Zhu, W.-Q., Kenrick P., 1999. A *Zosterophyllum*-like plant from the Lower Devonian of Yunnan Province, China. Review of Palaeobotany and Palynology 105, 111 – 118.

Chapter 4

Aliphatic and aromatic biomarkers from Carboniferous coal deposits at Dunbar (East Lothian, Scotland): Palaeobotanical and palaeoenvironmental significance

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4.1. Abstract

The immature Carboniferous (Visean) coals from Dunbar, East Lothian – Scotland contain well-preserved miospore and megaspore assemblages suggesting a lycopod-dominated forest ecosystem with some sphenopsids and pteridosperms. The immaturity and well defined flora form a good basis assessing lipids-environment relations for the Early Carboniferous. Our Rock-Eval and lipid analyses confirm the low maturity of the coals and indicate a fully terrestrial depositional environment. Although we also present and discuss a wide diversity of other lipid biomarkers (alkanes, hopanoids, steroids), we focus on the land-derived biomarkers. Of these, ionene and alkyldibenzofurans (pollen and lichens) as well as retene, cadalene, simonellite, tetrahydroretene and kaurane can be attributed to early Palaeozoic land plants. However, we also detected the terrestrial terpenoids abietane, phyllocladane, *ent*-beyerane and 4β(H)-eudesmane as well as land-plant-derived polycyclic aromatic hydrocarbons bisnorsimonellite, diaromatic totarane, diaromatic sempervirane and 2-methylretene. These components as yet had only been reported from conifers which do not appear until the Late Carboniferous. Conversely, the lower Carboniferous forest ecosystem can be identified as a new source for these components. Finally, the combustion-derived PAHs pyrene, fluoranthene, benzo[a]anthracene, chrysene and triphenylene indicate the occurrence of forest fire.

Keywords: terrestrial terpenoids, retene; 2-methylretene; phyllocladane; kaurane; beyerane; alkylnaphthalenes, alkyldibenzofurans, alkylphenanthrenes; Lower Carboniferous coals; land plant biomarkers; megaspores

4.2. Introduction

Aliphatic and aromatic hydrocarbons are the major components in organic extracts of coals, crude oils and sedimentary rocks (Tissot and Welte, 1984). Biomarkers in coals and other sediments can be combined with palynological observations, to better characterize the origin and type of the organic matter.

Although the major part of plant components in coals is degraded during coalification, the organic matter derived from Palaeozoic land plants is chemically best preserved in coals and related material (Tegelaar et al., 1989). For this reason, the recognition of saturated and aromatic biomarkers in Palaeozoic coals provides valuable information about the origin and nature of the organic matter and biogeochemical cycles involved. Accordingly, several lipid biomarkers for land-plants have been identified from these coals using gas chromatography and mass spectrometry (e.g. Murchison and Raymond, 1989).

A land-plant-derived biomarker is a well-recognised organic molecular compound considered to derive from terrestrial organic matter and classically used to reconstruct palaeoenvironments (Peters and Moldowan, 1993; Fleck et al., 2001, 2002; Hautevelle et al. 2006). The molecular biomarkers are important because they often can be detected even when micro- or macro-fossils have not been preserved (Peters and Moldowan, 1993). Typical land-plant signatures are an odd over even predominance of long-chain *n*-alkanes (>23 carbon atoms), some polycyclic aromatic hydrocarbons, and geoterpenoids derived from diagenesis of terpenoids common in vascular plant tissues and resins (Eglinton and Hamilton, 1967; Otto and Simoneit, 2001; Hautevelle et al. 2006).

The aim of this study is to identify and characterise aliphatic and aromatic biomarkers for Lower Carboniferous plants preserved in coals and to relate these to specific plant taxa. Biomarker analyses were performed on four Lower Carboniferous (Visean) coal samples from Dunbar, East Lothian – Scotland. The palynological content of these coal samples is rich and comprises abundant miospore and megaspore assemblages (Spinner, 1969; Spinner and Clayton, 1973). This gives us an opportunity to correlate the identified land plant biomarkers with the occurrence of land plant palynomorphs.

4.3. Geological setting and previous studies

The Midland Valley of Scotland is, in structural terms, a rift valley bounded by Highland Boundary and Southern Uplands Faults on the North and the South, respectively (Fig. 4.1; Murchison and Raymond, 1989; Underhill et al., 2008). This sedimentary basin evolved in response to crustal extension and especially contains Devonian to Carboniferous sediments and some igneous rocks (Murchison and Raymond, 1989; George, 1992; Underhill et al., 2008). The Carboniferous rocks in the Midland Valley of Scotland are only well exposed along coastlines (Murchison and Raymond, 1989). Coal samples were collected from two outcrop sections located at Dunbar, East Lothian, on the east coast of southern Scotland, some 40 km East of Edinburgh (Fig. 4.1). These sections show a group of alternating Lower Carboniferous (Visean) limestones, shales, sandstones and coals (Fig. 4.2.; Spinner, 1969; Spinner and Clayton, 1973).

SKT coal samples were collected around the bay containing Skateraw Harbour, approximately 6.4 km south-east of Dunbar, whereas WS coals were taken from rock successions exposed in White Sand Bay (Fig. 4.1). Both localities are clearly indicated on the geological map permanently exhibited just above the shoreline. In stratigraphic order, the lower seam is located immediately above the Middle Longcraig Limestone: samples WS-2 and WS-3 (Fig. 4.2); the upper one (samples SKT-E and SKT-D) occurs stratigraphically below the Chapel Point Limestone (Fig. 4.2). WS samples are equivalent to the Longcraig coal seam described by Spinner (1969). In contrast, SKT coals are more comparable to the sample horizon SC2 detailed by Spinner and Clayton (1973). In order to investigate the possible vertical stratigraphic variations, two samples were obtained from each coal seam

(Fig. 4.2). Accordingly, WS coals are separated by approximately 30 cm while SKT samples by 15 cm.

A fluvio-deltaic environment has been assigned to these Carboniferous coals which contain mainly land-plant derived organic matter (George, 1992). After deposition, these shallow-water deltaic deposits were mainly influenced by burial history and extensive volcanic, sill and dyke activities (Murchison and Raymond, 1989).

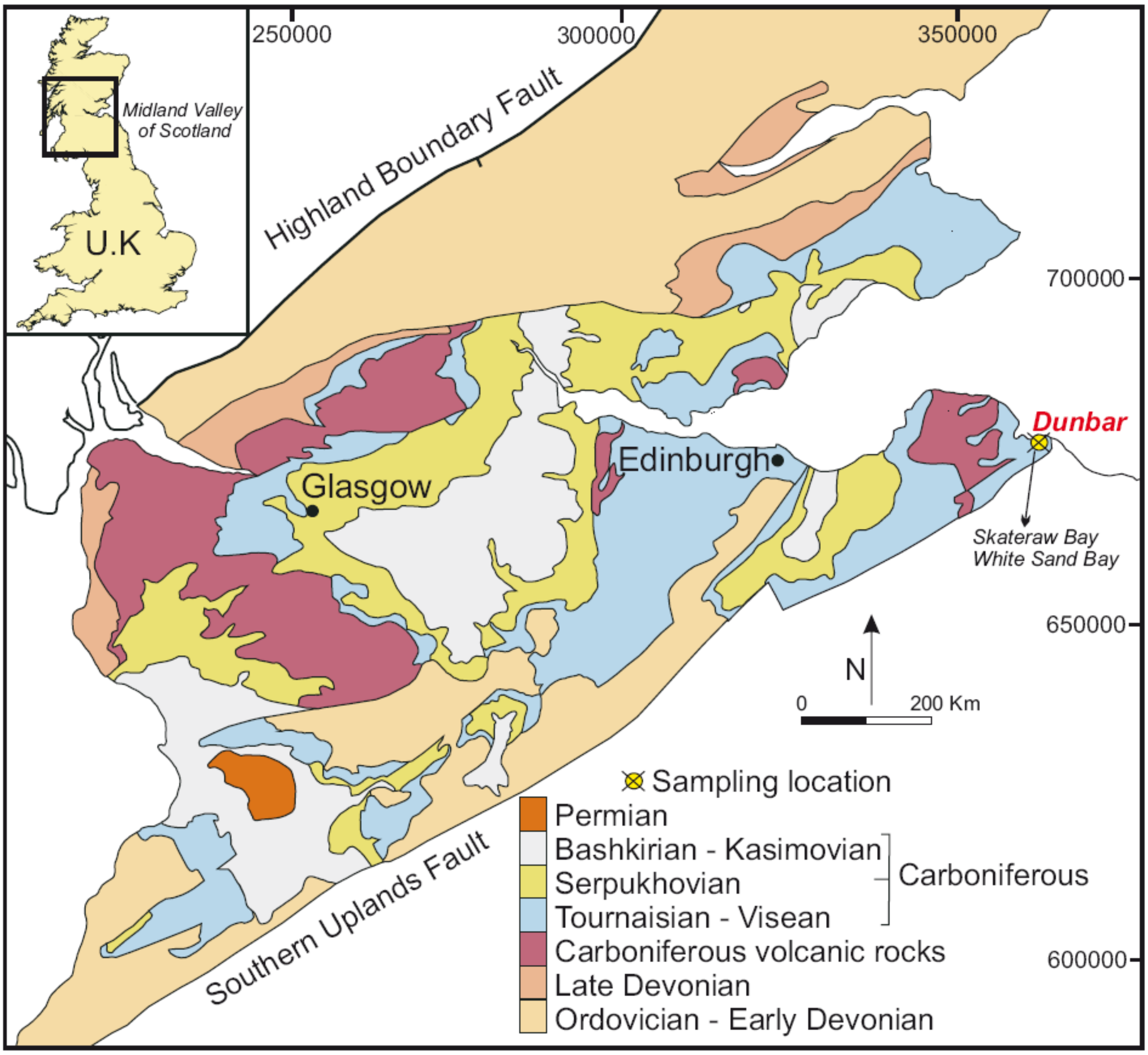

Figure 4.1: Simplified geologic map showing the outcrop sample positions at Dunbar (White Sand and Skateraw Bays) in the Midland Valley of Scotland (modified from Spinner and Clayton, 1973; Underhill et al., 2008)

The sampled outcrop successions were previously studied palynologically (Spinner, 1969; Spinner and Clayton, 1973). The WS coal interval is characterized by abundant and well preserved megaspores such as: *Zonalesporites fusinatus*, *Lagenicula subpilosa* and *Setosisporites* (Spinner, 1969). Miospores are less abundant in this lower interval and are dominated by the following taxa: *Lycospora pusilla*, *Calamospora*, *Densosporites* (Spinner, 1969). All megaspore specimens recognized in WS horizon (e.g. *Lagenicula subpilosa*, *Setosisporites* and *Zonalesporites fusinatus*) range through the SKT coal interval but the genus *Zonalesporites* is less abundant (Spinner and Clayton, 1973). This upper interval also shows a notable diversity of miospores represented by *Lycospora pusilla*, *Calamospora*, *Densosporites* and *Cingulizonates* (Spinner and Clayton, 1973). From the absence of plant megafossils, Spinner (1969) provides a megaspore-based flora reconstruction characterized by large arborescent lycopods with long leaves together with some more diminutive forms.

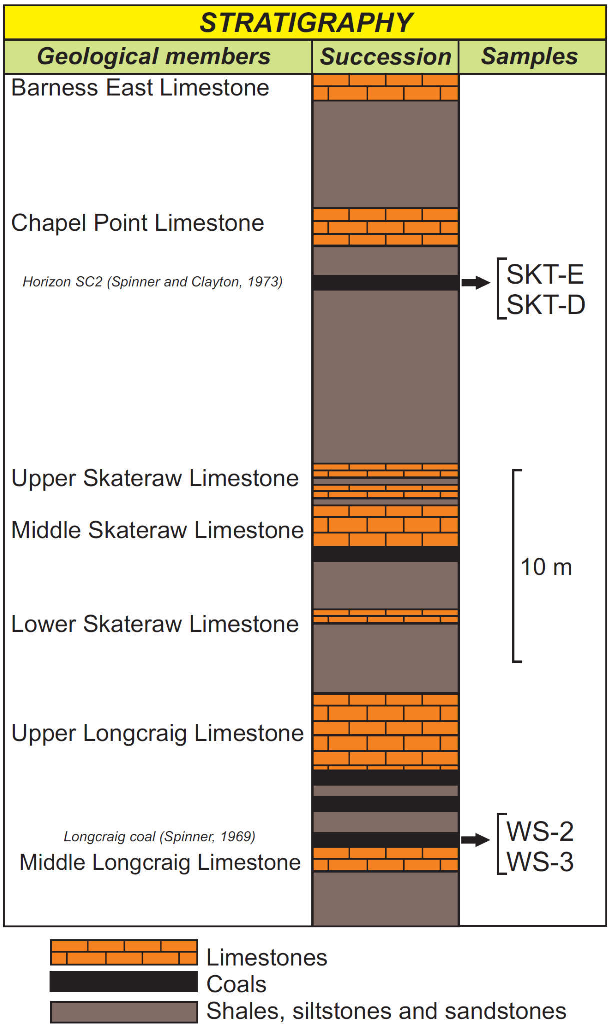

Figure 4.2: The outcrop geological succession showing alternating Carboniferous limestones, shales, sandstones and coals (modified from Spinner and Clayton, 1973)

4.4. Analytical methods

4.4.1. Experimental procedures

The four coal samples (Fig. 4.2) were studied by Rock-Eval pyrolysis and biomarker analysis. Rock-Eval pyrolysis was performed on 100 mg of ground rock with an Oil Show Analyser device at the University of Paris 6, France, using the conventional temperature program described in Espitalié et al. (1986). The pyrolysis oven temperature was programmed with 25°/min from 300°C (held 3 min) to 650°C (held 3 min). However, due to the poor estimation of the total organic carbon (TOC) content of coals by Rock-Eval analysis (Espitalié et al, 1986), their TOC content was additionally determined on 100 mg of powdered decarbonated sample using a LECO carbon analyser at the same university (Paris 6, France). The hydrogen index (HI) was calculated using the Rock-Eval S_2 and the LECO TOC values.

For biomarker analyses, rock fragments were extracted with dichloromethane (DCM) during 24 h in the refrigerator, in order to remove possible contamination on the sample surface. After this first extraction, the rock fragments were crushed to enable extraction of the lipids preserved inside the rock. Approximately 30 g of pulverised samples were extracted with a mixture of methanol (MeOH) and dichloromethane (DCM) (1/2, v/v) for 24 h with extensive stirring. This second extract was dried by means of Roto-evaporation and partly re-solubilized in cyclohexane. The cyclohexane-soluble fraction (maltenes) was further separated by column chromatography.

The apolar fraction was recovered from the maltenes by elution with cyclohexane on an activated silica column. Subsequent elution with a mixture of cyclohexane - DCM (2/1, v/v) recovered the aromatic fraction after which the polar fraction was recovered by elution with a mixture DCM – MeOH (2/1, v/v).

4.4.2. Gas chromatography – mass spectrometry (GC-MS)

The aliphatic and aromatic fractions were analysed by gas chromatography – mass spectrometry (GC-MS) using a Trace GC 2000 gas chromatograph and a DB5ht column (30 m length, 0.25 mm internal diameter, 0.1 µm film thickness). The oven temperature was programmed as follows 100 °C for 1 minute, 100 °C – 310°C at a rate of 4 °C/min followed by an isothermal period of 16.5 min at 310 °C. Helium was used as carrier gas. Samples were analysed in full scan (m/z 50 – 700; scan rate 1000 amu/s; scan speed 1.49/s, scan time 0.67 s). The organic compounds were identified by comparison of their mass spectra and retention times with available published data.

4.5. Results

4.5.1. Organic geochemical parameters

Bulk organic parameters obtained by Rock-Eval, LECO and aliphatic biomarker analyses are shown in Table 4.1. The TOC values for these coal samples range between 60.8 and 71.9%. HI and Tmax values vary between 144 to 218 mg HC/g TOC and 423 to 428°C, respectively (Fig. 4.3). The average Tmax is 426 °C. Based on the constructed HI vs. Tmax diagram (Fig. 4.3; Espitalié et al., 1986), coal samples plot in the type II – III organic matter sector. Table 4.1 also displays the selected biomarker ratios from the aliphatic fraction of coals and their values are described below in detail.

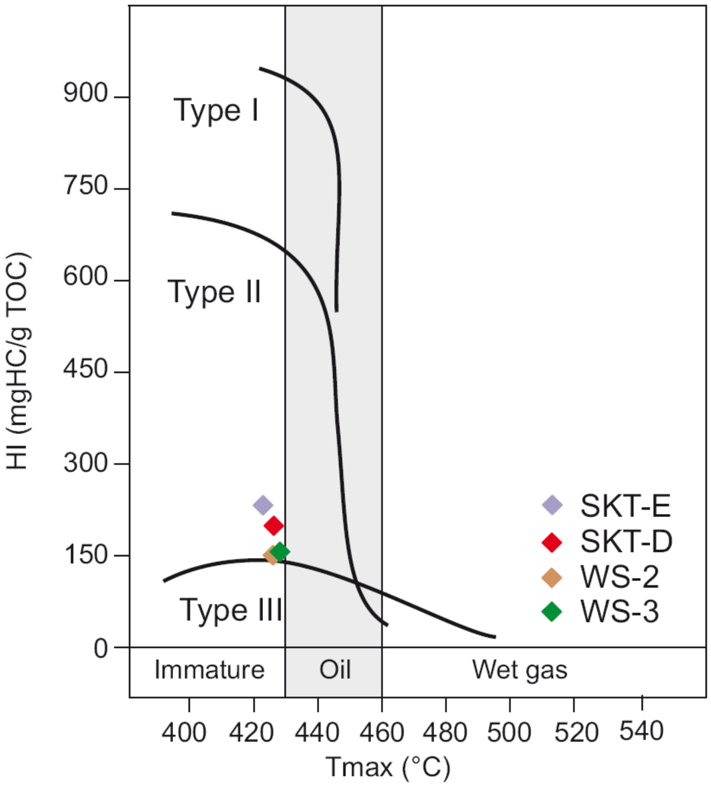

Figure 4.3: Plot of HI vs. Tmax values for Scottish coals (diagram from Espitalié et al., 1986)

Table 4.1: Bulk and molecular geochemical parameters from Scottish coals

	Samples			
Parameters	**SKT-E**	**SKT-D**	**WS-2**	**WS-3**
TOC (%)	69,4	70,7	60,8	71,9
Tmax (°C)	423	426	427	428
HI (mgHC/gTOC)	218	181	151	144
S_1 (mg/g)	5.0	5,3	6.8	5.6
S_2 (mg/g)	148.6	129.4	92.1	104.2
Pr/Ph$^{(1)}$	7.33	7.50	14.67	14.33
Pr/$C_{17}$$^{(2)}$	4.00	5.63	7.33	8.60
Ph/$C_{18}$$^{(3)}$	0.46	0.75	0.43	0.50
n-C_{18}/n-$C_{27}$$^{(4)}$	0.68	0.21	0.64	0.35
CPI$^{(5)}$	1.84	1.93	1.65	1.90
C_{27} Sterane (%)$^{(6)}$	32.0	27.9	37.0	29.6
C_{28} Sterane (%)$^{(6)}$	21.9	17.9	21.5	18.5
C_{29} Sterane (%)$^{(6)}$	46.2	54.3	41.5	51.9
C_{29}/C_{27} Steranes$^{(6)}$	1.07	1.52	0.83	1.38

(1) Pr/Ph = Pristane/Phytane; m/z = 183 (Didyk et al., 1978).

(2) Pr/n-C_{17}= Pristane/*n*-heptadecane; m/z = 183 (Didyk et al., 1978).

(3) Pr/n-C_{18}= Pristane/*n*-octadecane; m/z = 183 (Didyk et al., 1978).

(4) n-C_{18}/n-C_{27}= *n*-octadecane/*n*-heptacosane; m/z = 57 (Didyk et al., 1978).

(5) CPI= Carbon Preference Index = $\{[(n\text{-}C_{25}+n\text{-}C_{27}+n\text{-}C_{29}+n\text{-}C_{31}+n\text{-}C_{33})/(n\text{-}C_{26}+n\text{-}C_{28}+n\text{-}C_{30}+n\text{-}C_{32}+n\text{-}C_{34})]+[(n\text{-}C_{25}+n\text{-}C_{27}+n\text{-}C_{29}+n\text{-}C_{31}+n\text{-}C_{33})/(n\text{-}C_{26}+n\text{-}C_{28}+n\text{-}C_{30}+n\text{-}C_{32}+n\text{-}C_{34})]\}/2$; m/z = 57 (Bray and Evans, 1961).

(6) Calculated using peak height of ααα and αββ isomers on m/z 217 chromatogram.

4.5.2. Aliphatic hydrocarbons

4.5.2.1. Total aliphatic hydrocarbons

The total ion currents (TIC) of the aliphatic fractions of the coal-sample extracts (Fig. 4.4) are dominated by a series of *n*-alkanes ranging from C_{13} to C_{30}. The distribution of *n*-alkanes is relatively similar in all four samples and the most abundant are *n*-C_{27} and *n*-C_{29} (Fig. 4.5). Long chain C_{23} – C_{33} *n*-alkanes are characterized by an odd-over-even predominance with a maximum at *n*-C_{27} (Fig. 4.5). The carbon preference index ranges between 1.65 and 1.93 (CPI; Table 4.1). Series of C_{14} to C_{21} acyclic isoprenoids, dominated by norpristane (C_{18}), pristane (C_{19}) and phytane (C_{20}), were also detected (Fig. 4.5). Pristane is the most abundant compound in all the samples except SKT-D, where the *n*-C_{27} alkane is more abundant (Fig. 4.4). The pristane/phytane (Pr/Ph) ratio ranges from 7.33 to 14.67 (Table 4.1). The pristane/C_{17} *n*-alkane (Pr/*n*-C_{17}) ratios are more than 1, whereas the phytane/C_{18} *n*-alkane (Ph/*n*-C_{18}) ratio values are inferior to 1 (Table 4.1; Fig. 4.6). Branched alkanes without odd or even chain length predominance were also recognized in low abundance (Fig. 4.5). Steranes, hopanes, bicyclic alkanes and several diterpanoids were clearly detected in the aliphatic fractions (Fig. 4.4).

4.5.2.2. Hopanoids

Series of αβ-hopanes were detected by monitoring the m/z 191 ion abundance, in all the samples (Fig. 4.7). These compounds are dominated by 17α(H),21β(H)-hopanes (22R and 22S epimers) from C_{27} to C_{33}, with a maximum at C_{29} or C_{30} hopanes (Fig. 4.7).

Series of βα-moretanes (17β(H),21α(H)-moretanes) were also detected ranging from C_{27} to C_{32}, with a maximum at C_{30} moretane (Fig. 4.7). C_{29} ββ-hopane was present in all the samples (Fig. 4.7). Tricyclic terpanes and gammacerane were not observed.

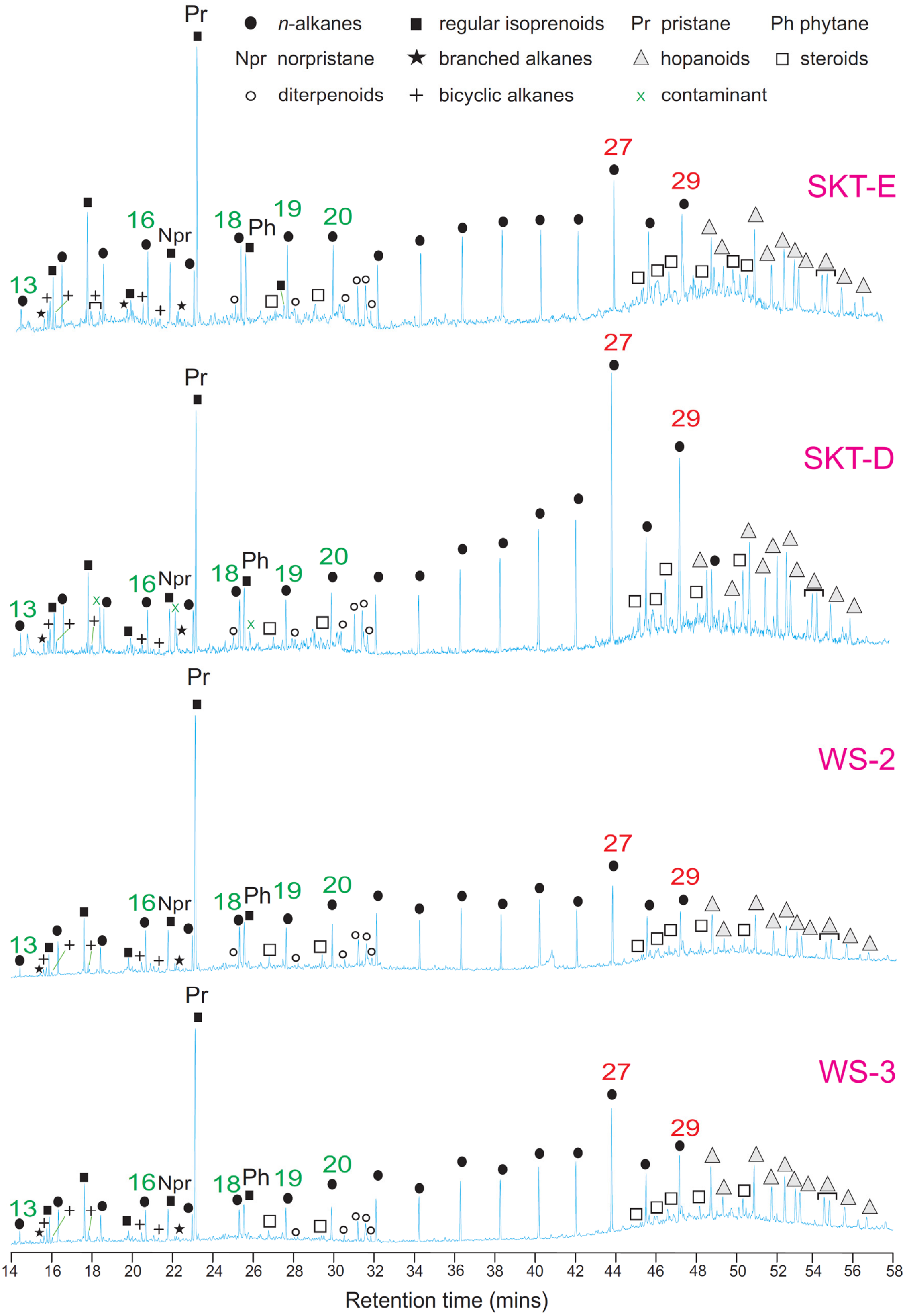

Figure 4.4: Total ion current chromatograms of the aliphatic fraction from coal extracts. Numbers above symbols denote to carbon number of *n*-alkane

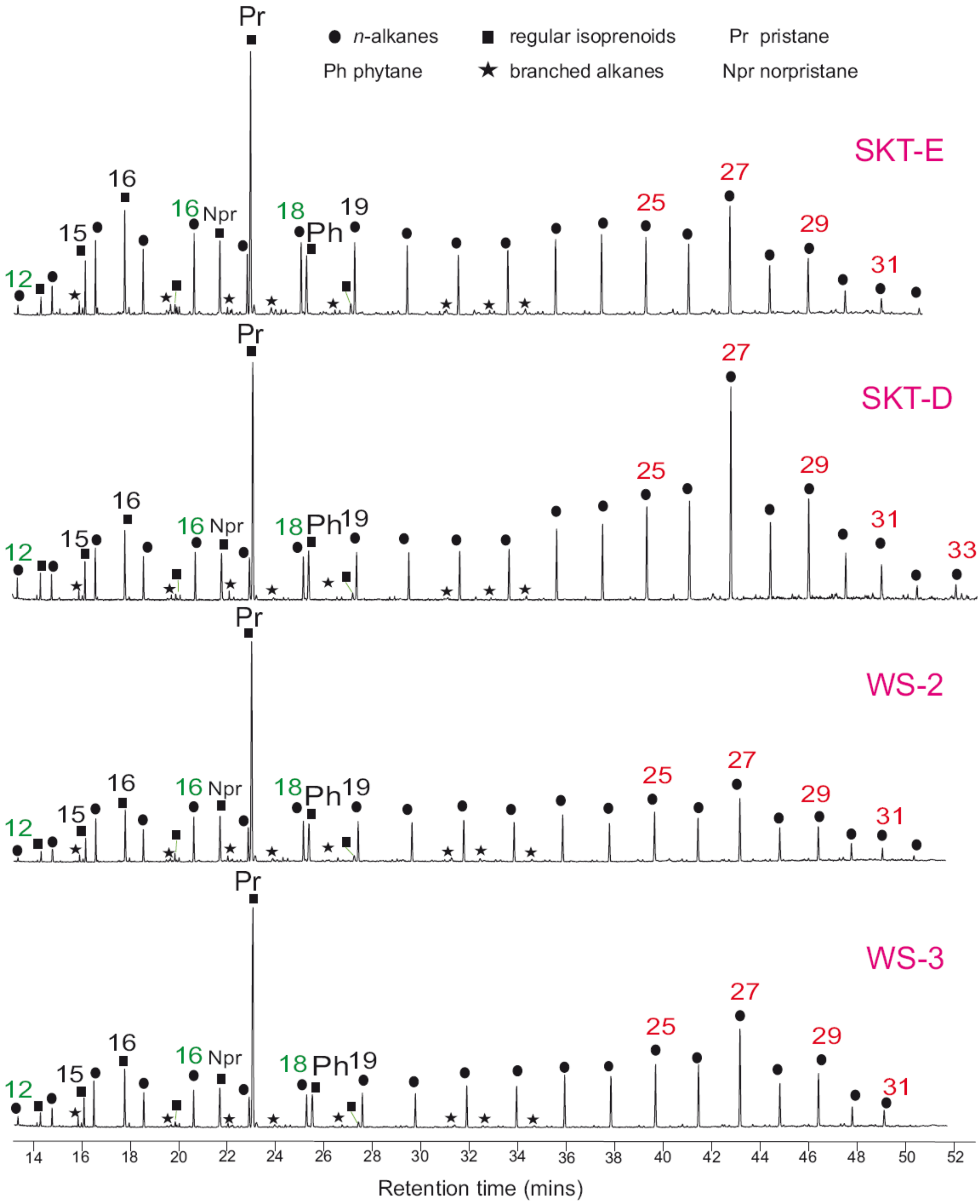

Figure 4.5: Mass chromatograms m/z 57 of aliphatic fractions from coal samples, showing the distribution of *n*-alkanes, isoprenoids and branched alkanes. Numbers above symbols indicate carbon number

Romero-Sarmiento, M.-F., et al. (In preparation)

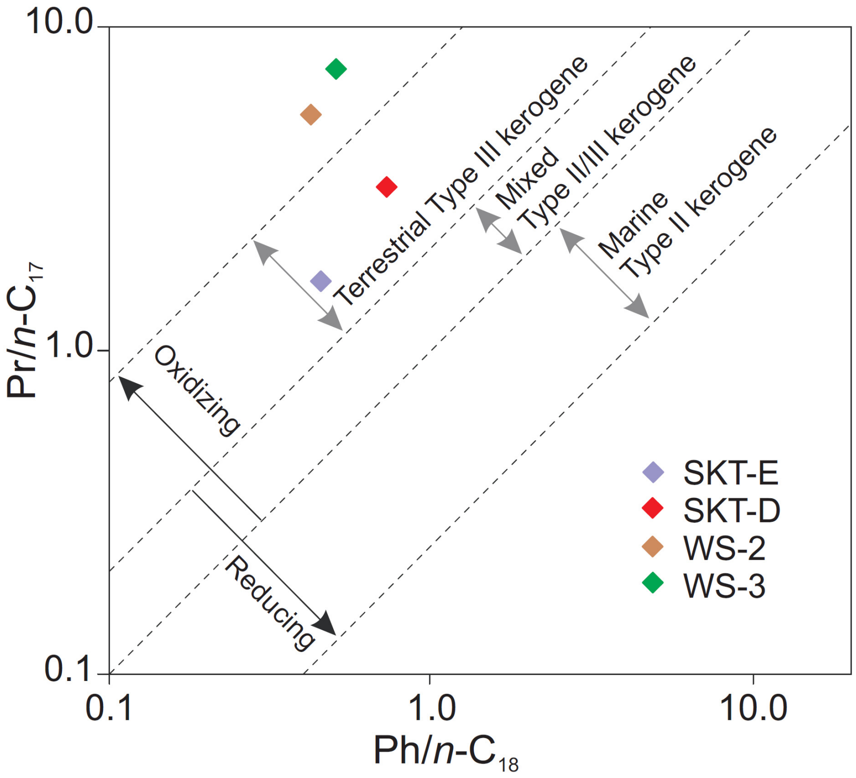

Figure 4.6: Plot of Pr/*n*-C_{17} vs. Ph/*n*-C_{18} for Scottish coals (diagram from Hunt, 1995)

4.5.2.3. Steroids

Steranes and diasteranes were detected in all the samples using the characteristic fragment at m/z 217 (Fig. 4.8). Steranes are more abundant than diasteranes and the distribution of these compounds is similar in all the samples (Fig. 4.8).

Steranes are dominated by the C_{29} 5α(H),14α(H),17α(H)-20R regular sterane (C_{29} ααα-sterane)(Fig. 4.8 and 4.9), followed by an important contribution of the αββ isomer (Fig. 4.8). Diasteranes are dominated by C_{29} βα-diasteranes. However, series of C_{27} to C_{29} αβ-diasteranes and C_{27} to C_{29} αββ-steranes have been also recognized in all the samples (Fig. 4.8). Additionally, short-chain steroids were also detected in low amounts. SKT coals show a marked predominance of short-chain C_{19} - C_{20} steroids in comparison with the content of these biomarkers in WS samples. The distribution of these compounds therefore allows separating WS coals from SKT samples (Fig. 4.8).

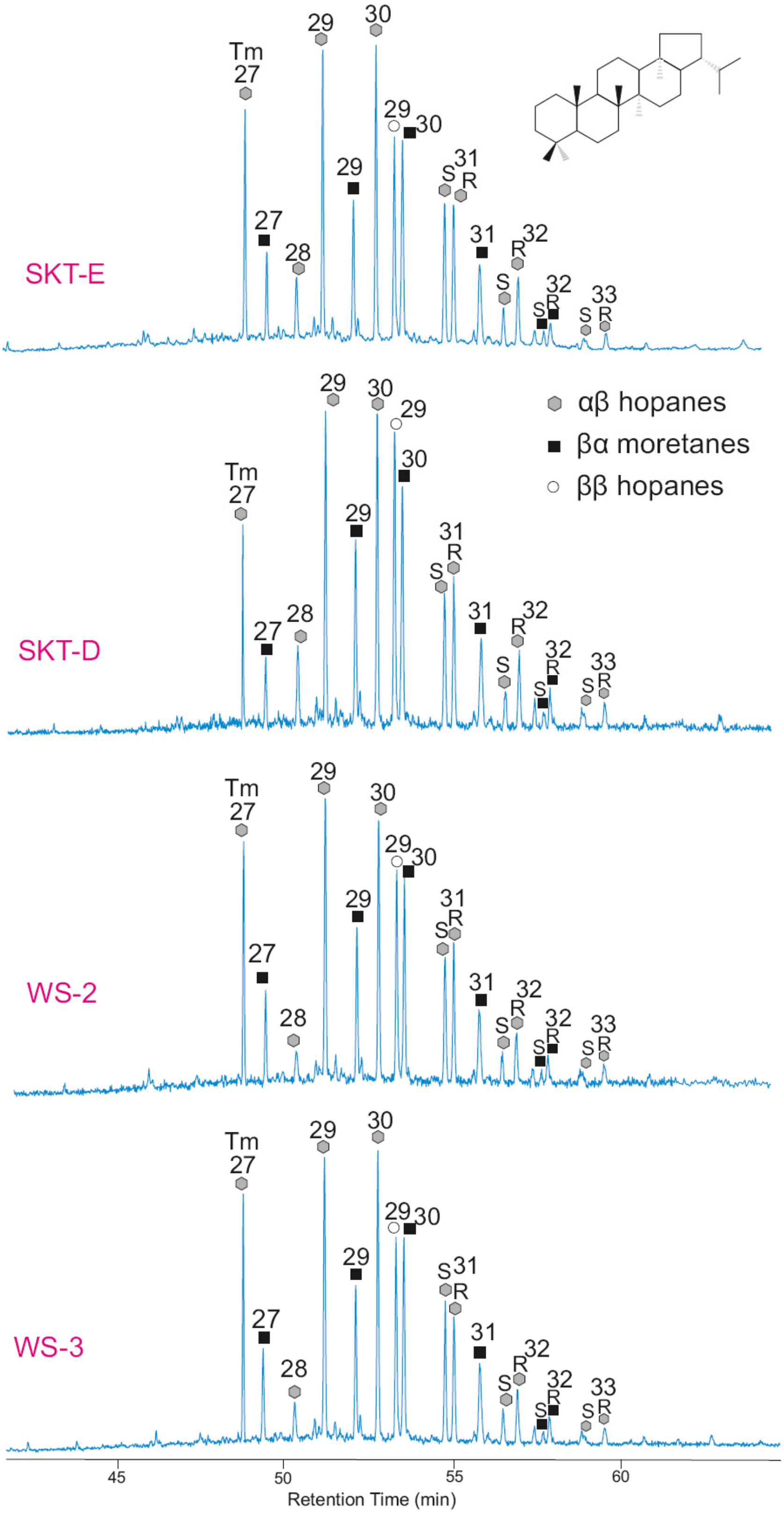

Figure 4.7: Mass chromatograms m/z 191 of aliphatic fractions from coal samples, showing the distribution of hopanes and moretanes. Numbers above symbols indicate carbon number. The molecular structure represents the standard hopane skeleton

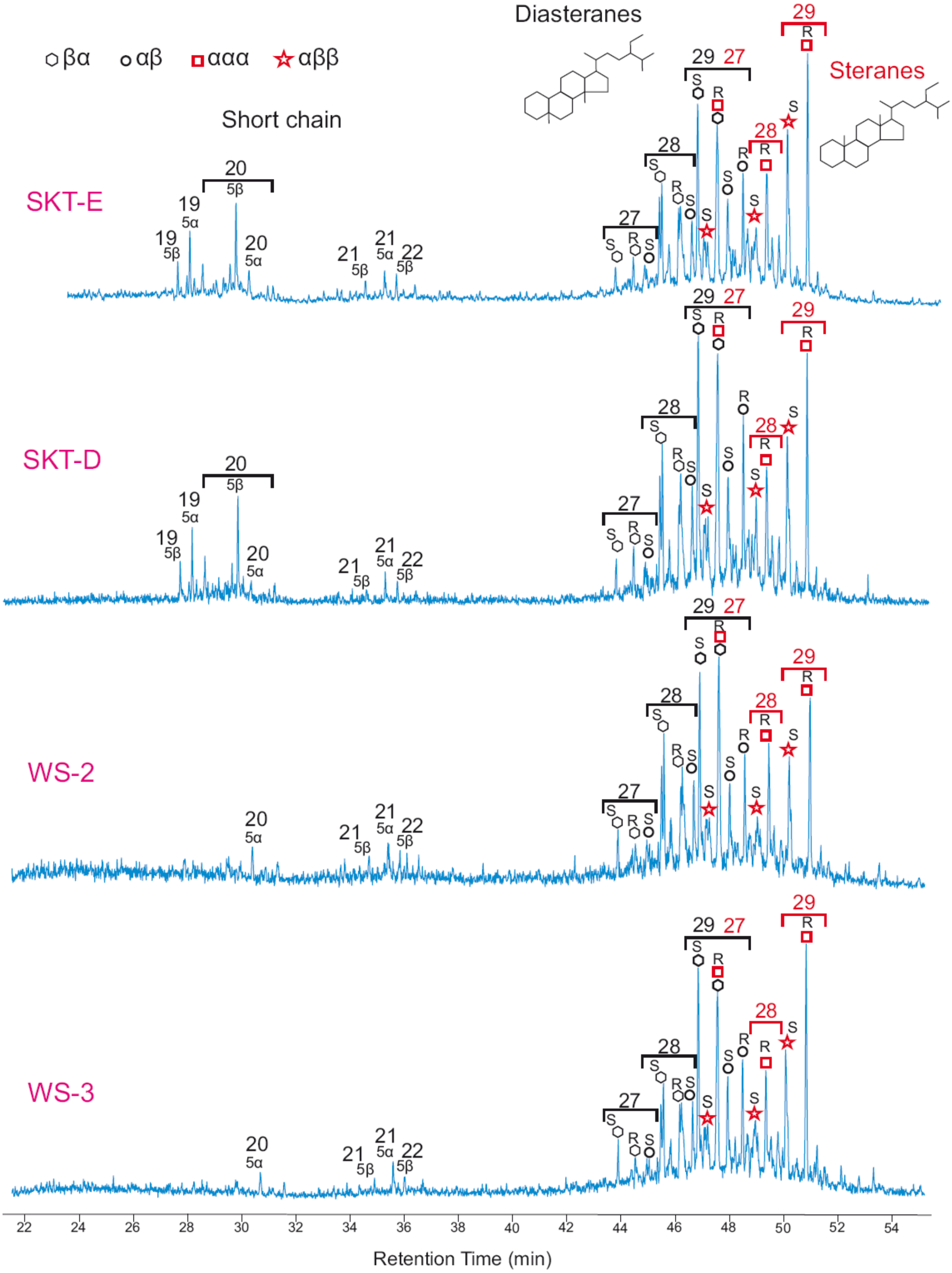

Figure 4.8: Mass chromatograms m/z 217 of aliphatic fractions from coal samples, showing the distribution of steroids. Numbers above symbols indicate carbon number

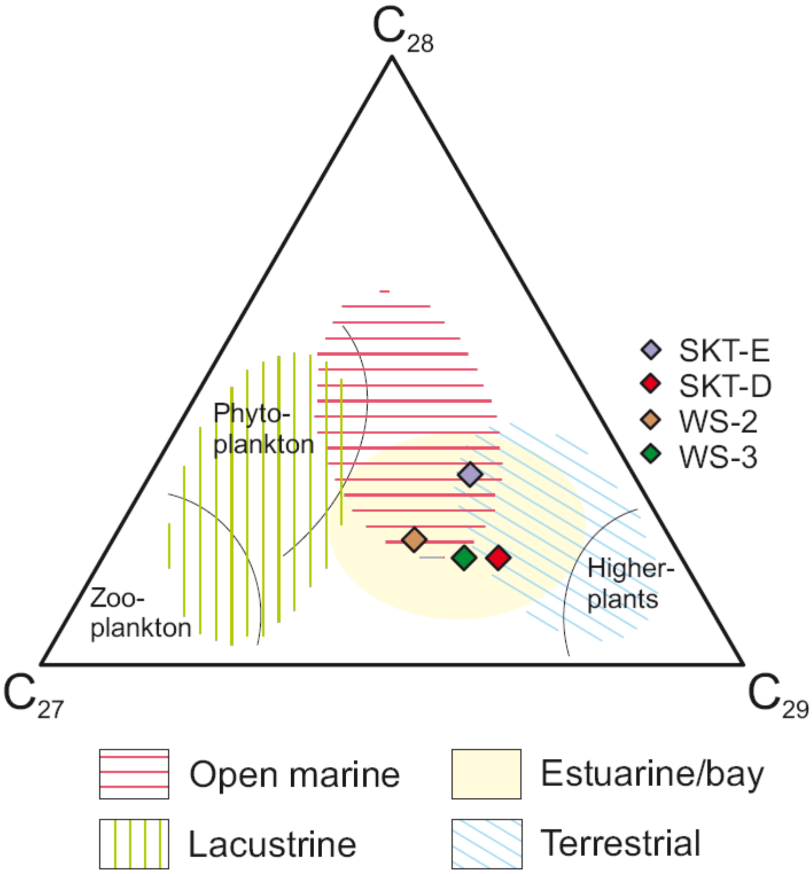

Figure 4.9: Steroids ternary plot.
The contributions of C_{27}, C_{28} and C_{29} steranes were calculated using the peak height of ααα (20R) and αββ (20S) isomers on m/z 217 fragmentograms.
Paleoenvironmental and source interpretation from Huang and Meinschein (1979)

4.5.2.4. Bicyclic alkanes

Seventeen bicyclic alkanes ranging from C_{14} to C_{16} carbon atoms have been identified in the aliphatic extracts of the Scottish coals, using the expanded m/z 109 + 123 + 179 + 193 mass fragmentograms (Fig. 4.10). Based on comparisons with the previously reported mass spectra and the retention times, bicyclic alkanes containing the eudesmane, drimane and homodrimane skeletons (4β(H)-eudesmane; 8β(H)-drimane; 8β(H)- and 8α(H)-homodrimanes) were clearly detected (Fig. 4.10; Noble, 1986; Noble et al., 1987). These compounds were not present as the major constituents of the aliphatic fractions (Fig. 4.4); however, they have been found in relative significant abundance (Fig. 4.10). Peak assignments for the bicyclic alkanes identified in our samples are summarized in Table 4.2.

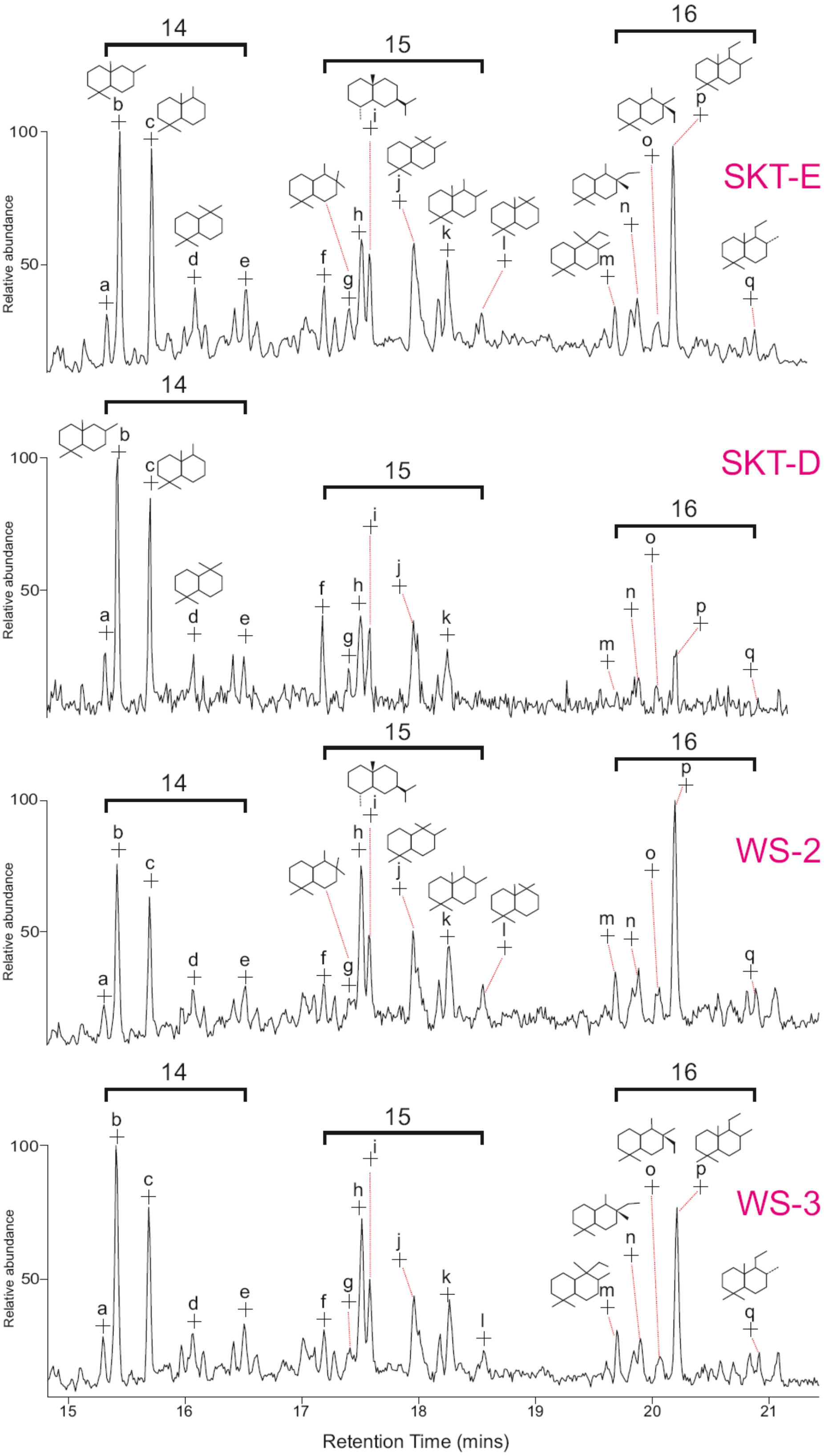

Figure 4.10: Partial m/z 109 + 123 + 179 + 193 mass fragmentograms showing the distribution of bicyclic alkanes (+) in the extracts of Scottish coals. Peak assignments are given in Table 4.2. Numbers denote carbon number

The distribution of these compounds is relatively similar in all four coals and the most abundant are C_{14} bicyclic alkanes (Fig. 4.10; e.g. Peaks **b** and **c**). However, 8β(H)-homodrimane (Fig. 4.10; Peak **p**) is also present in significant proportion in most samples except SKT-D, while the relative abundance of peak h allows differentiate WS coals from SKT samples. The relative abundance of 4β(H)-eudesmane and 8β(H)-drimane is similar in all samples (Fig. 4.10; Peaks **i** and **k**; respectively).

Table 4.2: Bicyclic alkanes identified in Carboniferous coals from Dunbar, Scotland.

Peak	m/z	MW	Compound	References
a	179	194	C_{14} bicyclic alkane? $^{+}$	-
b	179	197	C_{14} bicyclic alkane (Compound J)*	Noble, 1986
c	109	194	C_{14} bicyclic sesquiterpane	Philp, 1985
d	179	194	C_{14} bicyclic alkane (Compound K)	Noble, 1986
e	109	194	C_{14} bicyclic alkane? $^{+}$	-
f	193	208	C_{15} bicyclic alkane ? $^{+}$	-
g	193	208	C_{15} bicyclic alkane (Compound A)	Noble, 1986
h	109	208	C_{15} bicyclic alkane? $^{+}$	-
i	109	208	4β-eudesmane	Noble, 1986
j	193	208	C_{15} bicyclic alkane (Compound B)	Noble, 1986
k	123	208	8β(H)-drimane	Noble et al., 1987
l	123	208	C_{15} bicyclic alkane (Compound D)	Noble, 1986
m	109	222	C_{16} bicyclic alkane (Compound E)	Noble, 1986
n	109	222	C_{16} bicyclic alkane (Compound F)	Noble, 1986
o	109	222	C_{16} bicyclic alkane (Compound G)	Noble, 1986
p	123	222	8β(H)-homodrimane	Noble et al., 1987
q	123	222	8α(H)-homodrimane	Noble et al., 1987

(*) Names of compounds proposed by Noble (1986)

($^{+}$) Mass spectra are shown in Appendix A.6

4.5.2.5. Tricyclic and tetracyclic diterpenoids

The partial m/z 109 + 123 + 193 + 233 mass fragmentograms from the aliphatic fractions of Lower Carboniferous coals reveal the presence of eighteen tricyclic and tetracyclic diterpenoid hydrocarbons (Fig. 4.11). Peak assignments for these identified aliphatic diterpenoids are shown in Table 4.3.

The tetracyclic diterpenoids ($C_{20}H_{34}$) *ent*-beyerane, abietane, 16β(H)-phyllocladane, *ent*-16α(H)-kaurane, 16α(H)-phyllocladane and *ent*-16β(H)-kaurane were mainly recognized by comparison with the published mass spectra of the authentic compounds (Noble, 1986; Otto et al., 1997; also in Noble et al., 1985; Philp, 1985; Schulze and Michaelis, 1990).

The peak labelled **II** has a mass spectrum characterized by a strong molecular fragment at m/z 233 (Fig. 4.11. A). Based on its molecular fragment, compound **II** was tentatively identified as a C_{18} tricyclic hydrocarbon (Fig. 4.11). This latter compound is particularly present in significant proportions in SKT samples. To our knowledge, the molecular structures for most of the C_{18} – C_{19} diterpenoids identified in this study, have not been previously established.

In most samples, the tetracyclic diterpenoid distribution is dominated by kaurane and phyllocladane. Kaurane isomers, however, are relatively more abundant in SKT samples while phyllocladanes are more predominant in WS coals (Fig. 4.11). *ent*-Beyarane show a relatively similar contribution in all samples, and generally is present in low amounts in comparison to the other C_{20} diterpenoids.

The only tricyclic diterpenoid identified is abietane (Peak XIV; Fig. 4.11). It is present in relatively low abundance in all four samples. Its contribution is however more significant in WS coals (Fig. 4.11).

The molecular structures of *ent*-beyerane (Peak XII), abietane (Peak XIV), phyllocladane and kaurane skeletons (Peaks XIII; XV– XVII) are also shown in figure 4.11.

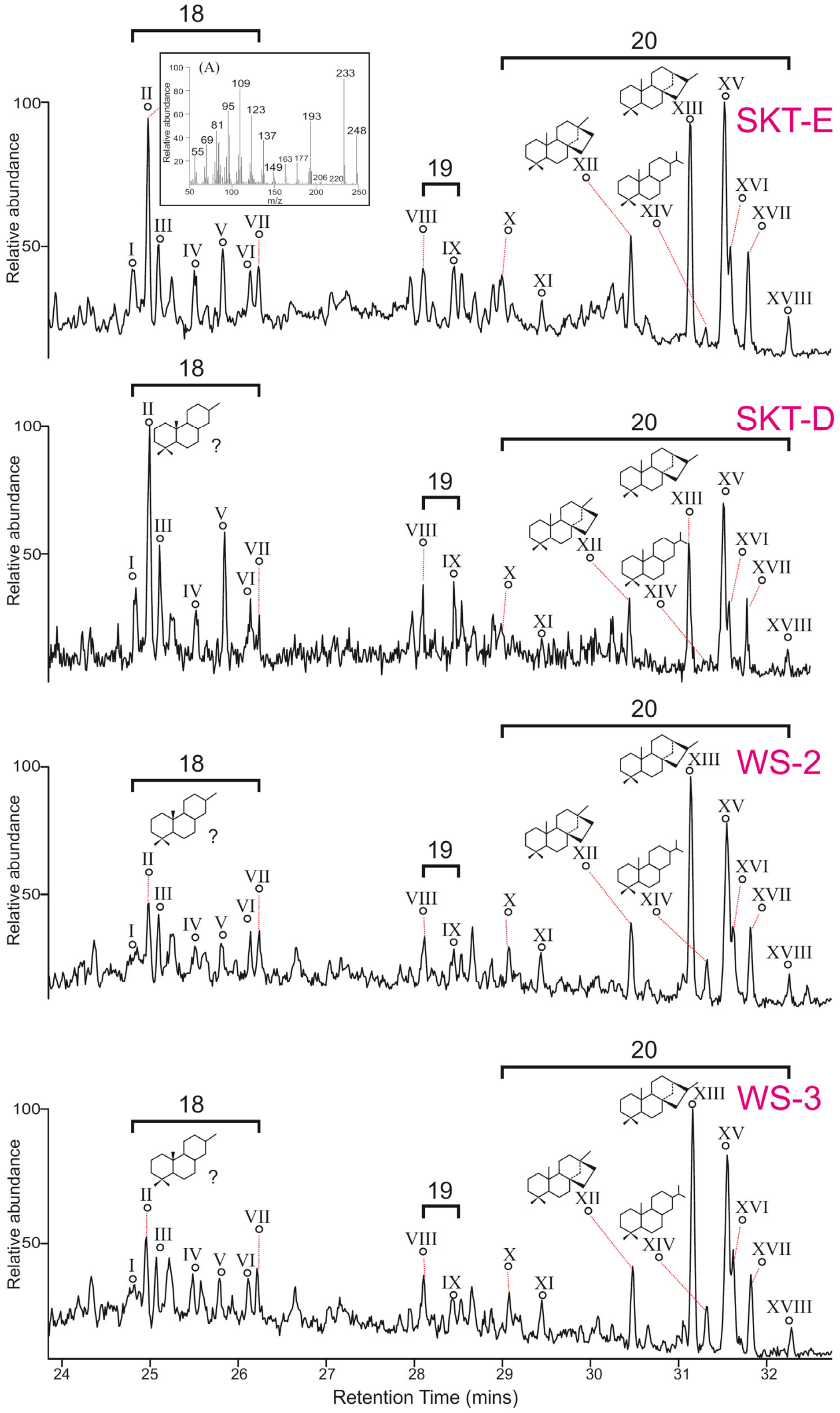

Figure 4.11: Partial m/z 109 + 123 + 193 + 233 mass fragmentograms showing the distribution of diterpenoid hydrocarbons (o) in the extracts of Scottish coals. Peak assignments are given in Table 4.3. Numbers denote carbon number. (A) Mass spectrum of compound identified as C_{18} tricyclic hydrocarbon (Peak **II**)

Table 4.3: Diterpenoid hydrocarbons identified in Carboniferous coals from Dunbar, Scotland

Peak	m/z	MW	Compound	References
I	193	248	C_{18} tricyclic hydrocarbon? ^{o}	-
II	233	248	C_{18} tricyclic hydrocarbon?	-
III	233	248	C_{18} tricyclic hydrocarbon? ^{o}	-
IV	233	248	C_{18} tricyclic hydrocarbon? ^{o}	-
V	193	248	C_{18} tricyclic hydrocarbon? ^{o}	-
VI	233	248	C_{18} tricyclic hydrocarbon? ^{o}	-
VII	233	248	C_{18} tricyclic hydrocarbon? ^{o}	-
VIII	123	262	C_{19} tricyclic hydrocarbon? ^{o}	-
IX	193	262	C_{19} tricyclic hydrocarbon? ^{o}	-
X	109	274	C_{20} tetracyclic diterpenoid? ^{o}	-
XI	109	274	C_{20} tetracyclic diterpenoid? ^{o}	-
XII	123	274	*ent*-beyerane	Noble, 1986
XIII	123	274	16β(H)-phyllocladane	Noble,1986;Otto et al.,1997
XIV	163	276	abietane	Philp, 1985
XV	123	274	*ent*-16α(H)-kaurane	Noble, 1986
XVI	123	274	16α(H)-phyllocladane	Noble,1986;Otto et al.,1997
XVII	123	274	*ent*-16β(H)-kaurane	Noble, 1986
XVIII	123	274	C_{20} tetracyclic diterpenoid? ^{o}	-

(^{o}) Mass spectra are shown in Appendix A.6

4.5.3 Aromatic fractions

4.5.3.1. Total aromatic hydrocarbons

Figure 4.12 displays the partial TIC of the aromatic fractions from SKT-E and WS-3 coal samples. The numbered chromatographic peaks are identified in Table 4.4. At least, ninety-seven aromatic compounds were detected by their characteristic mass fragments (Table 4.4).

Alkylnaphthalenes, alkylphenanthrenes, alkyldibenzofurans are the most abundant compound families observed in the aromatic fractions of the Lower Carboniferous coals (Fig. 4.12). The land-plant and combustion-derived polycyclic aromatic hydrocarbons (PAHs) have been also found in significant abundance. Alkylbiphenyls, alkylanthracenes and alkylpyrenes were recognized as minor contributors to the aromatic fractions (Fig. 4.12; Table 4.4). Dibenzothiophenes were not detected in our coal extracts.

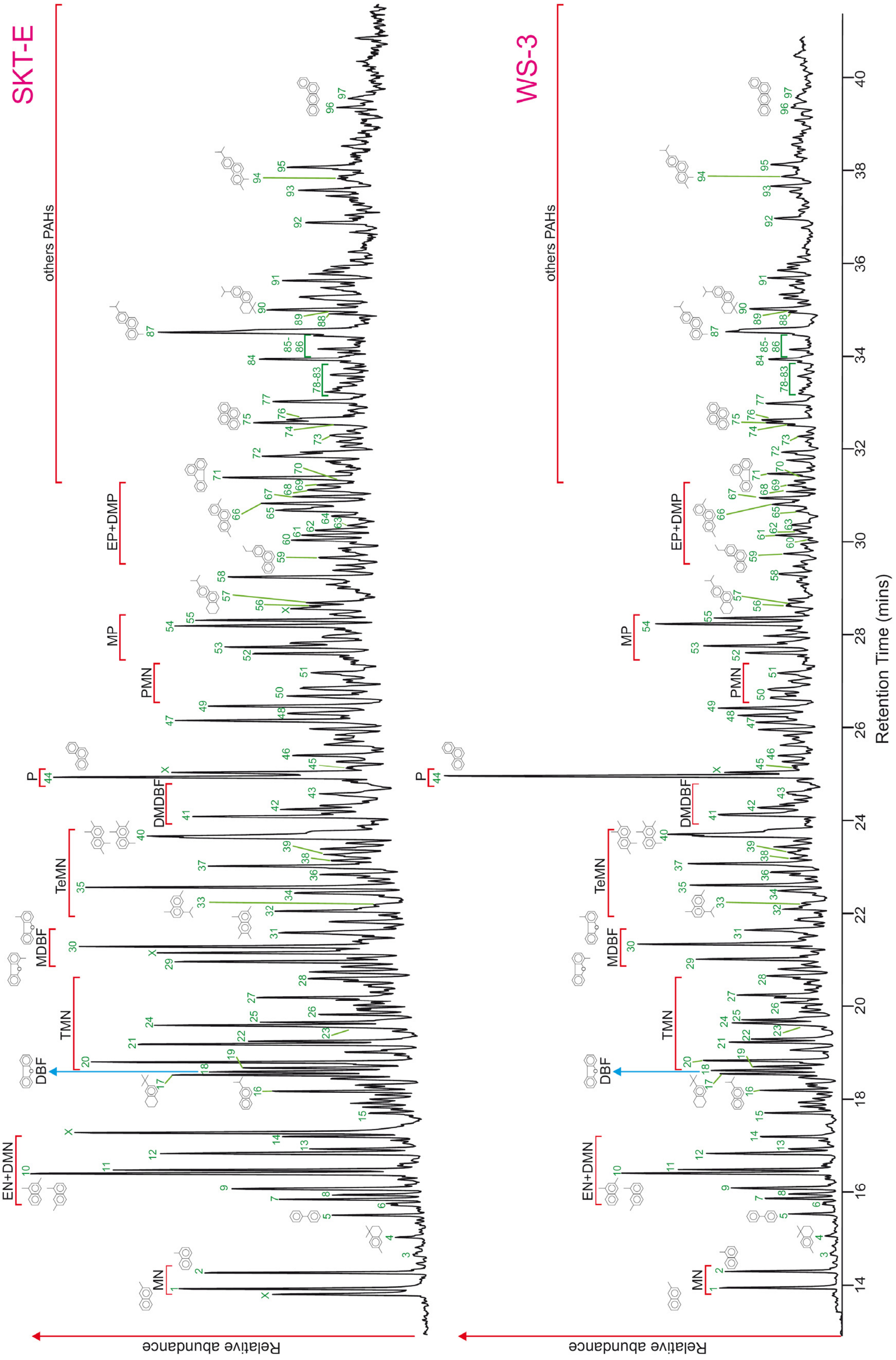

Figure 4.12: Total ion current chromatograms of aromatic fraction from extracts of two selected coal samples. Peak assignments are given in Table 4.4

Abbreviations: MN – methylnaphthalene; EN + DMN – ethyl- and dimethylnaphthalene; DBF – dibenzofuran; TMN – trimethylnaphthalene; MDBF – methyldibenzofuran; TeMN – tetramethylnaphthalene; DMDBF – dimethyldibenzofuran; P – phenanthrene; PMN – pentamethylnaphthalene; MP – methylphenanthrene; EP + DMP – ethyl– and dimethylphenanthrene; PAHs – polycyclic aromatic hydrocarbons; X – contaminant (polysiloxanes).

Table 4.4: Aromatic hydrocarbons identified in Carboniferous coals from Dunbar, Scotland.

Peak	m/z	Compound
1	142	2-MN
2	142	1-MN
3	159	methylated-tetrahydro napthalene?
4	159	1,2,3,4-tetrahydro-1,1,6-TMN (Ionene)
5	154	Biphenyl
6	159	methylated-tetrahydro napthalene?
7	156	2-EN
8	156	1-EN
9	156	2,6- + 2,7-DMN
10	156	1,3- + 1,7-DMN
11	156	1,6-DMN
12	156	1,4- + 2,3-DMN
13	156	1,5-DMN
14	156	1,2-DMN
15	168	2-methyl-biphenyl
16	155	2-isopropyl-naphthalene
17	173	1,2,3,4-tetrahydro-6-(1,1-dimethyl)-naphthalene
18	168	Dibenzofuran
19	170	1,3,7-TMN
20	170	1,3,6-TMN
21	170	1,4,6- + 1,3,5-TMN
22	170	2,3,6-TMN
23	170	1,2,7-TMN
24	170	1,6,7-TMN
25	170	1,2,6-TMN
26	170	1,2,4-TMN
27	170	1,2,5-TMN
28	170	1,4,5-TMN
29	182	4-MDBF
30	182	3- + 2-MDBF
31	182	1-MDBF
32	184	1,3,6,7-TeMN
33	183	Cadalene
34	184	1,2,4,6- + 1,2,4,7- + 1,4,6,7-TeMN
35	184	1,2,5,7-TeMN

Peak	m/z	Compound
36	184	2,3,6,7-TeMN
37	184	1,2,6,7-TeMN
38	184	1,2,3,7-TeMN
39	184	1,2,3,6-TeMN
40	184	1,2,5,6- + 1,2,3,5-TeMN
41	196	Dimethyldibenzofuran
42	196	Dimethyldibenzofuran
43	196	Dimethyldibenzofuran
44	178	Phenanthrene
45	178	Anthracene
46	212	Unknown
47	195	(M+210) Trimethyldibenzofuran ?
48	155	C_{17} Isohexylalkylnaphthalene
49	195	(M+210) Trimethyldibenzofuran ?
50	198	1,2,3,6,7-PMN
51	198	1,2,3,5,6-PMN
52	192	3-MP
53	192	2-MP
54	192	9-MP
55	192	1-MP
56	169	C_{18} Isohexylalkylnaphthalene
57	209	Bisnorsimonellite
58	204	2-phenyl-naphthalene?
59	206	3-EP
60	206	2- + 9-EP
61	206	1-EP
62	206	3,5- + 2,6-DMP
63	206	2,7-DMP
64	206	1,3- + 3,9- + 2,10- + 3,10-DMP
65	206	1,6- + 2,9- + 2,5-DMP
66	206	1,7-DMP
67	206	2,3-DMP
68	206	1,9- + 4,9- + 4,10-DMP
69	206	1,8-DMP
70	223	Tetrahydroretene
71	202	Fluoranthene
72	218	methylacephenanthrene ?
73	183	C_{19} Isohexylalkylnaphthalene
74	237	Simonellite
75	202	Pyrene
76	237	Totarane
77	223	Tetrahydroretene-derived isomer?
78	220	1,3,6- + 1,3,10- + 2,6,10-TMP
79	220	1,3,7- + 2,6,9- + 2,7,9-TMP
80	220	1,3,9- + 2,3,6-TMP
81	220	1,6,9- + 1,7,9- + 2,3,7-TMP
82	220	1,3,8-TMP
83	220	2,3,10-TMP
84	216	methyl-fluoranthene?

Peak	m/z	Compound
85	220	1,6,7-TMP
86	220	1,2,6-TMP
87	219	Retene + 4-methyl-pyrene
88	220	1,2,7-TMP
89	220	1,2,8-TMP
90	237	Sempervirane
91	216	1-methyl-pyrene
92	230	1-ethyl-pyrene ?
93	215	ethyl- or dimethylpyrene ?
94	233	2-methylretene
95	230	1,3-dimethyl-pyrene?
96	228	Benzo[a]anthracene
97	228	Chrysene + Triphenylene

Abbreviations: MN – methylnaphthalene, DMN – dimethylnaphthalene, TMN – trimethylnaphthalene, TeMN – tetramethylnaphthalene, PMN – pentamethylnaphthalene, EN – ethylnaphthalene, MDBF – methyldibenzofuran, MP – methylphenanthrene, EP – ethylphenanthrene, DMP – dimethylphenanthrene, TMP – trimethylphenanthrene.

4.5.3.2. Alkylnaphthalenes, alkylphenanthrenes and alkyldibenzofurans

The distributions of methylnaphthalene (MN), ethylnaphthalene (EN) and dimethylnaphthalene (DMN) isomers from all Scottish coals are shown in Fig. 4.13. The 2-MN is more abundant than 1-MN in all samples except SKT-D (Fig. 4.13; Table 4.5). The methylnaphthalene ratios (MNR) of the Scottish coals average about 1 (Table 4.5). In addition, a predominance of 2-EN over 1-EN is also noted (Fig. 4.13; Table 4.5). Regarding the dimethylnaphthalene distributions, the highest peaks correspond to 1,3- and 1,7-DMN, followed by 1,6-DMN (Fig. 4.13).

Figure 4.14 reveals an expanded section of the m/z 170 mass fragmentograms for the aromatic fraction of Scottish coals. The distribution of trimethylnaphthalene (TMN) homologues is similar in all four coals and the most predominant compound is 1,3,6-TMN (Fig. 4.14). The trimethylnaphthalene ratio values (TNR1, TNR2 and TMNr) are less than 1 (Table 4.5). The biomarker 1,2,5-TMN is present in all the samples. However, the abundance of 1,3,7-TMN is subtly superior to that of 1,2,5-TMN in all samples except WS-3 (Fig. 4.14).

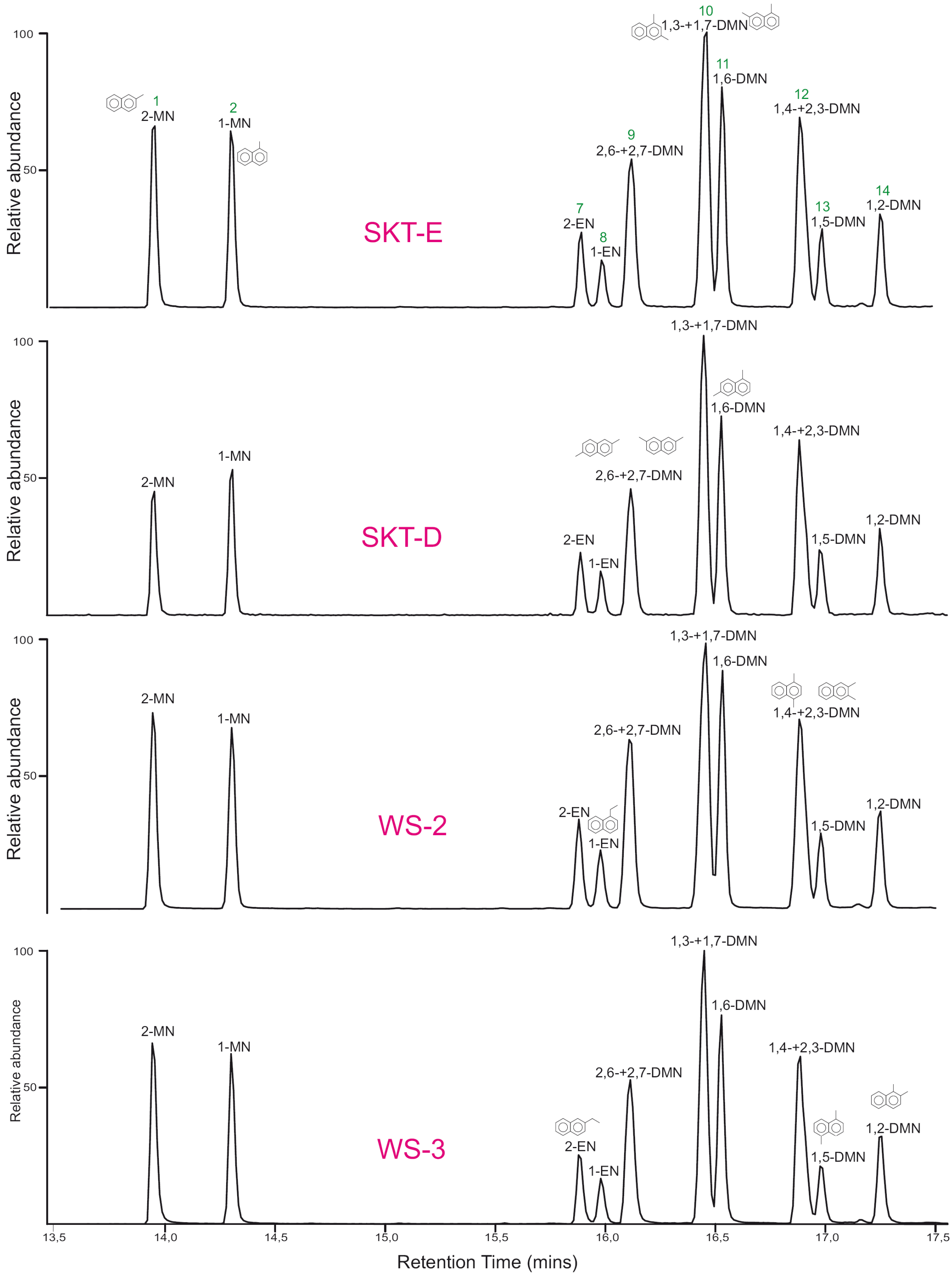

Figure 4.13: Partial m/z 142+156 mass fragmentograms showing the distribution of methyl-; ethyl- and dimethylnaphthalene isomers in the aromatic fractions of Scottish coals. ***Abbreviations:*** MN – methylnaphthalene; EN – ethylnaphthalene; DMN – dimethylnaphthalene. Peak assignments are given in Table 4.4

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Table 4.5: Maturity indicators from aromatic fractions of Carboniferous coal samples.

Abbreviations: MN – methylnaphthalene; EN – ethylnaphthalene; DMN – dimethylnaphthalene; TMN – trimethylnaphthalene; TeMN – tetramethylnaphthalene; P – phenanthrene; MP – methylphenanthrene

	Samples			
Parameters	**SKT-E**	**SKT-D**	**WS-2**	**WS-3**
MNR[(a)]	1.04	0.85	1.10	1.05
ENR[(b)]	1.71	1.43	1.78	1.67
DNR[(c)]	4.15	4.18	5.50	6.25
TNR1[(d)]	0.29	0.27	0.38	0.38
TNR2[(e)]	0.35	0.37	0.46	0.44
TMNr[(f)]	0.53	0.53	0.48	0.46
TeMNr[(g)]	0.43	0.41	0.23	0.24
MPR[(h)]	0.88	0.90	0.83	1.00
MPI 1[(i)]	0.52	0.59	0.39	0.36
MPI 2[(j)]	0.49	0.56	0.36	0.39

(a) MNR = [2-MN]/[1-MN]; m/z=142 (Radke et al., 1982; Radke et al., 1986).

(b) ENR = [2-EN]/[1-EN]; m/z=156 (Radke et al., 1982; Radke et al., 1986).

(c) DNR = {[2,6-DMN]+[2,7-DMN]}/[1,5-DMN]; m/z=156 (Radke et al., 1982; Radke et al., 1986).

(d) TNR1 = [2,3,6-TMN]/{[1,4,6-TMN]+[1,3,5-TMN]} ; m/z=170 (Alexander et al., 1985).

(e) TNR2 = {[1,3,7-TMN]+[2,3,6-TMN]}/{[1,3,5-TMN]+[1,3,6-TMN]+[1,4,6-TMN]} ; m/z=170 (Radke et al., 1986).

(f) TMNr = [1,3,7-TMN]/{[1,3,7-TMN]+[1,2,5-TMN]} ; m/z=170 (van Aarssen et al., 1999).

(g) TeMNr = [1,3,6,7-TeMN]/{[1,3,6,7-TeMN]+[(1,2,5,6+1,2,3,5)-TeMN]} ; m/z=170 (van Aarssen et al., 1999).

(h) MPR = [2-MP]/[1-MP]; m/z=178+192 (Radke et al., 1986).

(i) MPI 1 = {1.5([2-MP]+[3-MP])}/{[P]+[1-MP]+[9-MP]}; m/z=178+192 (Radke et al., 1986).

(j) MPI 2 = {3[2-MP]}/{[P]+[1-MP]+[9-MP]}; m/z=178+192 (Radke et al., 1986).

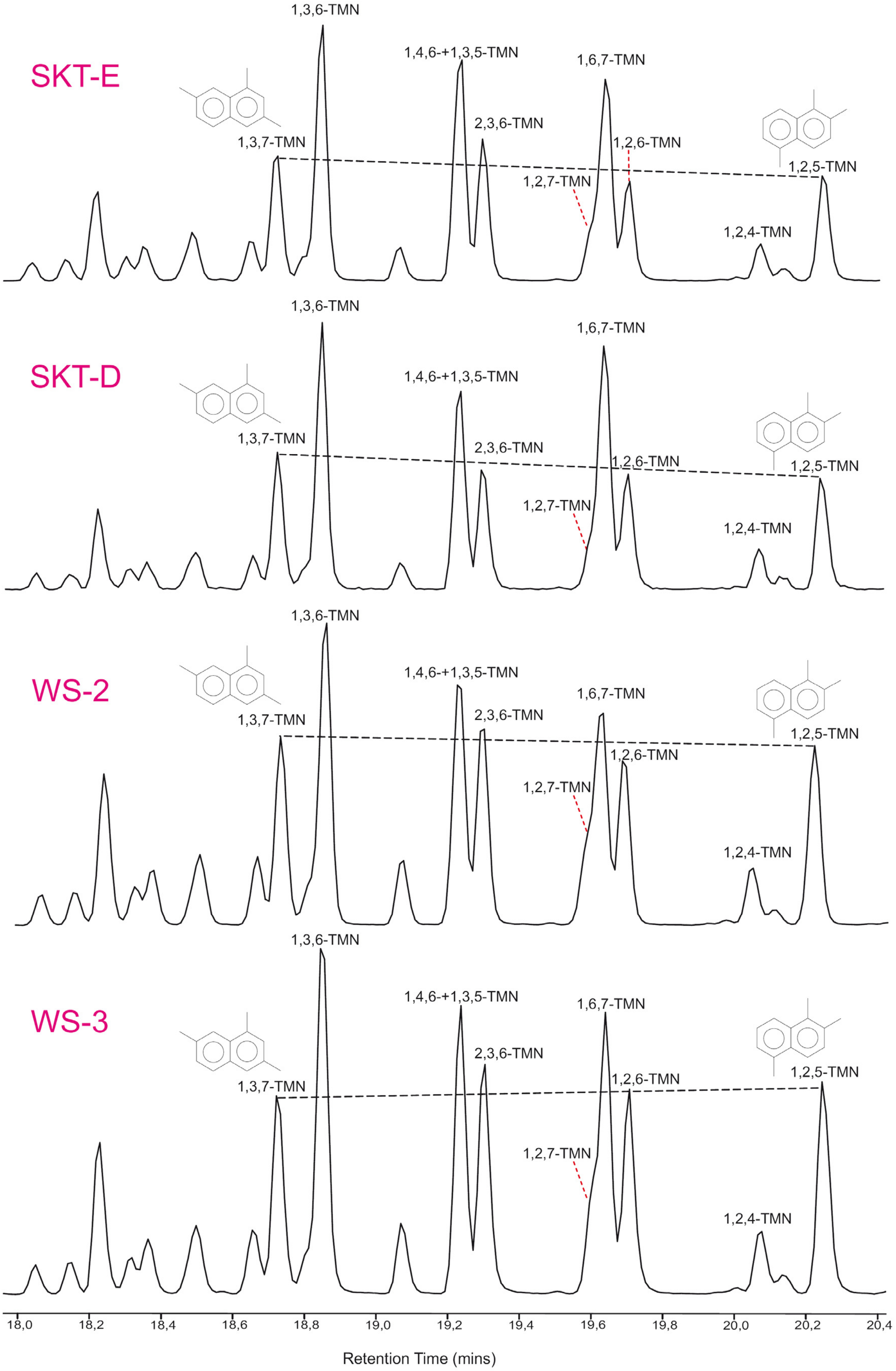

Figure 4.14: Partial m/z 170 mass fragmentograms showing the distribution of trimethylnaphthalene (TMN) isomers in the aromatic fractions of Scottish coals

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For the tetramethylnaphthalene (TeMN) homologues, 1,2,5,6-TeMN co-elutes with 1,2,3,5-TeMN and their abundances should be measured as one (Fig. 4.15; van Aarssen et al., 1999). Therefore, the most abundant isomers in the aromatic fractions of Scottish coals are 1,2,5,6- and 1,2,3,5-TeMN (Fig. 4.15). Additionally, 1,3,6,7-TeMN is also detected but in minor proportions compared to 1,2,5,6-TeMN (Fig. 4.15). The tetramethylnapthalene ratios (TeMNr) range from 0.23 to 0.43 (Table 4.5).

The distributions of phenanthrene (P), anthracene (A), and their methylphenanthrene (MP) and methylanthracene (MA) isomers from all aromatic fractions of Scottish coals are represented in Fig. 4.16. Based on these selected partial m/z 178+192 mass fragmentograms, phenanthrene is the most abundant compound whereas anthracene is the least abundant one. Sample SKT-D slightly differs from the other samples by the higher proportion of 2-MA (Fig. 4.16; Smith et al., 1995). Although 9-MP is the most predominant methylphenanthrene in all the samples, 2-MP and 3-MP are also observed and are relatively present in similar proportions (Fig. 4.16). The alkylphenanthrene indices (MPR, MPI 1 and MPI 2) are also given in Table 4.5. Figure 4.17 displays a partial m/z 206 mass fragmentograms showing the distribution of ethylphenanthrene (EP) and dimethylphenanthrene (DMP) isomers in the aromatic fractions of Scottish coals. Pimanthrene (1,7-DMP) is present in relatively high amounts in all the samples. Actually, it is the most abundant compound in SKT-D sample (Fig. 4.17).

Dibenzofuran (DBF) and its methyl derivatives (MDBF) have been also detected in the aromatic fractions (Fig. 4.12; Peaks 18, 29, 30 and 31, respectively). The distribution of MDBF is dominated by 2-MDBF (Peak 30) and 4-MDBF (Peak 29). The latter is the most thermodynamically stable isomer (Fig. 4.12; Radke et al., 2000). Several aromatic compounds with methylated-tetrahydronaphthalene skeletons (Fig. 4.12; Table 4.4) have also been determined. According to the published mass spectra, ionene (1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene) and 1,2,3,4-tetrahydro-6-(1,1-dimethyl)-naphthalene have been clearly identified (Fig. 4.12; Peaks 4 and 17, respectively). Finally, series of C_{17} to C_{19} isohexylalkylnaphthalenes have been also observed (Fig. 4.18). The contribution of the isohexylalkylnaphthalene (iHMN) compounds is relatively similar in all four samples and the most abundant is C_{17} iHMN except SKT-D, where C_{19} iHMN is more predominant (Fig. 4.18).

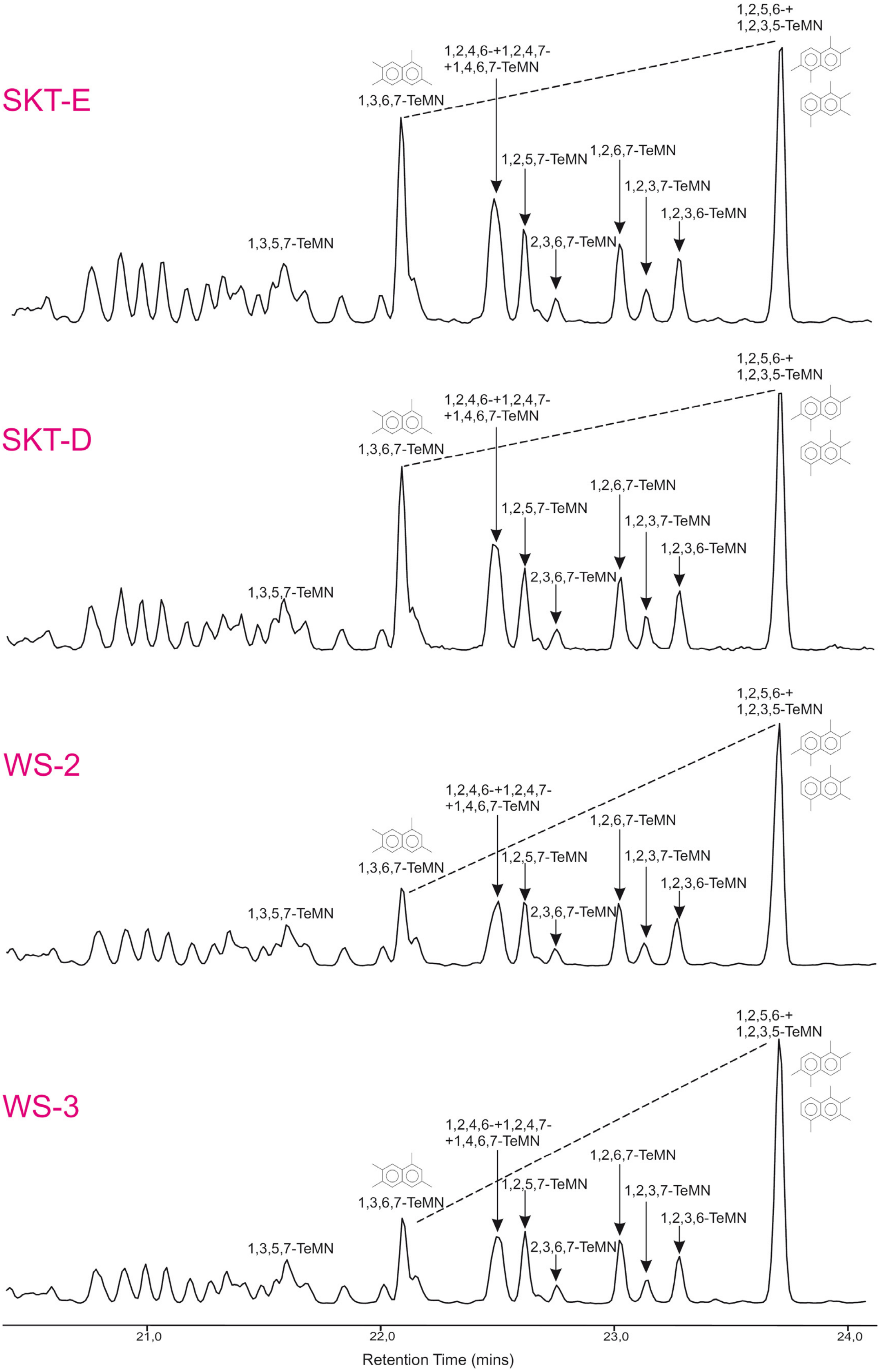

Figure 4.15: Partial m/z 184 mass fragmentograms showing the distribution of tretramethylnaphthalene (TeMN) isomers in the aromatic fractions of Scottish coals

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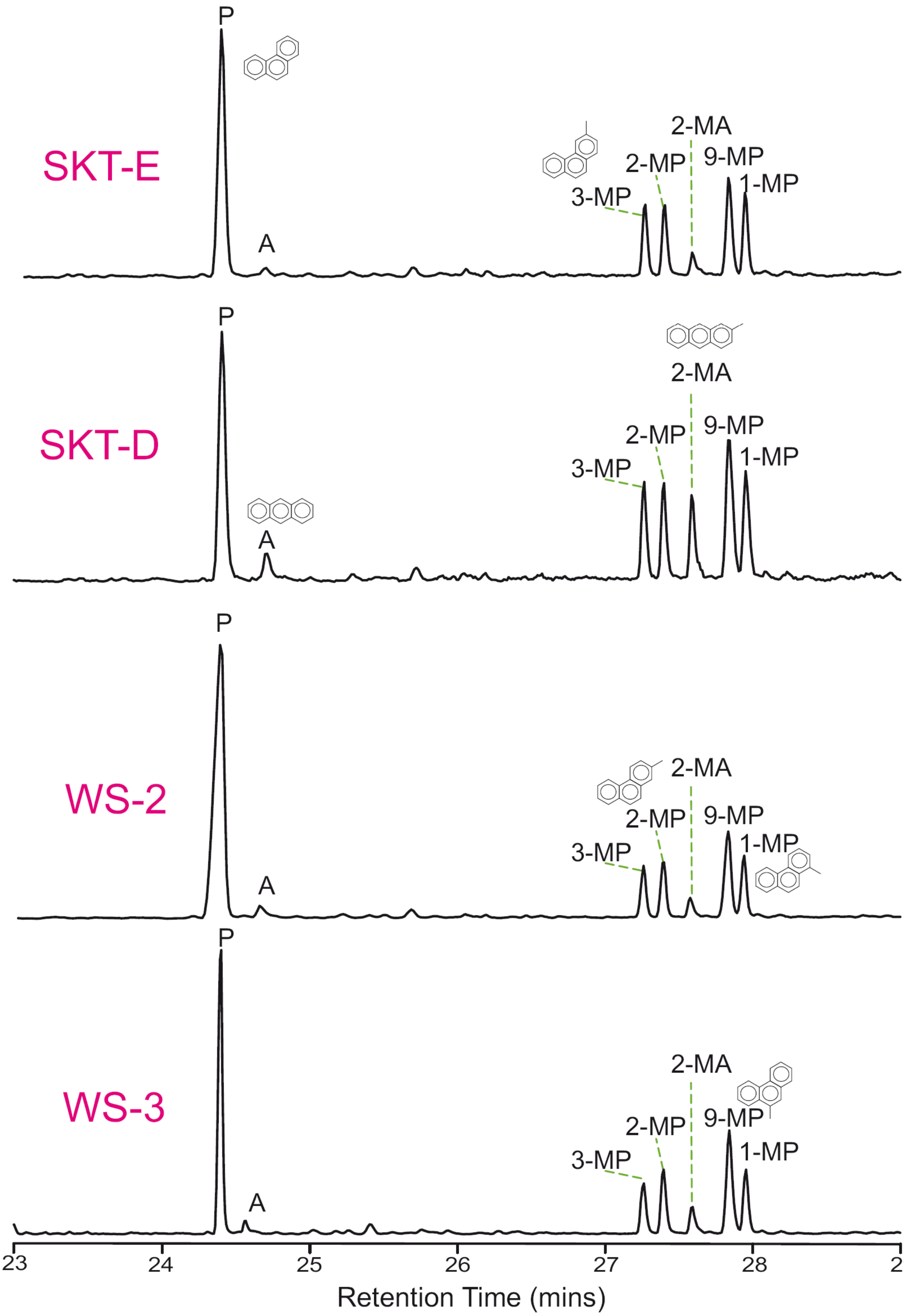

Figure 4.16: Partial m/z 178+192 mass fragmentograms showing the distribution of phenanthrene (P), anthracene (A), methylphenanthrene (MP) and methylanthracene (MA) isomers in the aromatic fractions of Scottish coals

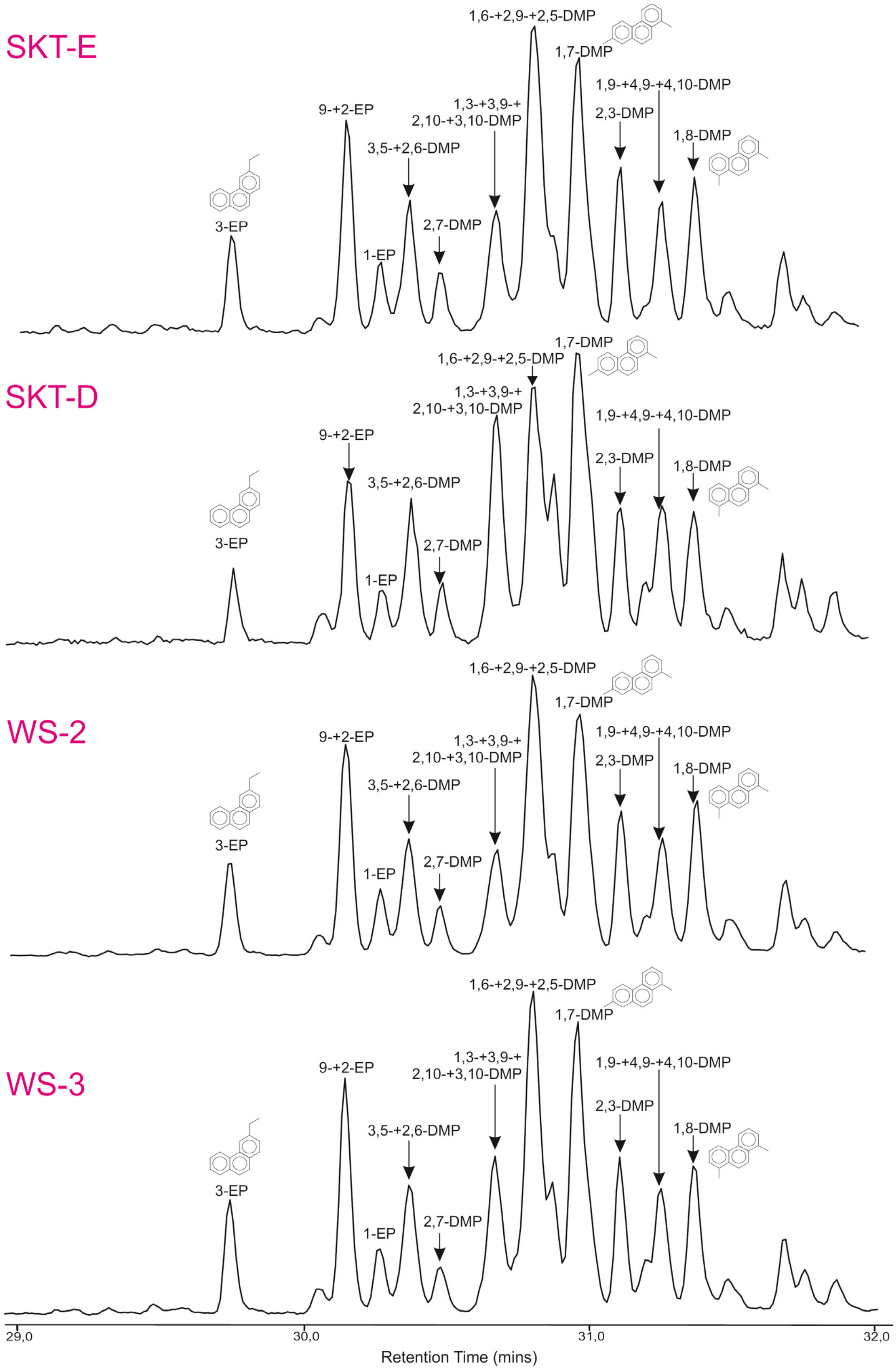

Figure 4.17: Partial m/z 206 mass fragmentograms showing the distribution of ethyl- and dimethylphenanthrene isomers in the aromatic fractions of Scottish coals. ***Abbreviations:*** EP – ethylphenanthrene, DMP – dimethylphenanthrene

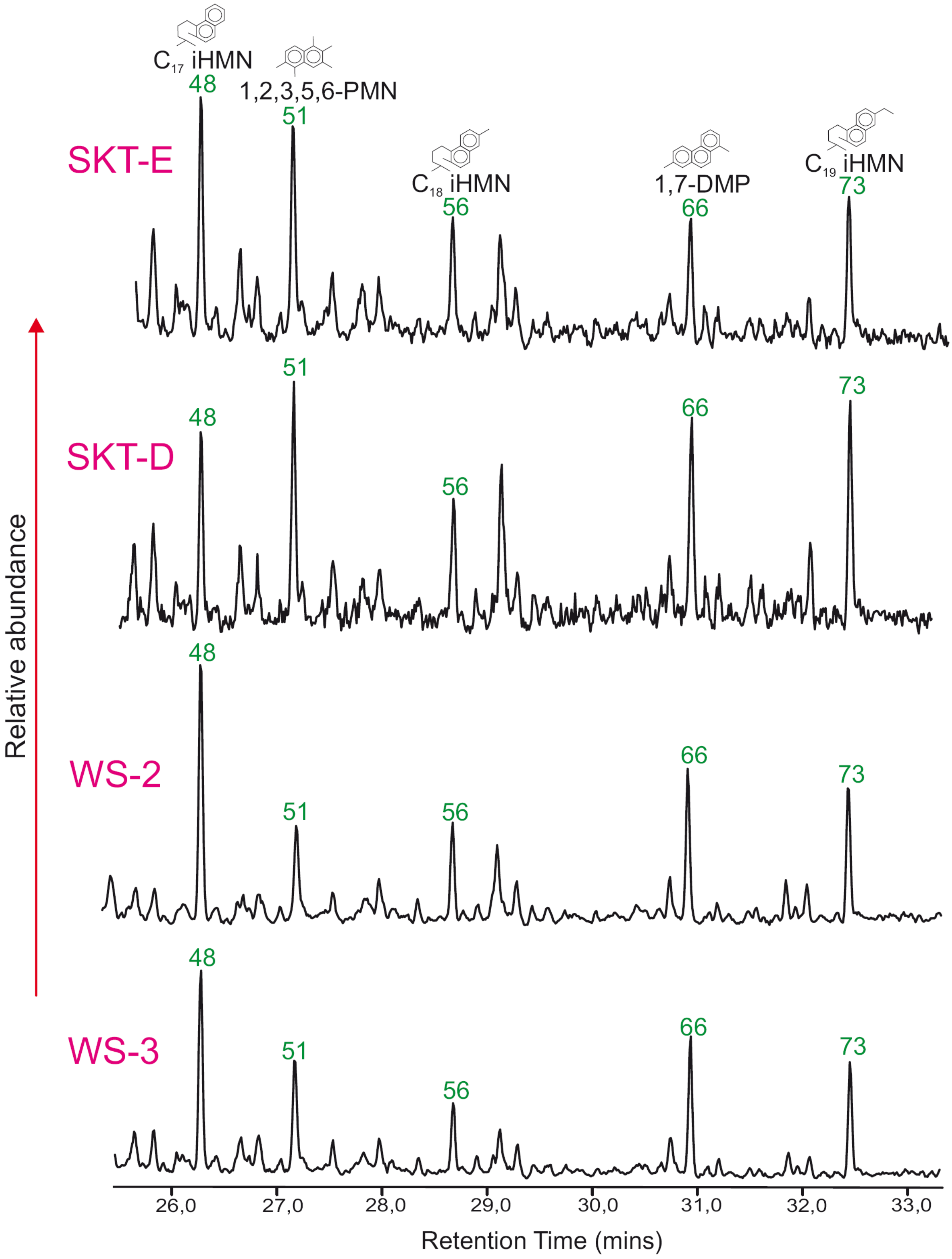

Figure 4.18: Partial m/z 155 + 169 + 183 mass fragmentograms showing a series of C_{17} to C_{19} isohexylalkylnaphthalenes in the aromatic fractions of Scottish coals. Peak assignments are given in Table 4.4

Abbreviations: iHMN – isohexylalkylnaphthalene, PMN – pentamethylnaphthalene, DMP – dimethylphenanthrene.

Romero-Sarmiento, M.-F., et al. (In preparation)

4.5.3.3. The land-plant and combustion-derived polycyclic aromatic hydrocarbons

Among the polycyclic aromatic hydrocarbons (PAHs), two particular groups have been recognized in these Scottish coals (Fig. 4.12; Table 4.4). The first one includes the land-plant-derived PAHs retene (87), cadalene (33), simonellite (74), bisnorsimonellite (57), tetrahydroretene (70), diaromatic totarane (76), diaromatic sempervirane (90) and 2-methylretene (94), whereas the second group comprises the combustion-derived PAHs pyrene (75), fluoranthene (71), benzo[a]anthracene (96), chrysene and triphenylene (97). These aromatic compounds have been determined using the available mass spectral data (Philp, 1985; Ellis et al., 1996, Otto et al., 1997; Jiang et al., 1998 and references therein; van Aarssen et al., 2000; Otto and Simoneit, 2001; Bastow et al., 2001, Tuo and Philp, 2005).

Simonellite (74), diaromatic totarane (76) and diaromatic sempervirane (90) (see also the expanded m/z 237 mass fragmentograms; Fig. 4.19) were clearly identified by comparison with the mass spectra published by Tuo and Philp (2005), but the elution pattern, however, slightly differs from one presented in Tuo and Philp (2005). Following the same features proposed by Tuo and Philp (2005), another family of diaromatic tricyclic hydrocarbons has been detected in the aromatic fractions (Fig. 4.19). These new supposedly also diaromatic hydrocarbons (Tre^1 and Tre^2) have very similar mass spectra to tetrahydroretene, exhibiting a base peak at m/z 223 and a molecular ion at m/z 238 (Fig. 4.19). Based on their mass spectra and elution times, comparison with the distribution of the established diaromatic tricyclic hydrocarbons simonellite, diaromatic totarane and diaromatic sempervirane (Otto et al., 1997; Otto and Simoneit, 2001; Tuo and Philp, 2005), and the identification of diaromatic totarane and sempervirane in our coals, these compounds were tentatively identified as tetrahydroretene isomers, based on the totarane and sempervirane skeletons, respectively. Compound Tre^1 could be the 1-methyl-1,2,3,4-tetrahydro-8-isopropylphenanthrene (totarane-derived) and Tre^2 the 1-methyl-1,2,3,4-tetrahydro-6-isopropylphenanthrene (sempervirane-derived; Fig. 4.19). Their molecular structures are also proposed in Figure 4.19.

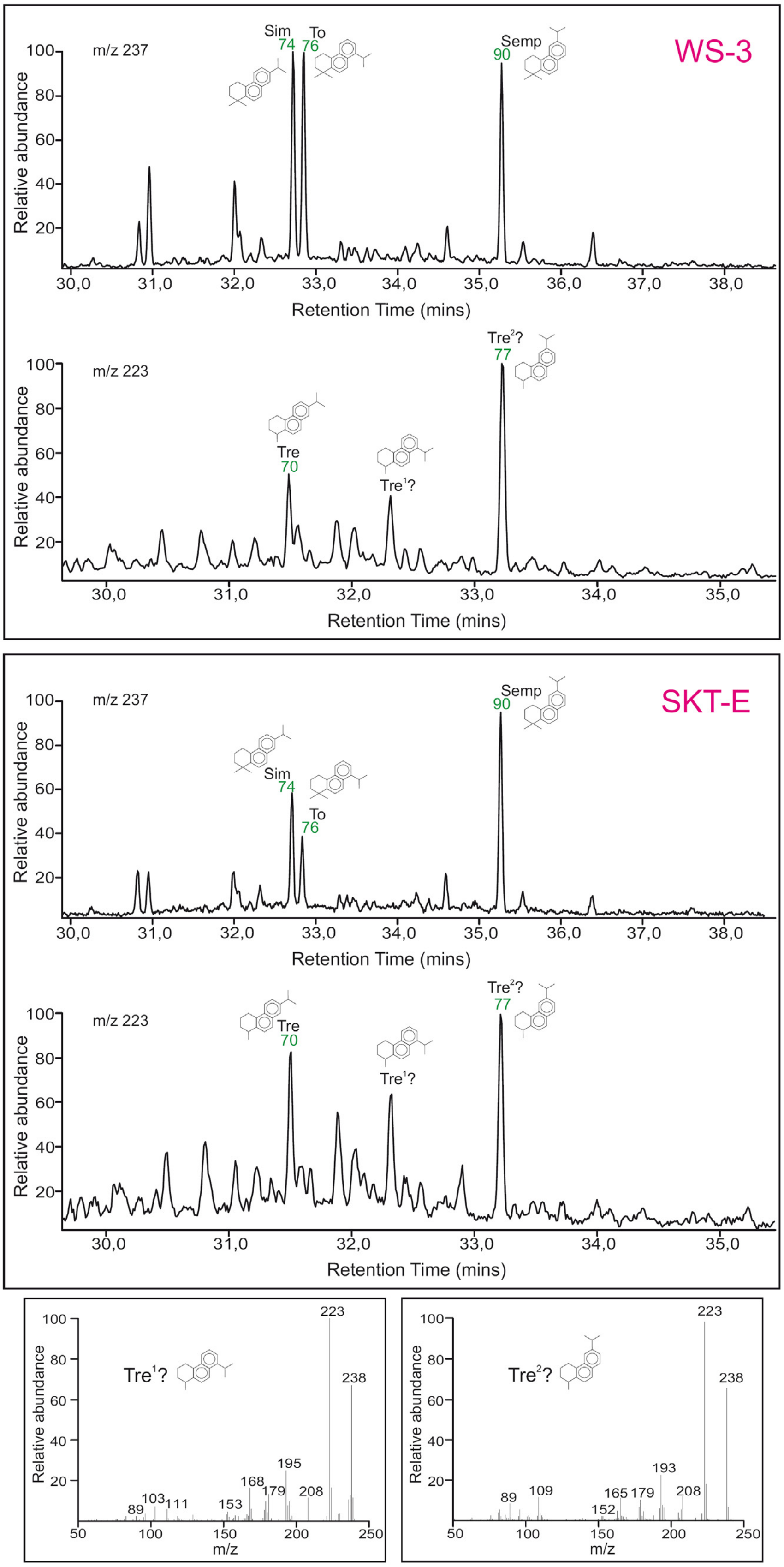

Figure 4.19: Partial m/z 237 and m/z 223 mass fragmentograms showing the distribution of diaromatic tricyclic hydrocarbons in the aromatic fractions of Scottish coals

Abbreviations: Sim – simonellite, To – diaromatic tricyclic totarane, Semp – diaromatic tricyclic sempervirane, Tre - tetrahydroretene. Peaks assignments are given in Table 4.4. Mass spectra of the two tentatively identified tetrahydroretene-dervived isomers are also showed shown (Tre^1 and Tre^2)

4.6. Discussion

4.6.1 Maturity of organic matter

According to LECO results, the TOC values for these Scottish samples are typical for coals (60.8 and 71.9%; Table 4.1). Based on the average Tmax (426 °C) from Rock-Eval analyses, the organic matter is generally in the immature stage of thermal evolution (Fig. 4.3). This interpretation is supported by other thermal maturity indicators from the aliphatic and aromatic fractions. First, the carbon preference index (CPI) is high (>1) due to odd preference (Table 4.1; Bray and Evans, 1961). Second, the thermally unstable C_{29} ββ hopane is observed in all samples (Fig. 4.7; Seifert and Moldowan, 1980). Third, the typical high thermal maturity marker in sediments 18α-22,29,30-trisnorneohopane (Ts) and αββ-steranes (Fig. 4.8) are absent or very low in abundance (Seifert and Moldowan, 1978, 1986). This is further attested by the aromatic hydrocarbon distribution of MN, EN, DMN, TMN, TeMN and MP isomers again indicating that the coals are immature (Table 4.5; Radke et al., 1982, 1986; Alexander et al., 1985; van Aarseen et al., 1999). For instance, the TeMN compounds show the predominance of 1,2,5,6- and 1,2,3,5-TeMN over the 1,3,6,7-TeMN (Fig. 4.15; van Aarssen et al., 1999). The distribution of methyl- and ethyl-naphthalene homologues with 2-MN and 2-EN dominating is typical for immature samples (Fig. 4.13; Table 4.5; Radke et al, 1982). The alkylphenanthrenes 9-MP and 1-MP are highly abundant, with 9-MP being predominant (Fig. 4.16) is another feature of immature samples (Radke et al., 1982). It is interesting to notice, however, that according to the chosen maturity index WS or SKT samples appears as more mature. WS samples are more mature with DNR, TNR1 and TNR2 while SKT samples are more mature with TMNr, TeMNr, MPI 1 and MPI 2 (Table 4.5). This feature probably reflects the control exerted by the source of the organic matter and depositional environment on these compounds at low maturity degree.

4.6.2. Biomarker distributions and palaeoenvironmental conditions

Like most coals, the investigated ones are typically terrestrial. This is evidenced by both biomarker distributions and palynology. In addition to the typical odd numbered long-chain *n*-alkanes (Eglinton and Hamilton, 1967), several aliphatic and aromatic compounds generally used as terrestrial biomarkers have been detected in our Carboniferous coal samples (Table 4.6).

The Carboniferous sedimentary rocks in the Midland Valley of Scotland consist of cyclical sequences of coals, oil shales, limestones, shales, mudstones, siltstones and sandstones (Murchison and Raymond, 1989; George, 1992). Coals are usually deposited in swampy terrestrial environments such as deltas and regions with poor drainage. Accordingly, a variety of depositional environments, all relatively shallow-water and predominantly deltaic, has been assigned to these Carboniferous rocks (Murchison and Raymond, 1989). In the case of a deltaic environment (such as in our case) marine intrusions may occur which may be evidenced by means of palynomorphs and lipids. Since marine palynomorphs and biomarkers (e.g. cheilantanes) are absent, the low proportion of short chain *n*-alkanes in our coals (Fig. 4.5) must be considered to be of bacterial rather than marine origin.

Other arguments for a purely terrestrial depositional environment are the high pristane/phytane (Table 4.1) and Pr/*n*-C_{17} vs. Ph/*n*-C_{18} ratios (Fig. 4.6; Hunt, 1995). These ratios characterize the Scottish coals as type III organic matter which is usually derived from terrestrial plants (Peters and Moldowan, 1993). From figure 4.6, these coals were deposited under oxidizing conditions, and in particular, WS coal level.

A bacterial contribution to the organic matter is further evidenced by the occurrence of branched alkanes (Figs. 4.5; e.g. Shiea et al., 1990), hopanoids (Figs. 4.4 and 4.7; Ourisson et al., 1979) and bicyclic alkanes of the drimane and homodrimane series (Figs. 4.4 and 4.10; Noble, 1986; Noble et al., 1987). In our samples, aromatic hydrocarbons often associated to a microbial source include 1,2,3,5,6-pentamethylnaphthalene (1,2,3,5,6-PMN; Fig. 4.12; Table 4.4; Bastow et al., 1998) and 1,3,6,7-tetramethylnaphthalene (1,3,6,7-TeMN; Fig.4.15; e.g. Jiang et al., 1998).

Table 4.6: List of target saturated and aromatic land plant biomarkers and their origin.

Biomarkers	Type	Origin	References
4β(H)-eudesmane	Bicyclic alkane	Angiosperms and gymnosperms	Noble, 1986; del Rio et al., 1994
		Bryophytes	Asakawa, 2004
ent-beyerane	Tetracyclic diterpenoid	All conifer families except Pinaceae	Noble, 1986; Sheng et al., 1992
phyllocladane	Tetracyclic diterpenoid	All conifer families except Pinaceae	Noble, 1986; Otto et al., 1997 Fabianska et al., 2003
Abietane	Tricyclic diterpenoid	All conifer families	Otto and Wilde, 2001 Cox et al, 2007
ent-kaurane	Tetracyclic diterpenoid	Vascular plants and non vascular plants	Noble, 1986; Sheng et al., 1992; Asakawa, 2004
Retene	Aromatic diterpene	All conifer families	van Aarssen et al., 2000
		Bryophytes	Romero-Sarmiento et al., 2010
Cadalene	Sesquiterpenoid	All kind of plants	Chopra and Kumra, 1988; Simoneit et al., 1986; Armstroff et al., 2006
Simonellite	Aromatic diterpene	All conifer families	Otto and Simoneit, 2001
Diaromatic totarane	Aromatic diterpene	*Podocarpaceae*, *Cupressaceae* and *Taxodiaceae* conifers	Otto and Wilde, 2001; Cox et al, 2007
Diaromatic sempervirane	Aromatic diterpene	*Podocarpaceae* and *Cupressaceae* conifers	Tuo and Philp, 2005 Cox et al, 2007
Tetrahydroretene	Aromatic diterpene	All conifer families	Otto and Simoneit, 2001
$C_{17} – C_{19}$ iHMN	Alkylnaphthalene	Terpenoids from (conifer ?) resins	Ellis et al., 1996
		Bryophytes	Romero-Sarmiento et al., 2010
2-methylretene	Aromatic diterpene	All conifer families	Bastow et al., 2001
1,6 – DMN	Alkylnaphthalene	Terrestrial plants; non-specific	Radke et al., 1994; Van Aarssen et al., 2000
1,2,5 – TMN	Alkylnaphthalene	Terrestrial plants; non-specific	Radke et al., 1994; van Aarssen et al., 2000
1,7 – DMP	Alkylphenanthrene	Terrestrial plants; non-specific	Radke et al., 1994; van Aarssen et al., 2000
MDBF	Alkyldibenzofuran	Lichen input	Radke et al., 2000
Ionene	Hydronaphthalene	Sporopollenins derived from higher plant pollen	Achari et al., 1973 Wang and Simoneit, 1990

Abbreviations: DMN – dimethylnaphthalene; TMN – trimethylnaphthalene; MDBF – methyldibenzofuran; DMP –dimethylphenanthrene; iHMN – isohexylalkylnaphthalene

Steroids have also been used to differentiate depositional settings (Fig. 4.8; Peters et al., 2005). Huang and Meinschein (1979) proposed a useful ternary diagram to identify the source of the organic matter which can be constructed by the distribution of C_{27}, C_{28} and C_{29} sterols (Fig. 4.9). Based on their results, the dominant source of C_{27} sterols is zooplankton, C_{28} sterols are generally components of phytoplankton and C_{29} sterols are mainly derived from terrestrial plants. There are many exceptions on these rules; for instance, C_{29} sterols are also found in marine diatoms and dinoflagellates (e.g. Rampen et al., 2010). Nevertheless, the method seemed reliable for other Palaeozoic (Middle Devonian) coals; which have a high predominance of C_{29} steranes (e.g. Fowler et al., 1991; Kashirtsev et al., 2010). Considering this, the dominance of C_{29} relative to C_{27} steranes in our coals further evidences their terrestrial origin (Table 4.1; Fig. 4.8). Consistent with the shallow-water environment proposed for Scottish coals (Murchison and Raymond, 1989), the steroids plot in a transitional estuarine-bay environment (Fig. 4.9). It is interesting to note that short-chain C_{19} - C_{20} steroids are abundant in SKT coals (Fig. 4.8). Although these compounds are not commonly described from coals, they have been reported in Devonian liptobioliths (Kashirtsev et al., 2010). The relative abundance of these C_{19} - C_{20} steroids may represent a typical characteristic of some primitive coals, i.e. they could be more specific biomarkers of early plants.

The occurrence of the combustion-derived polycyclic aromatic hydrocarbons (PAHs) pyrene, fluoranthene, benzo[a]anthracene, chrysene and triphenylene in our samples (Fig. 4.12) testify that fire events took place contemporarily with coal deposition. Such compounds have been previously observed in other coal samples from the Midland Valley of Scotland (Murchison and Raymond, 1989). These reports are consistent with the frequent occurrence of charred or fusainized debris in Scottish coals (Murchison and Raymond, 1989; Falcon-Lang, 2000). Fire events reports from coals are often related to lightning, but in the case of the Midland Valley of Scotland, volcanic activity also appears as a major cause of wildfires (Murchison and Raymond, 1989; Falcon-Lang, 2000).

4.6.3. Linking biomarkers, palynology and palaeobotany

Carboniferous coals and sediments are often characterized by the abundance of the megaspores *Lagenicula* and *Setosisporites* (Scott and Hemsley, 1996), which are generally considered to be lycophyte-derived (e.g. Wellman et al., 2009). The miospore and megaspore assemblages described in the Scottish coals by Spinner (1969) and Spinner and Clayton (1973) demonstrated that the Euramerican Coal Measure forest was globally dominated by a lycopod-flora. Lycopsids were abundant spore producers (DiMichele and Phillips, 1994), and this observation is consistent with the presence of ionene in our samples, which has been related to a pollen contribution (Fig. 4.12; Achari et al., 1973; Wang and Simoneit, 1990).

On the basis of the diverse palynomorph assemblages described by Spinner (1969) and Spinner and Clayton (1973), however, a more detailed reconstruction of the forest giving rise to the SKT and WS coals can be provided. The miospores *Densosporites* and *Cingulizonates* as well as the megaspores *Zonalesporites* are mostly produced by *Bodeodendron* (*Sporangiostrobus*) which is a well recognized Carboniferous heterosporous lycophyte (Wagner, 1989). *Setosisporites* megaspores are mainly derived by *Bothrodendron*, another arborescent lycopods (e.g. Phillips, 1979). Accordingly, it appears that SKT coals are mostly characterized by a lycopod-vegetation dominated by *Bodeodendron* and *Bothrodendron*. In contrast, WS coals are mainly marked by the abundance of *Lagenicula subpilosa* and *Lycospora pusilla* (Spinner, 1969). Both megaspore and miospore are particularly produced by the arborescent lycopods *Paralycopodites* (DiMichele, 1980). The occurrence of the miospores *Calamospora*, *Auroraspora* and *Remysporites* in the Scottish coals (Spinner and Clayton, 1973) also indicates the contribution of the sphenopsid *Calamites* to the flora. Several fern or pteridosperm miospores are also reported as well as a spore from *Sigillariaceae* (Spinner and Clayton, 1973). This floral assemblage is relatively more comparable in time and geographic setting to the Visean flora of East Kirkton, Scotland (Scott et al., 1993). For instance, at East Kirkton, abundant lycopsids have been recognized as well as sphenopsids (e.g. *Archaeocalamites*), *Lyginopteridaceae* pteridosperms, and *Botryopteris* ferns. However, typical spores of *Lyginopteridaceae* and of Medullosans (e.g. *Schulzospora*) have not been reported by Spinner (1969) and Spinner and Clayton (1973). Although there are some Viséan records of *Cordaites* (e.g. Wang, 1998), *Cordaites* remains were not reported from neither East Kirkton (Scott et al., 1993) nor *Cordaite* spores (e.g. *Florinites*) in the palynofacies from our Scottish coals (Spinner, 1969; Spinner and Clayton, 1973).

Some aromatic biomarkers detected in this study, can be related to specific plant taxa (Table 4.6). For instance, the presence of alkyldibenzofurans (MDBFs) indicates lichen input (Fig. 4.12; Radke et al., 2000). Although lichen fossils or specific lichen spores have not been reported in Scottish coals (Spinner, 1969; Spinner and Clayton, 1973), it can be safely assumed that Euramerican Coal Measure forests were a good potential habitat for lichens.

Among the less specific biomarkers, the detected combustion-derived PAH cannot be associated to specific kind of plants (Oros & Simoneit 2000a,b, Oros et al. 2006 ; Iinuma et al., 2007). It is also the case for 1,6-dimethylnaphthalene (1,6-DMN; Fig. 4.13), 1,2,5-trimethylnaphthalene (1,2,5-TMN; Fig. 4.14), pimanthrene (1,7-DMP; Fig. 4.15) and cadalene, which can be produced by aromatization of several different terpenoid structures and/or which precursors are widespread among terrestrial plants (Table 4.6). 4β(H)-eudesmane is another non-specific land plant biomarker. This compound has been rarely observed in Palaeozoic coals (del Rio et al., 1994; Dzou et al., 1995) and is considered to derive from evolved land plants such as angiosperms and gymnosperms (e.g. conifers) (Fig. 4.10; Noble, 1986). Its oldest reported occurrence in sediments, to our knowledge, is Middle Pennsylvanian (Dzou et al., 1995). However, bicyclic alkanes with an eudesmane skeleton have been also identified from recent bryophytes (Asakawa, 2004).

Most of the other aliphatic and aromatic compounds have been similarly related to more or less specific sources, especially to conifer families (Table 4.6). The oldest report of conifer megafossils is in the Late Carboniferous (Westphalian B; Scott, 1974). Accordingly, the presence of the conifer-derived biomarkers in our coals which predate the evolution of conifers (Scott, 1974), can be compared with the available palynological and botanical data in order to propose a different possible precursor for our components.

The diterpenoids *ent*-beyerane, abietane, *ent*-kaurane and phyllocladane have been previously described in Carboniferous (e.g. Schulze and Michaelis, 1990; Fleck et al., 2001; among others) and Permian coals (e.g. Noble, 1986; Noble et al., 1985). Abietane precursors occur in all conifer families (Otto and Wilde, 2001; Cox et al., 2007) and this compound has been recently described in the pyrolysate of Late Carboniferous amber (Bray and Anderson, 2009). Kaurane skeletons have been observed in different kinds of plants, and in particular bryophytes (e.g. Noble, 1986; Chopra and Kumra, 1988; Asakawa, 2004). The occurrence of this component in very early terrestrial organic matter (e.g. Sheng et al., 1992; Disnar and Harouna, 1994; Kashirtsev et al, 2010; Romero Sarmiento et al., 2010) can be therefore related to bryophytes, which represent the earliest land plants. Phyllocladane and *ent*-beyerane have been mostly associated to all conifer families except *Pinaceae* (e.g. Noble, 1986; Schulze and Michaelis, 1990; among others). However, the oldest recorded occurrences of phyllocladane and *ent*-beyerane are Serpukhovian (Fabianska et al., 2003; Izart et al., 2006) and Middle Devonian (Sheng et al. 1992), respectively. The occurrence of these compounds in these sediments that predate the evolution of conifers (Scott, 1974), has been related to the Voltziales (e.g. Schulze and Michaelis, 1990) and/or the close relatives *Cordaites* (e.g. Disnar and Harouna, 1994 and references therein).

It also seems that the relative abundance of tricyclic and tetracyclic diterpenoids in coals is mostly affected by the palaeobotanical and palaeoenvironmental conditions, and consequently by the available type of vegetation (e.g. Schulze and Michaelis, 1990; Fleck et al., 2001). In Permian coals, the dominance of tetracyclic diterpenoids, and in particular phyllocladane and kaurane isomers has been related to the pteridosperm *Glossopteris*, while a predominance of tricyclic terpanes (e.g. isopimarane, rimuane, fichtellite) can particularly indicate a *Medullosan* (pteridosperm) input (Noble, 1986). A predominance of kaurane has been also observed in Late Carboniferous sediments (Fabianska et al., 2003) whereas phyllocladane isomers are more predominant in Lower Carboniferous sediments (Visean; Disnar and Harouna, 1994).

In our coals, the abundance of tetracyclic phyllocladanes and kauranes compared to the tricyclic abietane (Fig. 4.11) indicates that non-Medullosan pteridosperms (e.g. Pteridospermopsida) can be considered as their potential biomarker precursors. The non-medullosans contribution is also attested by the palynofacies (Spinner and Clayton, 1973) and the observed results at East Kirkton (Scott et al., 1993). In addition, the abundance of *ent*-beyerane compared to *ent*-kaurane has been linked to a considerable contribution of *Cordaites* in late Carboniferous coals from France and Germany (e.g. Schulze and Michaelis, 1990; Vliex et al., 1994; Fleck et al., 2001; Auras et al., 2006). According to these previous studies, the low abundance of *ent*-beyerane in the Scottish coals can therefore indicate the absence of *Cordaites* inputs. As mentioned above, the absence of *Cordaites* contribution is indicated by the palynological record (Spinner, 1969; Spinner and Clayton, 1973; Scott et al., 1993), and supported by the absence of the arborane/fernane derivatives MATH, MAPH DAPH1 and DAPH2 which have been recently associated to *Cordaites* origin (Auras et al., 2006).

Similarly, retene, tetrahydroretene, bisnorsimonellite and simonellite (Figs. 4.12 and 4.19) have been widely accepted as conifer biomarkers (van Aarssen et al., 2000; Hautevelle et al., 2006). Actually, abietic acid, the major constituent of conifer resin, has been often considered as the biological precursor for retene and its related aromatic compounds (van Aarssen et al., 2000; Hautevelle et al., 2006). However, most of these compounds, except abietane and bisnorsimonellite, have been identified in the course of the present thesis work in upper Silurian to lower Devonian sediments and supposedly associated with early Palaeozoic bryophytes (Romero-Sarmiento et al., 2010). Following Ellis et al. (1996), the higher abundance of alkylphenanthrene compounds compared to isohexylalkylnaphthalenes in the Scottish coals (Figs. 4.12 and 4.18) could indicate derivation from (resin?) acids. The hypothesis that abietic acid synthesis had already evolved in early land plants, prior to the emergence of true conifers (Romero Sarmiento et al., 2010) gains therefore support with this observation. Similarly, Bray and Anderson (2009) concluded that biosynthetic mechanisms specific to angiosperms had already appeared in the Late Carboniferous, far before the emergence of true angiosperms. In addition, 2-methylretene occurs in our sediments, together with other land plant biomarkers such as retene. 2-methylretene is only known from Permian and younger sediments (Bastow et al., 2001). The diaromatic tricyclic totarane and sempervirane also typically co-occur with retene and related hydrocarbons. It has been generally accepted that these biomarkers derive from a restricted number of conifers families: mostly *Podocarpaceae* and *Cupressaceae*, (Otto and Wilde, 2001; Cox et al., 2007), though the totarane skeleton has been also observed in some angiosperms (e.g. Pinto et al, 1995; Clarkson et al., 2003) and bryophyte (e.g. Wu and Jong, 2001).

In summary, the occurrence of the different compounds generally related to evolved plants (e.g. 4β-eudesmane, abietane, 2-methylretene, diaromatic totarane and sempervirane) in the Early Carboniferous is therefore relatively unexpected since they occur in our sediments deposited prior to the supposed evolution of their conifer source organisms. *Cordaite*, a close relative to conifers, has been previously proposed as source for supposedly typical conifer biomarkers (e.g. Disnar and Harouna, 1994), however, no data support the presence of *Cordaites* in the flora that contributed to the Scottish coals. Alternative origin for these compounds therefore must be researched among the Visean Scottish flora which was dominated by arborescent lycopsids with a contribution from non-medullosan pteridosperms, Sphenopsids (e.g. *Calamite*) and ferns. Most of these groups of plants are extinct (Stewart and Rothwell, 1993), so that direct comparison with present-day plants in order to validate this assumption is not possible. Considering the fact that the distribution of tetracyclic diterpenoids in the Devonian Liptobiolith (Kashirtsev et al, 2010) is relatively similar to the one in the Scottish coals, that arborescent lycopsids dominate the Scottish flora, and that these latter plants already existed during the Devonian (Steward and Rothwell, 1993), arborescent lycopsids appear as the most likely source for these typical conifer biomarkers.

4.7. Conclusions

Although long chain *n*-alkanes, alkylnaphthalenes, methylated phenanthrenes and alkyldibenzofurans are the most abundant constituents of saturated and aromatic extracts from Early Carboniferous, the land-plant-derived PAHs retene, simonellite, tetrahydroretene, bisnorsimonellite, diaromatic totarane, diaromatic sempervirane and 2-methylretene as well as several other identified terrestrial terpenoids have been clearly detected in Visean coal samples. Classically, these compounds have been considered to be conifer-derived. However, micro and macrofossil evidence for conifers does not go further back than the Upper Carboniferous while palynological and paleobotanical data indicate that these coals mainly derive from lycopod-dominated forest (e.g. *Bodeodendron*, *Bothrodendron* and *Paralycopodites*). This discrepancy between the lipid and other fossil records leads to the following hypotheses:

1. Conifers had evolved already in the Early Carboniferous and this has now been evidenced with lipids.

2. The metabolic pathways giving rise to the observed biomarkers had evolved already in the Early Carboniferous in the direct ancestors of the conifers.

3. The identified biomarkers are derived from a different terrestrial source, not-giving rise to the conifers which implies the evolution of a separate possibly presently extinct or as yet unidentified metabolic pathway and associated products.

We already know that retene, simonellite and tetrahydroretene could also derive from degradation of kaurane-type compounds synthesised by the early bryophytes. The terrestrial terpenoids as well as the aromatic biomarkers were linked to the miospore and megaspore assemblages present in these Lower Carboniferous coals. The occurrence of alkyldibenzofurans and ionene in samples confirms their potential as terrestrial biomarkers.

Finally, most identified terrestrial terpenoids and aromatic biomarkers in our Lower Carboniferous coals can be related to a lycophyte arborescent flora in conjunction with some sphenopsids and pteridosperms which dominated the Euramerican Coal Measure forests at the time of deposition of coal seams in the Midland Valley of Scotland. These plants which are presently extinct can, in particular, be the source of the "typical conifer" biomarkers.

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4.9. References

Achari, R.G., Shaw, G., Holleyhead, R., 1973. Identification of ionene and other carotenoid degradation products from the pyrolysis of sporopollenins derived from some pollen exines, a spore coal and the Green River shale. Chemical Geology 12, 229 – 234.

Alexander, R., Kagi, R.I., Rowland, S.J., Sheppard, P.N., Chirila, T.V., 1985. The effects of thermal maturity on distribution of dimethylnaphthalenes and trimethylnaphthalenes in some ancient sediments and petroleums. Geochimica et Cosmochimica Acta 49, 385 – 395.

Armstroff, A., Wilkes, H., Schwarzbauer, J., Littke, R, Horsfield, B., 2006. Aromatic hydrocarbon biomarkers in terrestrial organic matter of Devonian to Permian age. Palaeogeography, Palaeoclimatology, Palaeoecology, 240, 253 – 274.

Asakawa, Y., 2004. Chemosystematics of the Hepaticae. Phytochemistry 65, 623 – 669.

Auras, S., Wilde, V., Scheffler, K., Hoernes, S., Kerp, H., Püttmann, W., 2006. Aromatized arborane/fernane hydrocarbons as biomarkers for *Cordaites*. Naturwissenschaften 93, 616 – 621.

Bastow, T., Alexander, R., Sosrowidjojo, I. B., Kagi, R.I., 1998. Pentamethylnaphthalenes and related compounds in sedimentary organic matter. Organic Geochemistry 28, 585 – 595.

Bastow, T., Singh, R., Van Aarssen, B., Alexander, R., Kagi, R. 2001. 2-methylretene in sedimentary material: a new higher plant biomarker. Organic Geochemistry 32: 1211 – 1217.

Bordoloi, M., Shukla, V.S., Nath, S.C., Sharma, R.P., 1989. Naturally occurring cadinenes. Phytochemistry 28, 2007 – 2037.

Bray, E.E., Evans, E.D., 1961. Distribution of *n*-paraffins as a clue to recognition of source beds. Geochimica et Cosmochimica Acta 22, 2 – 15.

Chopra, R.N., Kumra, K.P., 1988. Biology of Bryophytes. John Wiley & Sons, New York, 350 p.

Clarkson, C., Campbell, W.E., Smith, P., 2003. In vitro antiplasmodial activity of abietane and totarane diterpenes isolated from Harpagophytum procumbens (devil's claw). Planta Medica 69, 720 – 724.

Cox, R.E., Yamamoto, S., Otto, A., Simoneit, B.R.T., 2007. Oxygenated di- and tricyclic diterpenoids of southern hemisphere conifers. Biochemical Systematics and Ecology 35, 342 – 362.

del Río, J. C., Garcia-Molla, J., González-Vila, F. J., Martin, F., 1994. Composition and origin of the aliphatic extractable hydrocarbons in the Puertollano (Spain) oil shale. Organic Geochemistry, 21, 897 – 909.

Didyk, B.M., Simoneit, B.R.T., Brassell, S.C., Eglinton, G., 1978. Organic geochemical indicators of palaeoenvironmental conditions of sedimentation. Nature 272, 216 – 222.

DiMichele W.A., 1980. Paralycopodites Morey & Morey, from the Carboniferous of Euramerica — A Reassessment of Generic Affinities and Evolution of "Lepidodendron" brevifolium Williamson. American Journal of Botany 67, 1466 – 1476.

DiMichele, W.A., Phillips, T.L., 1994. Paleobotanical and paleoecological constraints on models of peat formation in the Late Carboniferous of Euramerica. Palaeogeography, Palaeoclimatology, Palaeoecology 106, 39 – 90.

Disnar, J.R., Harouna, M., 1994. Biological origin of tetra-cyclic diterpanes, n-alkanes and other biomarkers found in Lower Carboniferous Gondwana coals (Niger). Organic Geochemistry 21, 143 – 152.

Eglinton, G., Hamilton, R.J., 1967. Leaf epicuticular waxes. Science 156, 1322 – 1335.

Ellis, L., Singh, R., Alexander, R., Kagi, R., 1996. Formation of isohexyl alkylaromatic hydrocarbons from aromatization-rearrangement of terpenoids in the sedimentary environment: A new class of biomarker. Geochimica and Cosmochimica Acta 60, 4747 – 4763.

Espitalié, J., Deroo, G., Marquis, F., 1986. La pyrolyse Rock-Eval et ses applications. Revue de l'Institut Français du Pétrole 41, 73 – 89.

Fabianska, M. J., Bzowska, G., Matuszewska, A., Racka, A., Skret, U., 2003. Gas chromatography-mass spectrometry in geochemical investigation of organic matter of the Grodziec Beds (Upper Carboniferous), Upper Silesian Coal Basin, Poland. Geochemistry 63, 63 – 91.

Falcon-Lang, H. J., 2000. Fire ecology of the Carboniferous tropical zone. Palaeogeography, Palaeoclimatology, Palaeoecology 164, 339 – 355.

Fleck, S., Michels, R., Izart, A., Elie, M., Landais, P., 2001. Palaeoenvironmental assessment of Westphalian fluvio–lacustrine deposits of Lorraine (France) using a combination of organic geochemistry and sedimentology. International Journal of Coal Geology 48, 65 – 88.

Fleck, S., Michels, R., Ferry, S., Malartre, F., Elion, P., Landais, P., 2002. Organic geochemistry in a sequence stratigraphic framework. The siliciclastic shelf environment of Cretaceous series, SE France. Organic Geochemistry 33, 1533 – 1557.

Fowler, M.G., Goodarzi, F., Gentzis, T., Brooks, P.W., 1991. Hydrocarbon potential of Middle and Upper Devonian coals from Melville Island, Arctic Canada. Organic Geochemistry 17, 681 – 694.

Garrigues, P., de Sury, R., Angelin, M. L., Bellocq, J., Oudin, J. L., Ewald, M., 1988. Relation of the methylated aromatic hydrocarbon distribution pattern to the maturity of organic matter in ancient sediments from the Mahakam delta. Geochimica et Cosmochimica Acta 52, 375 – 384.

George, S.C., 1992. Effect of igneous intrusion on the organic geochemistry of a siltstone and an oil shale horizon in the Midland Valley of Scotland. Organic Geochemistry 18, 705 – 723.

Hautevelle, Y., Michels, R., Malartre, F., Trouiller, A., 2006. Vascular plant biomarkers as proxies for palaeoflora and palaeoclimatic changes at the Dogger/Malm transition of the Paris Basin (France). Organic Geochemistry 37, 610 – 625.

Huang, W.-Y., Meinschein, W.G., 1979. Sterols as ecological indicators. Geochimica et Cosmochimica Acta 43,739 – 745.

Hunt, J.M., 1995. Petroleum Geochemistry and Geology. W.H. Freeman and Co., New York.

Iinuma, Y., E. Brüggemann, T. Gnauk, K. Müller, M. O. Andreae, G. Helas, R. Parmar, and H. Herrmann, 2007. Source characterization of biomass burning particles: The combustion of selected European conifers, African hardwood, savanna grass, and German and Indonesian peat, Journal of Geophysical Research, 112, D08209.

Izart, A., Sachsenhofer, R. F., Privalov, V. A., Elie, M., Panova, E. A., Antsiferov, V. A., Alsaab, D., Rainer, T., Sotirov, A., Zdravkov, A., Zhykalyak, M. V., 2006. Stratigraphic distribution of macerals and biomarkers in the Donets Basin: Implications for paleoecology, paleoclimatology and eustacy. International Journal of Coal Geology, 66, 69 – 107.

Jiang, C., Alexander, R., Kagi, R.I., Murray, A. P., 1998. Polycyclic aromatic hydrocarbons in ancient sediments and their relationships to palaeoclimate. Organic Geochemistry 29, 1721 – 1735.

Kashirtsev, V.A., Moskvin, V.I., Fomin, A.N., Chalaya, O.N., 2010. Terpanes and steranes in coals of different genetic types in Siberia. Russian Geology and Geophysics 51, 404 – 411.

Murchison D. G., Raymond A. C., 1989. Igneous activity and organic maturation in the Midland Valley of Scotland. International Journal of Coal Geology 14, 47-82.

Noble, R. A., 1986. A geochemical study of bicyclic alkanes and diterpenoid hydrocarbons in crude oils, sediments and coals. Thesis. The University of Western Australia. 398 p.

Noble, R. A., Alexander, R., Kagi R.I., Knox, J., 1985. Tetracyclic diterpenoid hydrocarbons in some Australian coals, sediments and crude oils. Geochimica and Cosmochimica Acta 49, 2141 – 2147.

Noble, R. A., Alexander, R., Kagi R.I., 1987. Configurational isomerization in sedimentary bicyclic alkanes. Organic Geochemistry 11, 151 – 156.

Oros, D.R., Simoneit, B.R.T., 2001a. Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 1. Temperate climate conifers. Applied Geochemistry 16, 1513 – 1544.

Oros, D.R., Simoneit, B.R.T., 2001b. Identification and emission factors of molecular tracers in organic aerosols from biomass burning Part 2. Deciduous trees. Applied Geochemistry 16, 1545 – 1565.

Oros, D.R., Abas, M.R.b., Omar, N.Y.M.J., Rahman, N.A., Simoneit, B.R.T., 2006. Identification and emission factors of molecular tracers in organic aerosols from biomass burning: Part 3. Grasses. Applied Geochemistry 21, 919 – 940.

Otto, A., Walther, H., Püttmann, W., 1997. Sesqui- and diterpenoid biomarkers preserved in Taxodium-rich Oligocene Oxbow Lake Clays, Weisselster basin, Germany. Organic Geochemistry 26, 105 – 115.

Otto, A., Simoneit, B.R.D., 2001. Chemosystematics and diagenesis of terpenoids in fossil conifer species and sediment from the Eocene Zeitz Formation, Saxony, Germany. Geochimica et Cosmochimica Acta 65, 3505 – 3527.

Otto, A., Wilde, V., 2001. Sesqui-, di- and triterpenoids as chemosystematic markers in extant conifers - A review. The Botanical Review 67, 141 – 238.

Ourisson, G., Albrecht, P., Rohmer, M., 1979. The hopanoids. Palaeochemistry and biochemistry of a group of natural products. Pure and Applied Chemistry 51, 709 – 729.

Peters, K.E., Moldowan, J.M., 1993. The Biomarker Guide: Interpreting molecular fossils in petroleum and ancient sediments. Prentice Hall, Englewood Cliffs, New Jersey. 363 p.

Peters, K.E., Walters, C.W., Moldowan, J.M., 2005. The Biomarker Guide. Cambridge University Press. Cambridge -706 p.

Philp, R.P., 1985. Fossil Fuel Biomarkers Applications and Spectra. Elsevier, Amsterdam.

Phillps, T., 1979. Reproduction of heterosporous arborescent lycopods in the Mississippian - Pennsylvanian of Euramerica. Review of Palaeobotany and Palynology 27, 239 – 289.

Pinto A.C., Zocher D.H.T., Rezende C.M., Gottlieb H.E., 1995. A new diterpene with a totarane skeleton from Vellozia flavicans. Natural Product Research 6, 209 – 213.

Püttmann, W., Villar, H., 1987. Occurrence and geochemical significance of 1,2,5,6-tetramethylnaphthalene. Geochimica et Cosmochimica Acta 51, 3023 – 3029.

Radke, M., Willsch, H., Leythaeuser, D., 1982. Aromatic components of coal: relation of distribution pattern to rank. Geochimica et Cosmochimica Acta 46, 1831 – 1848.

Radke, M., Willsch, H., Welte, D.H., 1986. Maturity parameters based on aromatic hydrocarbons: influence of the organic matter type. Organic Geochemistry 10, 51 – 63.

Radke, M., Garrigues, P., Willsch, H., 1990. Methylated dicyclic and tricyclic aromatic hydrocarbons in crude oils from the Handil field, Indonesia. Organic Geochemistry 15, 17 – 34.

Radke, M., Rullkötter, J., Vriend, S. P., 1994. Distribution of naphthalenes in crude oils from the Java Sea: Source and maturation effects. Geochimica et Cosmochimica Acta 58, 3675 – 3689.

Radke, M., Vriend, S. P., Ramanampisoa, L. R., 2000. Alkyldibenzofurans in terrestrial rocks: Influence of organic facies and maturation. Geochimica et Cosmochimica 64, 275 – 286.

Rampen, S.W, Abbas, B.A., Schouten, S., Sinninghe Damsté, J.S., 2010. A comprehensive study of sterols in marine diatoms (Bacillariophyta): Implications for their use as tracers for diatom productivity. Limmonology and Oceanography 55, 91 – 105.

Romero-Sarmiento, M. F., Riboulleau, A., Vecoli, M. Versteegh., G., 2010. Occurrence of retene in upper Silurian – Lower Devonian sediments from North Africa: Origin and implications. Organic Geochemistry 41, 302 – 306.

Schulze, T., Michaelis, W., 1990. Structure and origin of terpenoid hydrocarbons in some German coals. Organic Geochemistry 16, 1051 – 1058.

Scott, A., 1974. The earliest conifer. Nature 25, 707 – 708.

Scott, A.C., Hemsley, A. R., 1996. Palaeozoic megaspores. Chapter 18G.in: Jansonius, J. & McGregor, D.C. (ed.) Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, Vol. 2, 629 – 639.

Scott A. C., Brown R., Galtier J., Meyer-Berthaud , B., 1993. Fossil plants from the Viséan of East Kirkton, West Lothian, Scotland. Transactions of the Royal Society of Edinburgh. Earth sciences 84, 249 – 260.

Seifert, W.K., Moldowan, J.M., 1978. Applications of steranes, terpanes and monoaromatics to the maturation, migration and source of crude oils. Geochimica et Cosmochimica Acta 42, 77 – 95.

Seifert, W.K., Moldowan, J.M., 1980. The effect of thermal stress on source rock quality as measured by hopane stereochemistry. In: Douglas, A.G., Maxwell, J.R. (Eds.), Advances in Organic Geochemistry, 1979. Pergamon Press, 229 – 237.

Seifert, W.K., Moldowan, J.M., 1986. Use of biological markers in petroleum exploration. In: Johns, R.B. (Ed.), Biological Markers in the Sedimentary Record, Methods in Geochemistry and Geophysics, vol. 24. Elsevier, Amsterdam, pp. 261–290.

Sheng, G., Simoneit, B. R. T., Leif, R. N., Chen, X., Fu, J., 1992. Tetracyclic terpanes enriched in Devonian cuticle humic coals. Fuel, 71, 523 – 532.

Shiea, J., Brassell, S.C., Ward, D.M., 1990. Mid-chain branched mono- and dimethyl alkanes in hot spring cyanobacterial mats: a direct biogenic source for branched alkanes in ancient sediments? Organic Geochemistry 15, 223 – 231.

Simoneit, B. R. T., Grimalt, J. O., Wang, T. G., Cox, R. E., Hatcher, P. G., Nissenbaum, A., 1986. Cyclic terpenoids of contemporary resinous plant detritus and of fossil woods, ambers and coals. Organic Geochemistry 10, 877 – 889.

Smith, J.W., George, S.C., Batts, B.D., 1995. The geosynthesis of alkylaromatics. Organic Geochemistry 23, 71 – 80.

Spinner, E., 1969. Megaspore assemblages from Visean deposits at Dunbar, East Lothian, Scotland. Palaeontology 12, 441 – 458.

Spinner, E., Clayton, G., 1973. Visean spore assemblages from Skateraw, East Lothian, Scotland. Pollen et Spores XV, 139 – 165.

Tissot, B., Welte, D.M., 1984. Petroleum Formation and Occurrence. Springer-Verlag, Berlin, 699 p.

Tegelaar, E.W., de Leeuw, J.W., Derenne, S., Largeau, C., 1989. A reappraisal of kerogen formation. Geochimica et Cosmochimica Acta 15, 3103 – 3106.

Thomas, B. R., 1969. Organic Geochemistry. Methods and results, chapter Kauri resins-modern and fossil. Springer Verlag, Berlin, 599 – 618.

Tuo, J., Philp, R. P., 2005. Saturated and aromatic diterpenoids and triterpenoids in Eocene coals and mudstones from China. Applied Geochemistry 20, 367 – 381.

Underhill, J. R., Monaghan, A. A., Browne, M. A. E., 2008. Controls on structural styles, basin development and petroleum prospectivity in the Midland Valley of Scotland. Marine and Petroleum Geology 25, 1000 – 1022.

van Aarssen, B.K.G, Bastow, T.P., Alexander, R., Kagi, R., 1999. Distributions of methylated naphthalenes in crude oils: indicators of maturity, biodegradation and mixing. Organic Geochemistry 30, 1213 – 1227.

van Aarssen, B.K.G., Alexander, R., Kagi, R.I, 2000. Higher plant biomarkers reflect palaeovegetation changes during Jurassic times. Geochimica et Cosmochimica Acta 64, 1417 – 1424.

Vliex, M., Hagemann, H.W., Püttmann, W., 1994. Aromatized arborane/fernane hydrocarbons as molecular indicators of floral changes in Upper Carboniferous/Lower Permian strata of the Saar-Nahe Basin, southwestern Germany. Geochimica et Cosmochimica Acta 58, 4689 – 4702.

Wagner, R.H., 1989. A late Stephanian forest swamp with Sporangiostrobus fossilized by volcanic ash fall in the Puertollano Basin, central Spain. International Journal of Coal Geology 12, 523 – 552.

Wang, S. J., 1998. The cordaitean fossil plants from Cathaysian area in China. Acta Botanica Sinica 40, 573 – 579.

Wang, T.G., Simoneit, B.R.T., 1990. Organic geochemistry and coal petrology of Tertiary brown coal in the Zhoujing mine, Baise Basin, South China. Fuel 69, 12 – 20.

Wellman, C. H., Arioli, C., Spinner, E., Vecoli, M., 2009. Morphology and wall ultrastructure of the megaspore *Lagenicula* (Triletes) mixta (Winslow 1962) comb. nov. from the Carboniferous (Early Mississippian: mid Tournaisian) of Ohio, USA. Review of Palaeobotany and Palynology 156, 51 – 61.

Wu C.-L., Jong J.-R., 2001. A Cyclic Peroxide of Clerodenoic Acid from the Taiwanese Liverwort Schistochila acuminata. Journal of Asian Natural Products Research 3, 241 – 246.

Chapter 5

Conclusions

The major conclusions obtained throughout this work are listed below:

1. Several molecular biomarkers of terrestrial origin were extracted from sediments of Late Ordovician to Early Carboniferous age. The first occurrences of these identified biomarkers were revised in this study; in particular from sediments of Late Ordovician to Early Devonian, confirming that the molecular signature of the earliest land plants was already recognizable. Although the presence of land plants on terrestrial environment was principally documented by spores during the investigated period, our results contributed to fill the gap existing between the molecular and fossil records of land plants (Fig. 5.1).

2. The terrestrial biomarkers detected from sediments of Late Ordovician to Early Devonian (Tunisia, North Africa) and coals of Early Carboniferous age (Dunbar, Scotland) have been compared to the available palynological and palaeobotanical data. This therefore allowed relating the biomarkers to different groups of early land plants:

    - Retene, tetrahydroretene, simonellite, isohexylalkylnaphthalenes and norabietane in upper Silurian to lower Devonian sediments can be related to bryophytes and, more likely, to primitive tracheophytes (e.g. *Cooksonia*, lycophytes and zosterophylls). The potential of perylene as a marker for higher plant-degrading fungi is supported in this thesis. It was only observed in the most terrestrial-influenced samples from Tunisia (Ghadamis Basin).

- The Early Carboniferous (Visean) flora which contributed to the formation of the coal deposits at Dunbar was dominated by arborescent lycopods (e.g. *Bodeodendron*, *Bothrodendron* and *Paralycopodites*), sphenopsids (e.g. *Calamites*) and pteridosperms (e.g. *Lyginopteridaceae*). These groups of plants can therefore be considered as the major precursors for the identified terrestrial biomarkers in this period, in particular abietane, phyllocladane, bisnorsimonellite, diaromatic totarane, diaromatic sempervirane and 2-methylretene. Retene, tetrahydroretene, simonellite, isohexylalkylnaphthalene were also observed in these Scottish coals.

- The contribution of lichen input to the coals was not documented by the available fossil flora, but its contribution was indicated by the presence of dibenzofuran and its methylated-isomers. In contrast, the potential of ionene as a specific marker for sporopollenin was clearly confirmed by its occurrence in the Scottish coals, where abundant spores were present.

- Several non-specific terrestrial biomarkers (e.g. kaurane; cadalene; eudesmane) have been recognized in the organic matter of Late Ordovician to Lower Carboniferous age. These biomarkers cannot be particularly associated to specific kind of plants since their precursors are widely distributed in the plant kingdom. However, some of these compounds can be eventually related to the bryophytes, since they occur, for instance, in the upper Ordovician to lower Silurian samples (e.g. long-chain *n*-alkanes). This led to us suggesting that retene probably derived from the maturation of compounds with a kaurane skeleton.

- Combustion-derived polycyclic aromatic hydrocarbons were detected in most samples indicating that fire events took place on emerged lands as soon as they were colonized by plants. However, they cannot be linked to specific plant taxa.

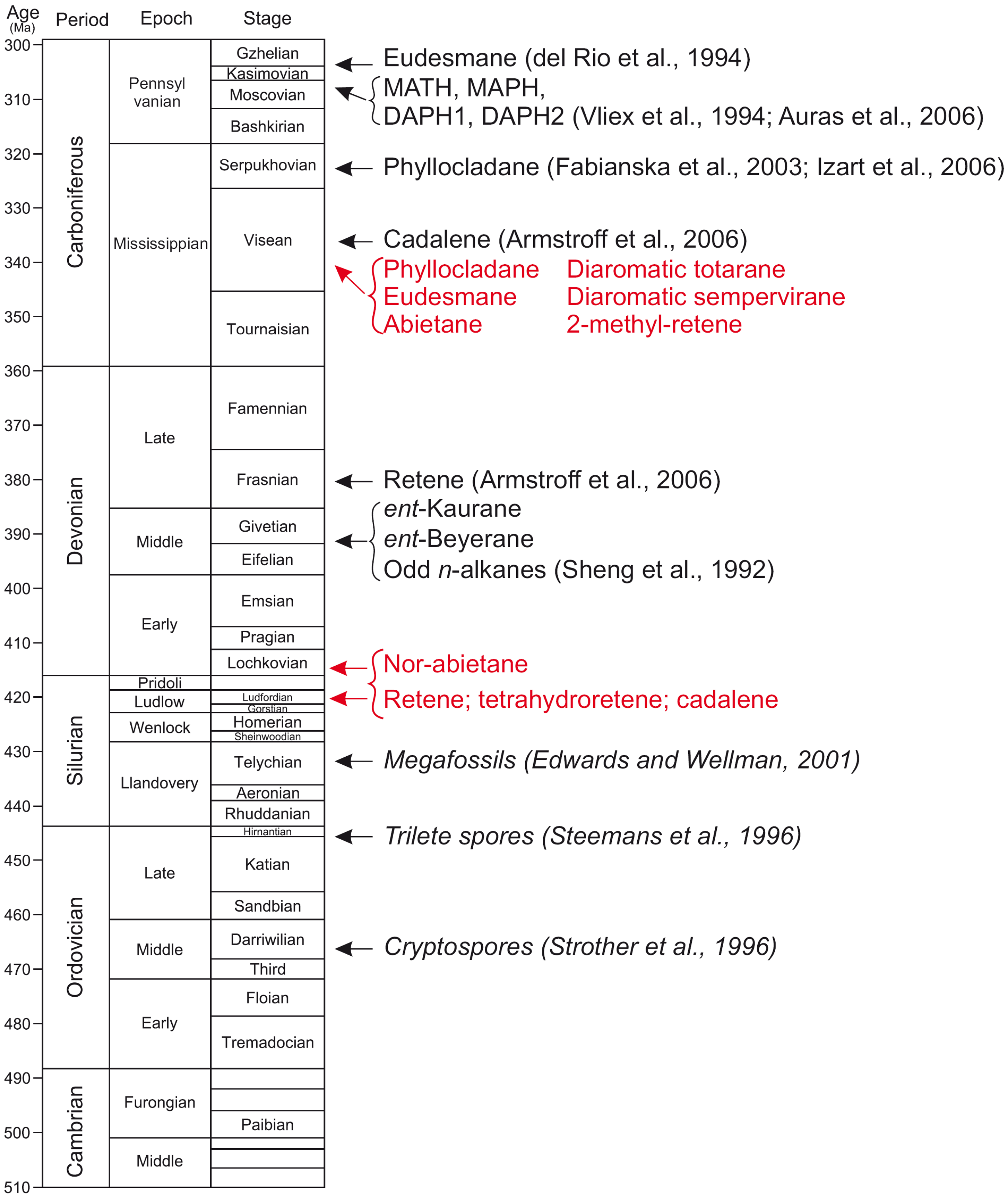

Figure 5.1: Stratigraphic position of the detected terrestrial biomarkers in this study (in red)
Geologic time scale from the International Commission on Stratigraphy (ICS)

3. In order to contribute to the knowledge of early land plant evolution during the Palaeozoic, the major result obtained in this study is that earliest land plants are also characterized by a molecular signature generally considered as typical of conifers: e.g. retene, abietane, phyllocladane, totarane and sempervirane. If retene can derive from the aromatisation of several terpenoids and appear to not be that specific for conifers, abietane, phyllocladane, totarane and sempervirane are presently mainly observed in conifers. Samples here studied however predate the appearance of true conifers in the Late Carboniferous. These observations can be explained in different ways:

   - These compounds characterize plants which were related to conifers: conifer ancestors (Voltziales), or a sister group (Pteridosperms). Biosynthetic pathways typical of conifers, such as the synthesis of abietic acid, would therefore have already appeared before conifers acquire their morphological characteristics.

   - These compounds characterize plants which were not particularly related to conifers but are currently extinct (for instance arborescent lycopods). These molecules could therefore have been produced by biosynthetic pathways which remained characteristic of these plants, or these plants could represent a first attempt for some biosynthetic pathways (abietic acid synthesis?), which later became a characteristic feature of conifers.

References

Armstroff, A., Wilkes, H., Schwarzbauer, J., Littke, R, Horsfield, B., 2006. Aromatic hydrocarbon biomarkers in terrestrial organic matter of Devonian to Permian age. Palaeogeography, Palaeoclimatology, Palaeoecology, 240, 253 – 274.

Auras, S., Wilde, V., Scheffler, K., Hoernes, S., Kerp, H., Püttmann, W., 2006. Aromatized arborane/fernane hydrocarbons as biomarkers for *Cordaites*. Naturwissenschaften 93, 616 – 621.

del Río, J. C., Garcia-Molla, J., González-Vila, F. J., Martin, F., 1994. Composition and origin of the aliphatic extractable hydrocarbons in the Puertollano (Spain) oil shale. Organic Geochemistry, 21, 897 – 909.

Edwards, D., Wellman, C. H., 2001. Embryophytes on land: The Ordovician to Lochkovian (Lower Devonian) record. In: Gensel, P. G. and Edwards, D. (eds). Plants invade the land: Evolutionary and environmental perspectives. Columbia University Press, New York. 3 – 28.

Fabianska, M. J., Bzowska, G., Matuszewska, A., Racka, A., Skret, U., 2003. Gas chromatography-mass spectrometry in geochemical investigation of organic matter of the Grodziec Beds (Upper Carboniferous), Upper Silesian Coal Basin, Poland. Geochemistry 63, 63 – 91.

Izart, A., Sachsenhofer, R. F., Privalov, V. A., Elie, M., Panova, E. A., Antsiferov, V. A., Alsaab, D., Rainer, T., Sotirov, A., Zdravkov, A., Zhykalyak, M. V., 2006. Stratigraphic distribution of macerals and biomarkers in the Donets Basin: Implications for paleoecology, paleoclimatology and eustacy. International Journal of Coal Geology, 66, 69 – 107.

Sheng, G., Simoneit, B. R. T., Leif, R. N., Chen, X., Fu, J., 1992. Tetracyclic terpanes enriched in Devonian cuticle humic coals. Fuel, 71, 523 – 532.

Steemans, P., Le Hérissé, A., Bozdogan, N., 1996. Ordovician and Silurian cryptospores and miospores from Southeastern Turkey. Review of Palaeobotany and Palynology 93, 35 – 76.

Strother, P.K., Al-Hajri, S., Traverse, A., 1996. New evidence for land plants from the lower Middle Ordovician of Saudi Arabia. Geology 24, 55 – 58.

Vliex, M., Hagemann, H.W., Püttmann, W., 1994. Aromatized arborane/fernane hydrocarbons as molecular indicators of floral changes in Upper Carboniferous/Lower Permian strata of the Saar-Nahe Basin, southwestern Germany. Geochimica et Cosmochimica Acta 58, 4689 – 4702.

Appendix A.1

Di-*iso*-propylnaphthalenes: environmental contaminants of increasing importance for organic geochemists

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1. Abstract

Di-*iso*-propylnaphthalenes (DIPNs) have distinctive mass spectra, including m/z 197 base peaks and m/z 212 molecular ions, and on the apolar columns typically used in organic geochemical applications resolve into 5 main peaks, although there are ten possible isomers. DIPNs have been recognized in rock matrix and solid bitumen samples from Oklo (Gabon), and in subsurface samples of Silurian – Devonian clastic sediments from southern Tunisia (borehole MG1, Ghadamis Basin). DIPNs have synthetic/anthropogenic origins and are used as plant growth regulators and in the manufacture of printed papers. The most likely source of the DIPNs in these organic geochemical samples is tissue paper used to wrap the samples. It is suggested that they are preferentially adsorbed by solid bitumens, and hence are not released during solvent extraction of surfaces. Although DIPNs have been reported in environmental and food samples, this appears to be their first report in organic geochemical samples.

Keywords: Di-*iso*-propylnaphthalenes, diisopropylnaphthalenes, contamination, paper, solvent extraction, Franceville Basin, Oklo, Ghadamis Basin, GC-MS

2. Introduction

Contamination of geological samples prior to or during organic geochemical analyses is an on-going problem for molecular compositional studies, particularly those involving trace amounts of biomarkers. It has long been known that phthalate esters, distinguished during gas chromatography-mass spectrometry (GC-MS) by m/z 149 ions, are compounds typically found in plasticizers that can be leached from plastic bottles used to store samples or solvent extracts (e.g., Bowers et al., 1981). Unfortunately, contamination is often ignored or passed over in publications, as it has an essentially negative impact on geochemical interpretations (although see Brocks et al., 2008) and is often very difficult to identify and trace to its point of origin. However, a recent comprehensive study of organic contaminants encountered during a marine survey highlights the care that needs to be taken in handling samples destined for GC-MS analysis (Grosjean and Logan, 2007). In this study, sunscreen cream was shown to be the source of the UV adsorbers octabenzone and octyl methoxycinnamate, and plastic sampling bags the source of fatty acid amides and butylated hydroxytoluene. A series of branched alkanes with quaternary carbons (BACQs) are also derived from plastic bags, together with various methylalkanes, alkylcyclopentanes and alkylcyclohexanes (Grosjean and Logan, 2007). The latter three groups also occur naturally in geological samples, but it appears that BACQs are exclusively derived from plastic bags (Grosjean and Logan, 2007; Brocks et al., 2008).

Precautions such as wrapping organic geochemical samples in aluminium foil after sampling, non-use of plastic bags in contact with rock samples, the use of diamond saws free of oil-based fluids, and the removal of the outside surface of samples prior to crushing and solvent extraction can eliminate some of these issues. Comprehensive series of outside-rinse, procedural and system blanks can help understand contaminant distribution (e.g., George et al., 2008). Interior/exterior sub-sampling by sawing has also been advocated, in order to understand the past migration of contaminants deeper into core (Brocks et al., 2003; 2008). On occasion, however, samples of rare material arrive at an organic geochemistry laboratory that may have been collected many years ago, have an uncertain storage history and may be too small for removal of outside surfaces prior to analysis. These are the circumstances in which samples from the FB Formation Proterozoic rocks from the Franceville Basin in Gabon (Dutkiewicz et al., 2007) arrived at the CSIRO Laboratories. In this note, the identification and distribution of di-*iso*-propylnaphthalenes (DIPNs) discovered in these and other organic geochemical samples are shown, and their source and implications are discussed.

3. Methods

3.1. Samples

Two samples were obtained from the vicinity of the Oklo natural fission reactors within the Franceville Basin in Gabon. Sample #59 is from 147.7 m depth in borehole 212 and comprises the 2.1 Ga old FB black shale of the Franceville Series (Mossman et al., 2005). It contains a 3 mm wide veinlet of microscopically homogeneous solid bitumen, which obliquely cuts the black shale. The solid bitumen has a maximum reflectance in oil of 5.3% and a $\delta^{13}C$ value of –23.02‰ (Mossman et al., 2001). Sample SD90 is from near reactor #13, south of a major dolerite dyke, and is composed of “normal” (common) low-grade uranium ore (0.1 to 1.0%) (Gauthier-Lafaye and Weber, 1989). Solid bitumen in this sample has an H/C ratio of <0.5% and occupies secondary porosity in the sandstone caused by extensive microfracturing. For further information on the geological setting and geochemistry of the solid bitumen and associated fluid inclusions, see Mossman et al. (2001; 2005) and Dutkiewicz et al. (2007).

Eleven core samples come from borehole MG1, which was drilled by SEREPT from July 1958 to April 1959, and penetrates a Silurian-Devonian succession near the northern margin of the Ghadamis Basin in southern Tunisia (Romero-Sarmiento et al., 2010; Spina and Vecoli, 2009). These samples have been stored in plastic sampling bag under uncertain conditions for a long time (tens of years). For further information on the geological setting and geochemistry of the Tunisian clastic sediments, see Romero-Sarmiento et al. (2010).

3.2. Experimental procedure

The outside surfaces of the two Oklo samples (#59: 50.7 g; SD90: 21 g) were ultrasonicated in dichloromethane (DCM): methanol (MeOH) (93:7) for 10 minutes. Small portions of solid bitumen (2.1 and 1.4 g, respectively) were manually scraped and cut from the parent samples, crushed with a pestle and mortar and analysed separately. The remaining matrix of each sample (which contained some solid bitumen) was crushed in a Tema mill (39.2 and 29.0 g, respectively). Sub-samples of the solid bitumen were ultrasonically extracted for 40 minutes (5 minute bursts) using 3 mL DCM:MeOH (93:7) in a 4 mL vial. The extracts were filtered on a short silica column. The matrix samples were Soxhlet extracted for 72 hours using DCM:MeOH (93:7). Any elemental sulfur present was removed from the four extracts using activated copper, and the extracts analysed by gas chromatography-mass spectrometry (GC-MS) on a Hewlett Packard 5890 gas chromatograph (DB5MS, 60 m x 0.25 mm i.d, 0.25 µm film thickness) interfaced to a VG AutospecQ Ultima mass spectrometer (for further instrument method details, see George et al., 2007).

Biomarker analyses were performed on the samples from borehole MG1 (Romero-Sarmiento et al., 2010). The Tunisian samples were crushed in coarse fragments and soaked in DCM for contaminant removal (extract 0). The samples were then pulverised, extracted using DCM:MeOH (1/2, v/v), dried and separated into aliphatic and aromatic fractions that were analysed by GC-MS using a Trace GC 2000 gas chromatograph (DB5ht, 30 m x 0.25 mm i.d, 0.1 µm film thickness). For further analytical details, see Romero-Sarmiento et al. (2010).

4. Results

The Oklo matrix sample #59 total ion chromatogram (TIC) is dominated by a cluster of five major peaks eluting between *n*-C_{16} and *n*-C_{18}, as well as peaks due to *n*-alkanes (C_{10} to C_{30}), alkylbenzenes, phthalates, benzothiazole and diethyltoluamide (Fig. 1a). The separated solid bitumen from sample #59 is even more strongly dominated by the five major peaks (Fig.

1b). Expansion of this area using the m/z 197 chromatogram (Fig. 1c), shows that the first two peaks are a double, the three later peaks are a triplet, and that *n*-C_{17} elutes shortly after the doublet on the DB5MS column phase used. These five peaks have identical mass spectra (Fig. 1d) consistent with $C_{16}H_{26}$ hydrocarbons, including m/z 197 base peaks, m/z 212 molecular ions and significant ions at m/z 155. NIST Library searching indicates these are likely to be di-*iso*-propylnaphthalenes. The SD90 matrix sample also contains major phthalates and DIPNs, with subordinate n-alkanes (C_{12} to C_{30}) and some elemental sulphur that was not fully removed from the sample by the initial treatment with activated copper (Fig. 1e). The separated solid bitumen from sample SD90 has a similar compound distribution, except that toluene and the early eluting non-identified peaks are more abundant (Fig.1f).

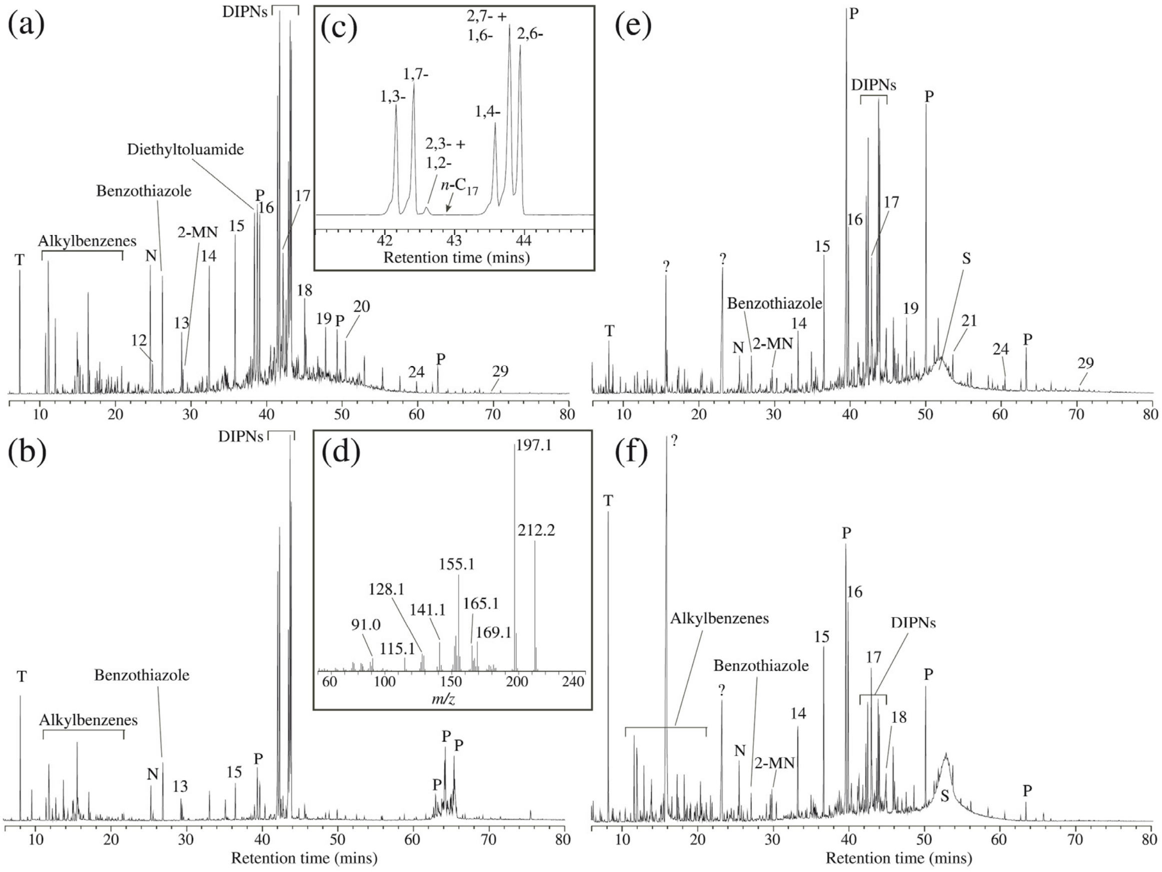

Figure 1: Total ion chromatograms (TICs) of (a) sample #59 matrix, (b) sample #59 solid bitumen, (e) sample SD90 matrix and (f) sample SD90 solid bitumen. (c) Partial m/z 197 mass chromatogram of the sample #59 solid bitumen, showing the elution order of the di-*iso*-propylnaphthalenes relative to *n*-C_{17}. (d) Mass spectra of 1,3-di-*iso*-propylnaphthalene in sample #59 solid bitumen. Peak identifications in TICs: numbers = *n*-alkanes, T = toluene, N = naphthalene, MN = methylnaphthalene, P = phthalate, S = elemental sulphur, and DIPNs = di-*iso*-propylnaphthalenes

Seven of the Tunisian sample aromatic fractions contain DIPNs in the m/z 197 mass chromatogram, generally in low amounts, except for sample MG1-2519.3 in which DIPNs are the major peaks (Fig. 2). This sample comes from the Ouan Kasa Formation, and consists of a shallow-marine sandstone of Early Devonian age (Romero-Sarmiento et al., 2010). At least 8 possible DIPN isomers were detected in this sample (Fig. 2), eluting between the retention time positions of *n*-C_{16} and *n*-C_{18}.

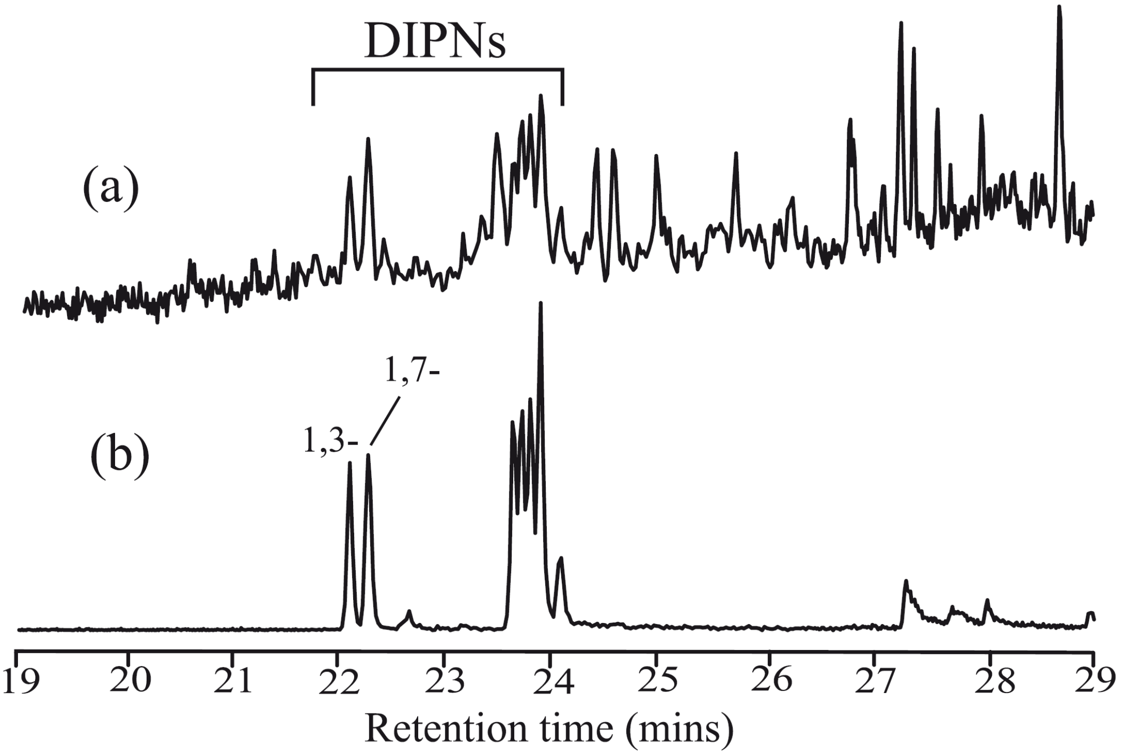

Figure 2: Total ion chromatogram (a) and partial m/z 197 mass chromatogram (b) of the aromatic fraction of sample MG1-2519.3 (Early Devonian Ouan Kasa Formation), showing the identification of DIPNs

The retention time characteristics of DIPNs have recently been unambiguously determined (Franke et al., 2007; Bouvier et al., 2009), confirming and extending earlier reports (Sturaro et al., 1994; Brzozowski et al., 2002). There are ten possible DIPN isomers, but one (1,8-) is extremely sterically hindered and very rare (Franke et al., 2007). The other nine DIPN isomers are fully resolvable on polar columns such as INNOWAX and CP-SIL-88, but only partially resolvable on apolar columns. A GC×GC method to fully separate DIPNs has recently been published, allowing quantitative analysis of the DIPN isomers even in complex reaction mixtures (Bouvier et al., 2009). The DIPN retention time pattern reported by Franke et al. (2007) on apolar columns is very similar to that found on DB5MS (Fig. 1b): 1,3- and 1,7-DIPN form the early eluting doublet, the next minor peak is due to co-eluting 1,2- and 2,3-DIPN (sterically hindered and hence typically in low abundance), and the triplet eluting after n-C_{17} is composed of 1,4-DIPN, then co-eluting 2,7- and 1,6-DIPN, then 2,6-DIPN (1,5-DIPN would elute slightly later, and it appears not to be present). However, on DB5ht the resolution over the triplet region appears to be superior (Fig. 2). The DIPN isomer distribution is similar in all samples analysed, and the mass spectra only vary slightly as previously noted.

5. Discussion and conclusions

The DIPNs are not known to be naturally occurring hydrocarbons in the environment, but certainly have synthetic/anthropogenic origins. Single DIPN isomers, especially 2,6-DIPN, are used in agriculture as plant growth regulators (Everest-Todd, 1988; Lewis et al., 1997). This is based on the structural and functional similarity of 2,6-DIPN to naturally occurring plant growth regulators found in plant tissues, such as 1-isopropyl-4,6-dimethylnaphthalene (USEPA, accessed 2009). Therefore, one possible source of this compound is customs spraying of geochemical samples. However, it is unlikely that this is the source of the contaminants in these samples, because at least six isomers can be detected (Figs. 1c and 2).

“Technical” DIPN consists mainly of a mixture of seven isomers (1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 2,6-, and 2,7-) and is used in a variety of applications as a replacement for polychlorinated biphenyls, especially in the manufacture of printed papers (Terasaki et al., 2008) and food packaging materials (e.g., Nerín and Asensio, 2007). This is the most likely source of the DIPNs in the Proterozoic rocks from Gabon, which prior to receipt in the organic geochemistry lab had been wrapped in tissue paper and stored in a cardboard box. This could not be confirmed as these materials were previously discarded, but is supported by the higher abundance of DIPNs relative to other hydrocarbons in the solid bitumen compared to the matrix extract in sample #59.

Regarding the Tunisian samples, since extract 0 and the polar fraction were not analysed, the contaminant origin of the detected DIPNs cannot be proven. However, the absence of phthalates or BACQ in the aliphatic and aromatic fractions indicates that these were not contaminated since fractionation. Interestingly, core sample MG1-2519.3 where DIPNs are the most abundant (Fig. 2) has a very different Rock-Eval pyrogram to that of the other samples (Romero-Sarmiento et al., 2009), with two S_2 peaks: the first and smaller one has a Tmax similar to that of the other samples from core MG1 (around 435°C), and the second larger peak has a Tmax at 543°C, revealing the presence of another non-identified organic phase, possibly solid bitumen.

The higher amounts of DIPNs in samples associated with solid bitumen, and their presence despite solvent extraction of external surfaces, suggests that DIPNs migrated into the samples from the storage media, and penetrated more deeply than just the surface of the samples, tending to be preferentially adsorbed by solid bitumens. Once strongly adsorbed on solid bitumens by covalent bonding, solvent extraction of sample surfaces would not yield them; they would only be released during later crushing followed by solvent extraction.

DIPN mixtures are becoming more commonly detected in rivers and aquatic sediments as environmental contaminants (Terasaki et al., 2008; Mansuy-Huault et al., 2009). The lack of published reports to 2009 of DIPNs in organic geochemical samples is probably due to a combination of (1) their non-identification because of low abundance or analysis by single ion monitoring (SIM), (2) their mis-identification or non-identification because it was not know what the mysterious peaks with large m/z 197 ions were, and (3) their recognition and identification, but an unwillingness to disclose contamination issues.

Feedback at an IMOG09 poster (George et al., 2009) suggested that DIPNs have been noticed in organic geochemical samples, but not published. For example, DIPNs have been noted in Australian oils and source rock extracts (Ben van Aarssen, pers. comm.) As more organic geochemists acquire m/z 197 mass chromatograms during SIM so as to identify 6-isopropyl-1-isohexyl-2-methylnaphthalene (ip-iHMN) (Ellis et al., 1996; van Aarssen et al., 2000), the presence of DIPNs may become more apparent. It is likely that in the future other organic geochemistry groups will detect these compounds in modern and ancient samples.

6. Acknowledgements

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7. References

Bouvier, C., Reumkens, N., Buijs, W., 2009. Separation of di-*iso*-propylnaphthalene isomers. Journal of Chromatography A 1216, 6410 – 6416.

Bowers, M.D., Parsons, M.L., Clement, R.E., Eiceman, G.A., Karasek, F.W., 1981. Trace impurities in solvents commonly used for gas chromatographic analysis of environmental samples. Journal of Chromatography A 206, 279 – 288.

Brocks, J.J., Buick, R., Logan, G.A., Summons, R.E., 2003. Composition and syngeneity of molecular fossils from the 2.78 to 2.45 billion-year-old Mount Bruce Supergroup, Pilbara Craton, Western Australia. Geochimica et Cosmochimica Acta 67, 4289 – 4319.

Brocks, J.J., Grosjean, E., Logan, G.A., 2008. Assessing biomarker syngeneity using branched alkanes with quaternary carbon (BAQCs) and other plastic contaminants. Geochimica et Cosmochimica Acta 72, 871 – 888.

Brzozowski, R., Skupiński, W., Jamróz, M.H., Skarżyński, M., Otwinowska, H., 2002. Isolation and identification of di-*iso*-propylnaphthalene isomers in the alkylation products of naphthalene. Journal of Chromatography A.

Dutkiewicz, A., George, S.C., Mossman, D., Ridley, J., Volk, H., 2007. Oil and its biomarkers associated with the Palaeoproterozoic Oklo natural fission reactors, Gabon. Chemical Geology 244, 130 – 154.

Ellis, L., Singh, R.K., Alexander, R., Kagi, R.I., 1996. Formation of isohexyl alkylaromatic hydrocarbons from aromatization-rearrangement of terpenoids in the sedimentary environment - a new class of biomarker. Geochimica et Cosmochimica Acta 60, 4747 – 4763.

Everest-Todd, S., 1988. Plant growth regulators. Patent Cooperation Treaty, International Patent Application WO 88/ 08249.

Franke, S., Grunenberg, J., Schwarzbauer, J., 2007. The isomer-specific analysis of di-isopropylnaphthalenes. International Journal of Environmental and Analytical Chemistry 87, 437 – 448.

Gauthier-Lafaye, F., Weber, F., 1989. The Francevillian (Lower Proterozoic) uranium deposits of Gabon. Economic Geology 84, 2267 – 2285.

George, S.C., Volk, H., Ahmed, M., 2007. Geochemical analysis techniques and geological applications of oil-bearing fluid inclusions, with some Australian case studies. Journal of Petroleum Science and Engineering 57, 119 – 138.

George, S.C., Volk, H., Dutkiewicz, A., 2009. Di-*iso*-propylnaphthalenes: environmental contaminants of increasing importance for organic geochemists. In: Book of abstracts, 24th International Meeting on Organic Geochemistry, P-99. IMOG2009 Secretary, University of Bremen - MARUM, Bremen, Germany.

George, S.C., Volk, H., Dutkiewicz, A., Ridley, J., Buick, R., 2008. Preservation of hydrocarbons and biomarkers in oil trapped inside fluid inclusions for > 2 billion years. Geochimica et Cosmochimica Acta 72, 844 – 870.

Grosjean, E., Logan, G.A., 2007. Incorporation of organic contaminants into geochemical samples and an assessment of potential sources: Examples from Geoscience Australia marine survey S282. Organic Geochemistry 38, 853 – 869.

Lewis, M., Kleinkopf, G., Shetty, K., 1997. Dimethylnaphthalene and diisopropylnaphthalene for potato sprout control in storage: 1. Application methodology and efficacy. American Journal of Potato Research 74, 183 – 197.

Mansuy-Huault, L., Regier, A., Faure, P., 2009. Analyzing hydrocarbons in sewer to help in PAH source apportionment in sewage sludges. Chemosphere 75, 995 – 1002.

Mossman, D.J., Gauthier-Lafaye, F., Jackson, S.E., 2001. Carbonaceous substances associated with the Paleoproterozoic natural nuclear fission reactors of Oklo, Gabon: paragenesis, thermal maturation and carbon isotopic and trace element compositions. Precambrian Research 106, 135 – 148.

Mossman, D.J., Gauthier-Lafaye, F., Jackson, S.E., 2005. Black shales, organic matter, ore genesis and hydrocarbon generation in the Paleoproterozoic Franceville Series, Gabon. Precambrian Research 137, 253 – 272.

Nerín, C., Asensio, E., 2007. Migration of organic compounds from a multilayer plastic–paper material intended for food packaging. Analytical & Bioanalytical Chemistry 389, 589 – 596.

Romero-Sarmiento, M. F., Riboulleau, A., Vecoli, M. Versteegh., G., 2010. Occurrence of retene in upper Silurian – Lower Devonian sediments from North Africa: Origin and implications. Organic Geochemistry 41, 302 – 306.

Spina, A., Vecoli, M., 2009. Palynostratigraphy and vegetational changes in the Siluro-Devonian of the Ghadamis Basin, North Africa. Palaeogeography, Palaeoclimatology, Palaeoecology 282, 1 – 18.

Sturaro, A., Parvoli, G., Rella, R., Doretti, L., 1994. Gas chromatographic–mass spectrometric, high performance liquid chromatographic–UV and gas chromatographic–Fourier transform IR responses to an industrial mixture of di-*iso*-propylnaphthalenes. Journal of Chromatography A 688, 211 – 219.

Terasaki, M., Fukazawa, H., Tani, Y., Makino, M., 2008. Organic pollutants in paper-recycling process water discharge areas: First detection and emission in aquatic environment. Environmental Pollution 151, 53 – 59.

USEPA, accessed 2009. 2,6-di-*iso*-propylnaphthalene. US Environmental Protection Agency Office of Pesticide Programs, Biopesticides Registration Action Document.

van Aarssen, B.G.K., Alexander, R., Kagi, R.I., 2000. Higher plant biomarkers reflect palaeovegetation changes during Jurassic times. Geochimica et Cosmochimica Acta 64, 1417 – 1424.

Appendix A.2

Photographic plate: Borehole TT1

TT1-1313.6

TT1-1300.8

TT1-1299.4

TT1-1296.7

TT1-1291.1

TT1-1286.9

TT1-1282.0

TT1-1280.0

TT1-1273.0

TT1-1267.0

TT1-1247.0

TT1-1243.0

TT1-1239.0

Appendix A.3

Photographic plate: Borehole MG1


MG1-3711.0


MG1-3710.3


MG1-3230.6


MG1-3183.0


MG1-3097.0


MG1-3052.5


MG1-3039.0


MG1-3037.6


MG1-2942.0


MG1-2892.0


MG1-2890.0


MG1-2870.0


MG1-2635.0


MG1-2519.3

Appendix A.4

Photographic plate: Scottish coals

WS-2

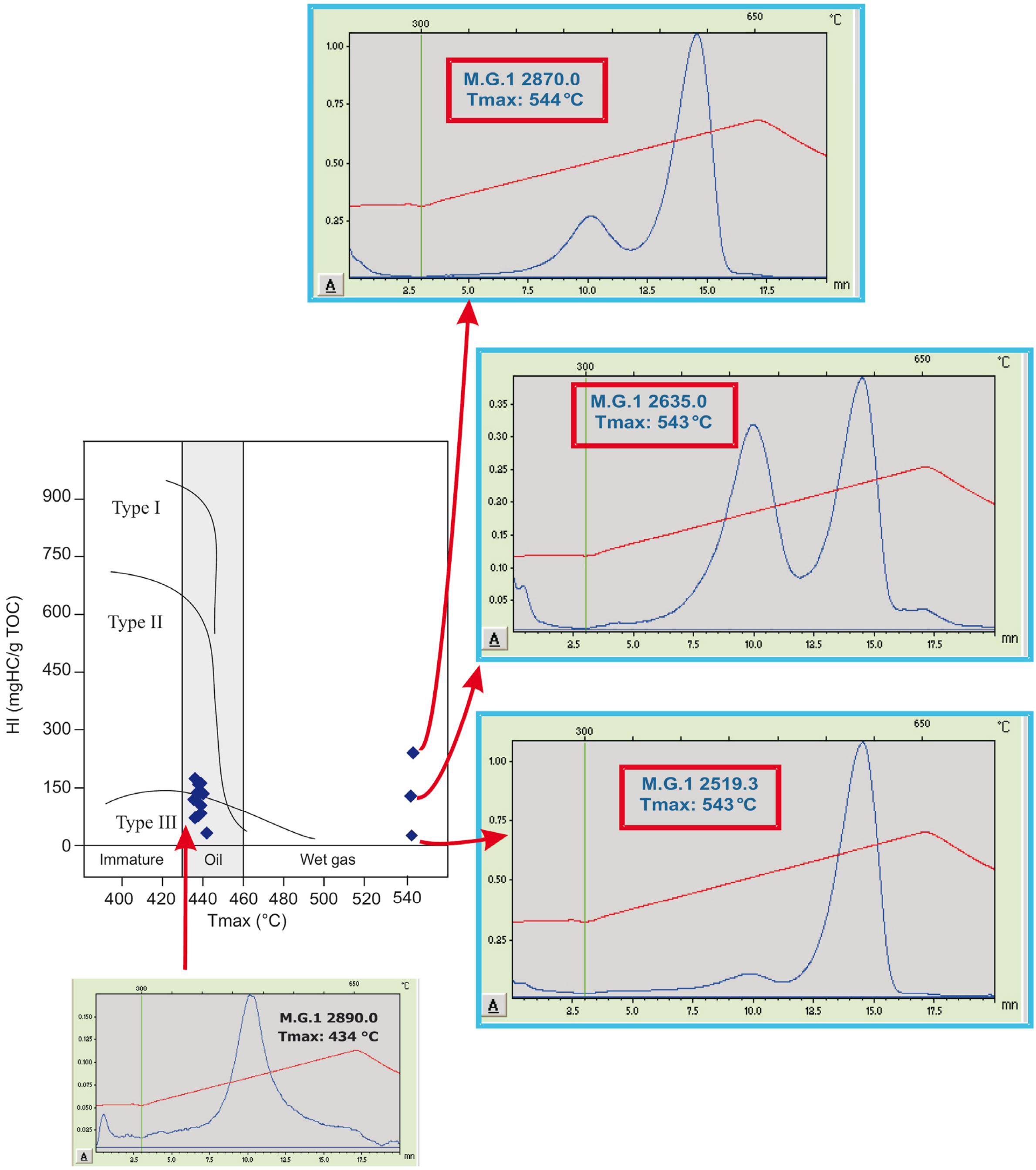
SKT-E

WS-3

SKT-D

Appendix A.5

Rock-Eval pyrograms: Borehole MG1

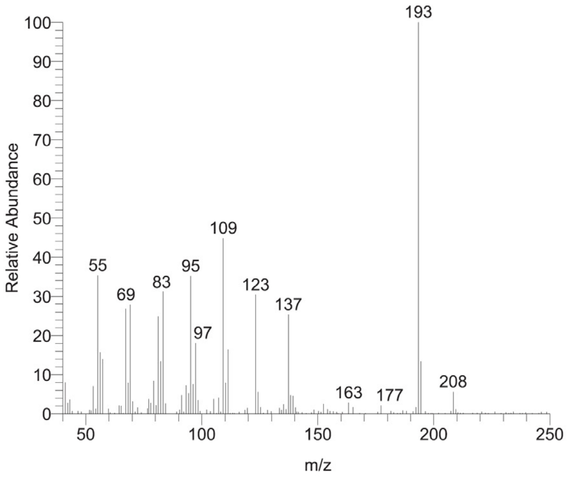


For most samples, the Rock-Eval pyrograms show only one peak of hydrocarbon generation which Tmax values (435 – 437°C). However, three samples (MG1-2519.3; MG1-2635.0; MG1-2870.0) have very different Rock-Eval pyrograms. Their pyrograms show two S_2 peaks of which the smaller first one has a Tmax similar to that of the other samples from core MG1 (~ 436°C), and the second, larger, peak has a Tmax between 543 and 544°C). This particular behaviour may indicate the presence of another organic phase such as solid bitumen (George et al., subm.; Appendix A.1).

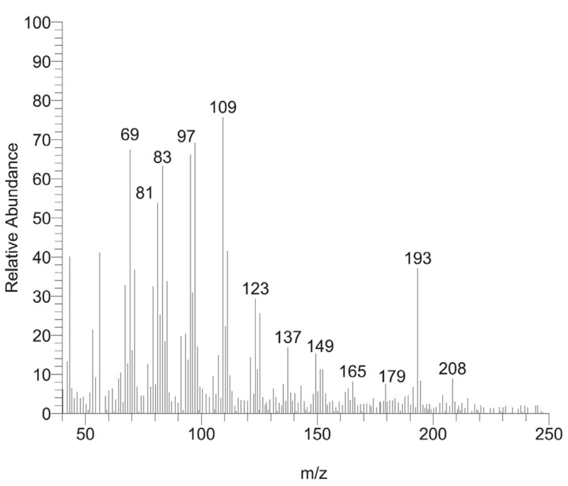
Appendix A.6

Mass Spectra: Unknown compounds

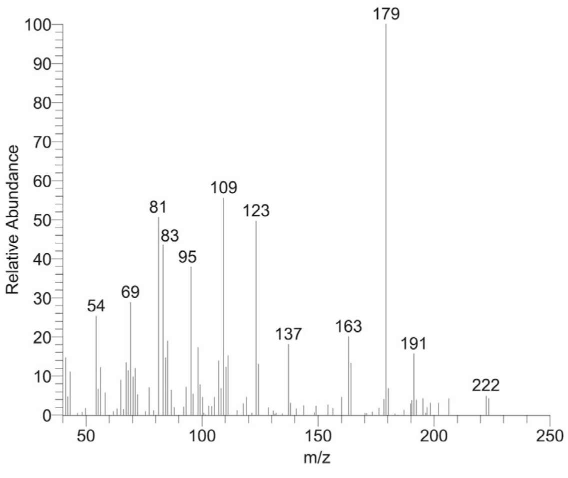
Peak assignments are given in Table 3.3 (Chapter 3)



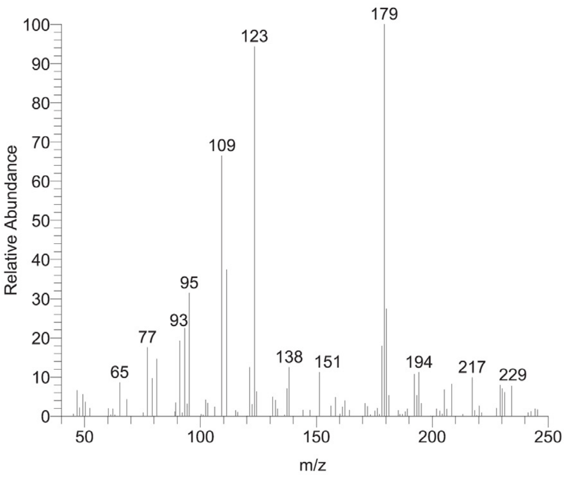
Peak a: C_{15} bicyclic alkane?



Peak c: C_{15} bicyclic alkane?



Peak g: C_{16} bicyclic alkane?

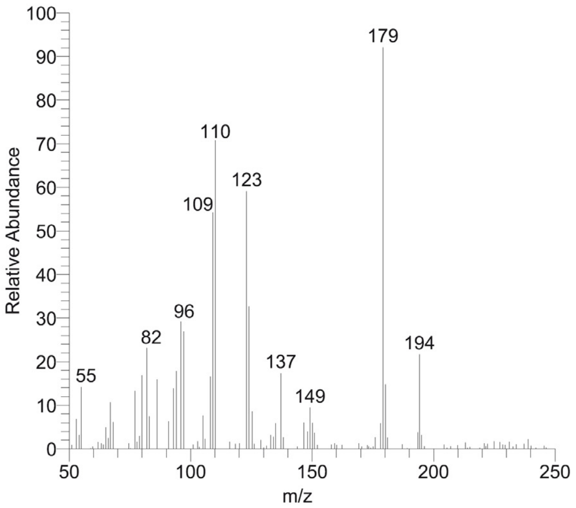


Peak k: Unknown compound?

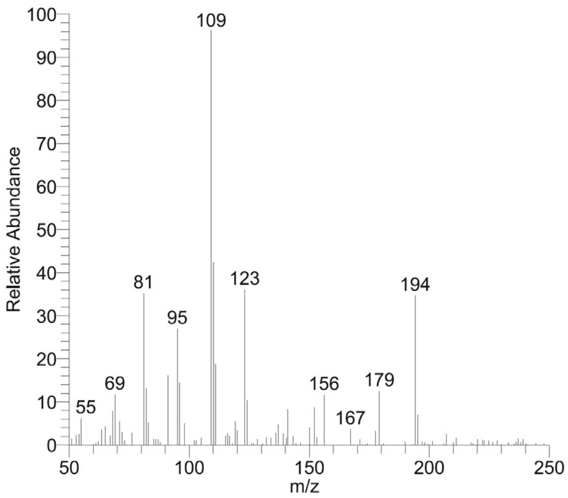
Appendix A.6

Mass Spectra: Unknown compounds

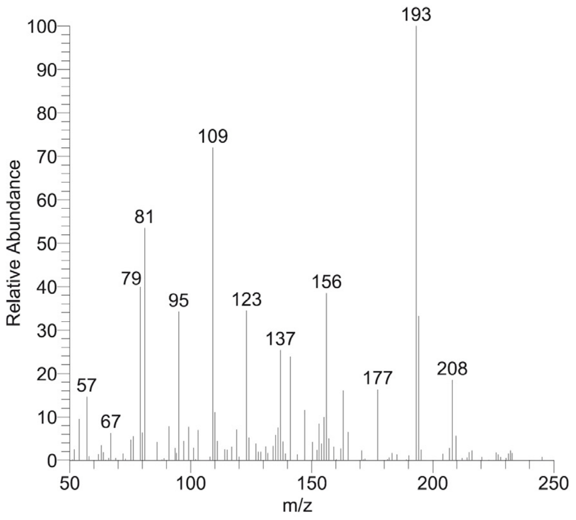
Peak assignments are given in Table 4.2 (Chapter 4)



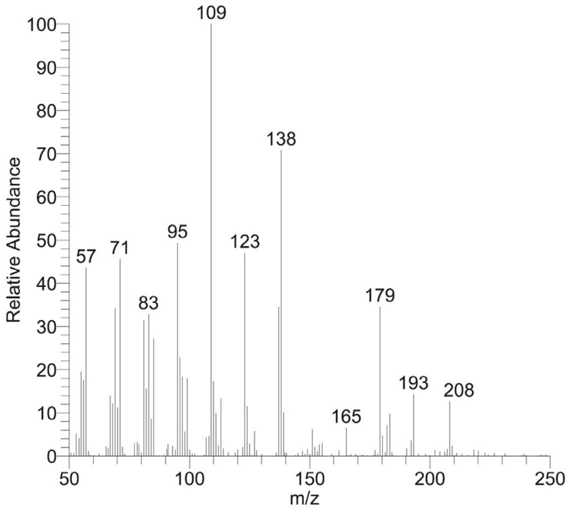
Peak a: C_{14} bicyclic alkane?



Peak e: C_{14} bicyclic alkane?



Peak f: C_{15} bicyclic alkane?

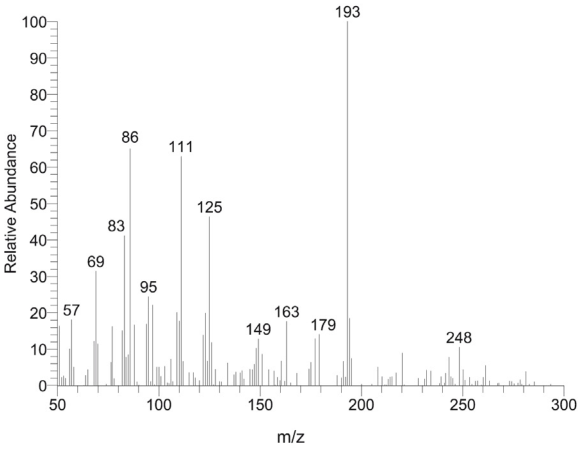


Peak h: C_{15} bicyclic alkane?

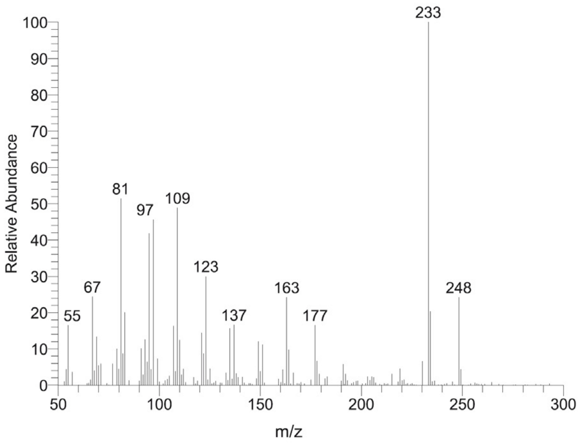
Appendix A.6

Mass Spectra: Unknown compounds

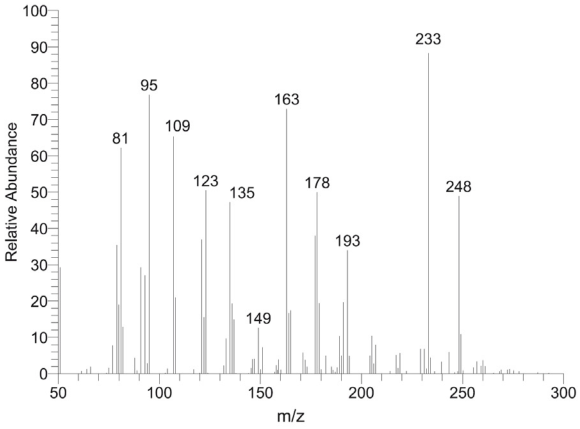
Peak assignments are given in Table 4.3 (Chapter 4)



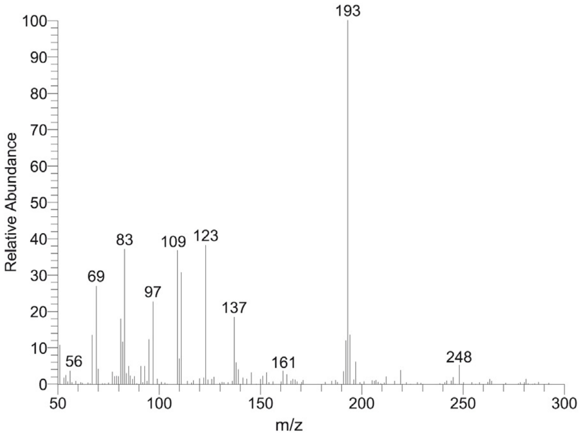
Peak I: C_{18} tricyclic hydrocarbon?



Peak III: C_{18} tricyclic hydrocarbon?



Peak IV: C_{18} tricyclic hydrocarbon?

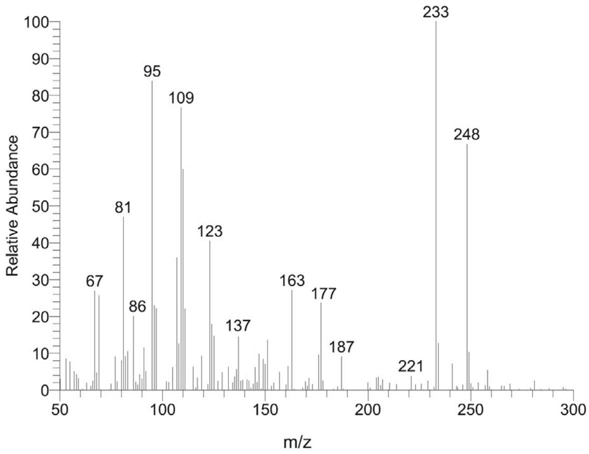


Peak V: C_{18} tricyclic hydrocarbon?

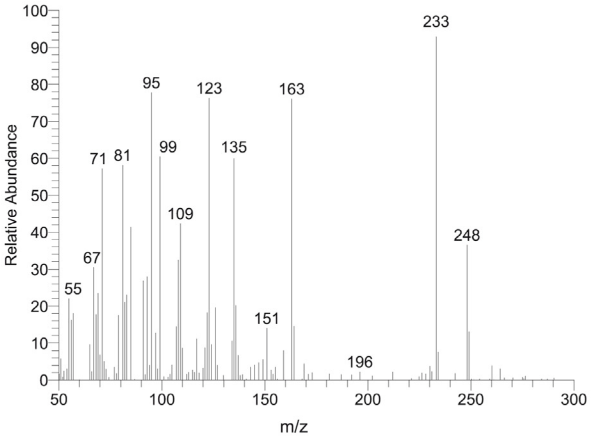
Appendix A.6

Mass Spectra: Unknown compounds

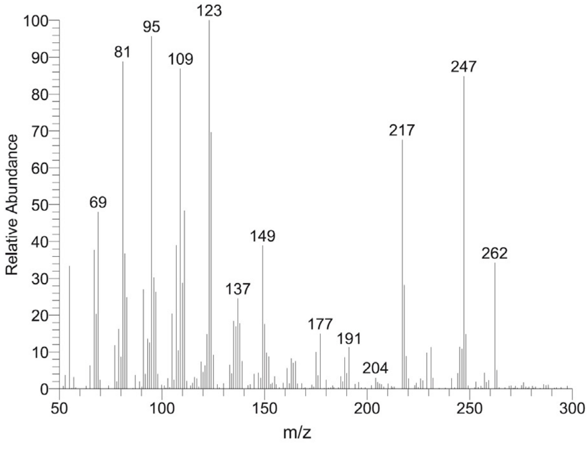
Peak assignments are given in Table 4.3 (Chapter 4)



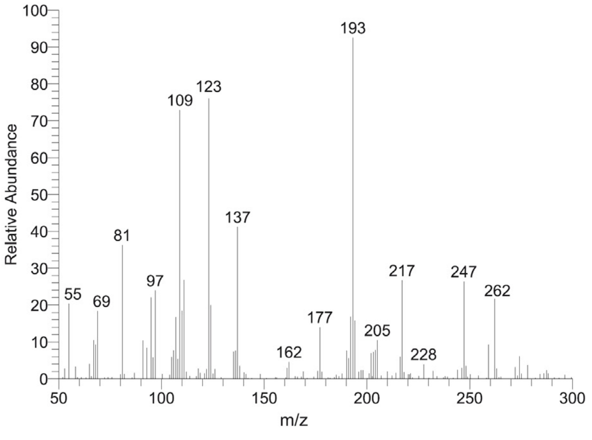
Peak VI: C_{18} tricyclic hydrocarbon?



Peak VII: C_{18} tricyclic hydrocarbon?



Peak VIII: C_{19} tricyclic hydrocarbon?

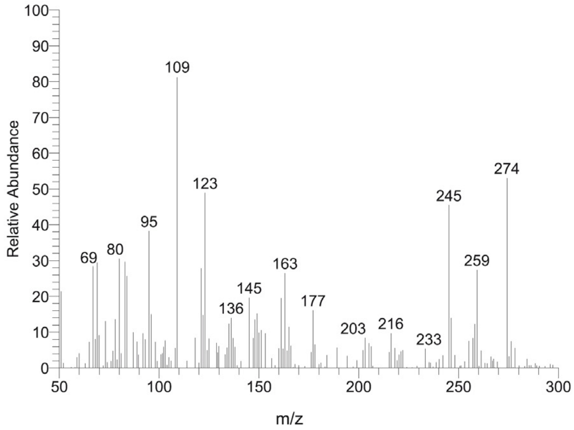


Peak IX: C_{19} tricyclic hydrocarbon?

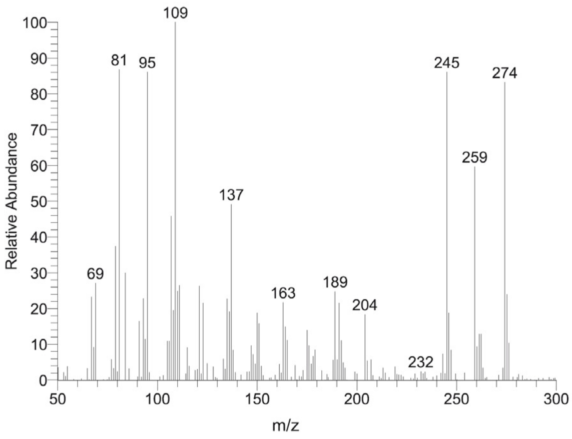
Appendix A.6

Mass Spectra: Unknown compounds

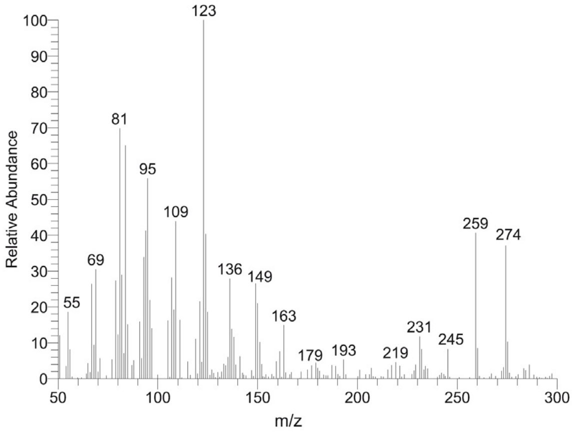
Peak assignments are given in Table 4.3 (Chapter 4)



Peak X: C_{20} tretracyclic diterpenoid?



Peak XI: C_{20} tetracyclic diterpenoid?



Peak XVIII: C_{20} tetracyclic diterpenoid?

Contribution of molecular biomarkers to the knowledge of terrestrial plants development during the Palaeozoic

Abstract

The aliphatic and aromatic biomarker content from terrestrial and marine sediments of Late Ordovician to Early Carboniferous age have been related to their palynomorph assemblages (e.g. acritarchs, prasinophytes, chitinozoans, cryptospores, trilete spores and megaspores) in order to contribute to the knowledge of land plant evolution during the Palaeozoic. This investigation is therefore focused on the land-derived biomarkers and their attributions to specific kind of plants. The biomarker record of middle Silurian – lower Devonian sediments from southern Tunisia, Ghadamis Basin (Gondwana) reveals the presence of retene, cadalene, kaurane, norabietane, tetrahydroretene, C_{19} isohexylalkylnaphthalene and simonellite. The early Palaeozoic bryophytes and tracheophytes (e.g. *Cooksonia*, lycophytes and zosterophylls) may therefore be considered as potential precursors for retene and its related molecular compounds in sediments of Middle Silurian to Early Devonian age. In contrast, the Early Carboniferous flora formed by arborescent lycopods, sphenopsids and pteridosperms have been suggested here as a possible terrestrial source for phyllocladane, abietane, *ent*-beyerane, bisnorsimonellite, diaromatic totarane, diaromatic sempervirane and 2-methylretene in the Lower Carboniferous (Viséan) coal deposits at Dunbar (East Lothian, Scotland). Among the other biomarkers detected in our samples, ionene, alkyldibenzofurans, perylene and combustion-derived polycyclic aromatic hydrocarbons (PAHs) indicate pollen, lichens, fungi and vegetation fire contributions, respectively. Most of the biomarkers identified here had been so far generally associated to conifers, though conifers only evolved during Late Carboniferous. These compounds therefore are also characteristic of early land plants.

Keywords: Land plant biomarkers, Ghadamis Basin, The Midland Valley of Scotland, Silurian, Devonian, Lower Carboniferous, terpenoids, retene, phyllocladane, kaurane, beyerane, alkyldibenzofurans, alkylphenanthrenes, cryptospores, trilete spores, megaspores.

Apports des biomarqueurs moléculaires à la connaissance du développement des plantes terrestres au cours du Paléozoïque

Résumé

Le contenu en biomarqueurs aliphatiques et aromatiques de sédiments d'origine terrestre et marine de l'Ordovicien supérieur au Carbonifère inferieur a été comparé aux assemblages de palynomorphes (acritarches, prasinophytes, chitinozoaires, cryptospores, spores trilètes et mégaspores) afin de contribuer à la connaissance de l'évolution des plantes terrestres au cours du Paléozoïque. Cette étude est donc basée sur les biomarqueurs d'origine terrestre et leur attribution à une espèce de plantes. L'enregistrement des biomarqueurs dans les successions clastiques du Silurien moyen – Dévonien inferieur de Tunisie méridionale, dans le basin de Ghadamis (Gondwana) révèle la présence de rétène, cadalène, kaurane, norabiétane, tetrahydroretene, C_{19} isohexylalkylnaphthalene et simonellite. Les premières bryophytes et les trachéophytes du Paléozoïque (par exemple *Cooksonia*, lycophytes et zosterophylles) peuvent donc être considérés comme de potentiels précurseurs pour le rétène et ses composés moléculaires associés dans les sédiments du Silurien Moyen au Dévonien Inférieur. En contrepartie, la flore du Carbonifère inferieur formée principalement de lycopodes arborescents, sphenopsides et pteridospermes est proposée comme une possible source pour le phyllocladane, abiétane, *ent*-béyerane, bisnorsimonellite, totarane diaromatique, sempervirane diaromatique et 2-méthylrétène dans les dépôts de charbon du Carbonifère inférieur (Viséan) de Dunbar (Est Lothian, Ecosse). Parmi les autres biomarqueurs identifiés dans nos échantillons, ionène, alkyldibenzofuranes, pérylène et les hydrocarbures aromatiques polycycliques (HAPs) dérivés de combustion indiquent la contribution de pollens, lichens, champignons et végétation carbonisée, respectivement. La plupart des biomarqueurs identifiés ici ont été généralement associées aux conifères, qui ne se sont développés qu'après le Carbonifère supérieur. Il apparait donc que ces composés sont également caractéristiques des premières plantes terrestres.

Les mots clés: biomarqueurs de plantes terrestres, Basin du Ghadamis, Midland Valley of Scotland, Silurien, Dévonien, Carbonifère inférieur, terpénoïdes, rétène, phyllocladane, kaurane, béyerane, alkyldibenzofuranes, alkylphenanthrènes, cryptospores, spores trilètes, mégaspores.