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Hydropyrolysis of algae, bacteria, archaea and lake sediments; insights into the origin of nitrogen compounds in petroleum

Barry Bennett [a,*], Arnaud Lager [a], Christopher A. Russell [b], Gordon D. Love [c], Stephen R. Larter [a]

[a] *PRG, Department of Geology and Geophysics, University of Calgary, 2500 University Drive NW, Calgary, AB, T2N IN4, Canada*
[b] *Nottingham Fuel and Energy Centre, School of Chemical, Environmental and Mining Engineering, The University of Nottingham, UK*
[c] *Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA 02139-4307, USA*

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Abstract

Nitrogen compounds are ubiquitous in fossil fuels and yet our understanding of their origins in the geosphere is limited. In this study, high hydrogen pressure pyrolysis was performed on sample material representing potential contributors to sedimentary organic matter (algae, bacteria and archaea) and sediments representing early diagenetic accumulations from Lake Pollen (Norway) and Priest Pot (UK). Previous workers demonstrated the structurally conservative nature of high hydrogen pressure pyrolysis in that the technique maximizes the yields of covalently bound hydrocarbon biomarkers from organic matter without adversely affecting their stereochemistry (Love et al., 1995). Release of covalently bound biomarkers in high yields from kerogen via catalytic hydropyrolysis. In this study, the types and distributions of organic nitrogen compounds in the hydropyrolysates were characterised under similar conditions to such experiments where biomarker hydrocarbons undergo minimal rearrangement. Compounds identified by gas chromatography–mass spectrometry included alkyl-substituted indoles, carbazoles, benzocarbazoles, quinolines and benzoquinolines.

Indoles are present in all hydropyrolysates, suggesting a common origin. A potential source of indoles is represented by tryptophan which was shown to degrade through a series of alkylated intermediates to indole. Carbazole, quinoline and benzoquinoline were also found in the hydropyrolysates of algae, bacteria and archaea. The presence of these petroleum-related nitrogen compounds in hydropyrolysates generated from biomass suggests an early origin for petroleum nitrogen compounds. A potential source of naturally occurring nitrogen compounds such as that in the alkaloids has yet to be realised.

Benzocarbazoles were absent from hydropyrolysates of algae, bacteria and archeae, but present in those from recent sediments, suggesting their presence may be related to processes occurring during early diagenesis at, or immediately below, the sediment–water interface. In sediments from Lake Pollen, changes in the benzocarbazole ratio [*a*]/([*a*] + [*c*]) ratio coincides with the interval described as a transition from fjord to lake environment, suggesting that benzocarbazoles are sensitive to changes in depositional environment and may have potential to act as a marker for environmental conditions.

* Corresponding author. Tel.:+1 403 220 5841; fax: +1 403 284 0074.
E-mail address: bennettb@ucalgary.ca (B. Bennett).

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1. Introduction

Nitrogen is ubiquitous in fossil fuels, but its concentration by weight is low. Tissot and Welte (1984) reported that about 90% of crude oils contain less than 0.2%. Nitrogen in petroleum is found to be predominantly present as heterocyclic aromatic structures (Snyder and Buell, 1965; Bett et al., 1983; Kendall, 1978; Yamamoto et al., 1991; Schmitter et al., 1983; Baxby et al., 1994). These nitrogen compounds have been classified by Richter et al. (1952) into two different groups: neutral pyrrolic types and basic pyridinic types. The neutral nitrogen species usually comprise pyrrole, indole, carbazole and their higher alkylated and benzylated analogues. Meanwhile, basic nitrogen species usually include pyridines, quinolines, benzoquinolines and their alkylated derivatives.

Within the last decade, research and interest in the nitrogen compounds has grown considerably. Despite this interest and proposed uses (e.g., application as indicators of relative petroleum migration distances [Larter et al., 1996]), surprisingly little is known about their biological origin and how such compounds (or related precursor compounds), become incorporated into the macromolecular organic matter in sediments during early diagenesis. After intensive studies of nitrogen species in Californian oils, Snyder (1965) proposed that many of the alkylcarbazoles and alkylbenzocarbazoles in petroleum may be derived from alkaloids with an indole nucleus, often distributed in higher plants and also in blue green algae. However, Li et al. (1995) suggested that plant alkaloids could not be the major source of pyrrolic nitrogen compounds since nitrogen species in high-wax oils derived from higher plant material did not differ significantly from those oils of lacustrine and marine origins.

Humic acids appear to be important for the inclusion of organic nitrogen compounds in source rocks and petroleum. The ultimate source of marine-derived humic acids is primarily protein-rich phytoplankton, while terrestrial humic acids are derived from lignin-rich higher plant material (Tissot and Welte, 1984). During diagenesis, amine groups are lost, but, some amine groups may survive through incorporation within the humic acid complex, while further consolidation into heterocyclic molecules (Bakel and Philp, 1990) may lead to pyrrolic and pyridinic forms, which may proceed via further reactions to produce larger heterocyclic molecules, such as carbazoles or quinolines. Pyridinic and pyrrolic structures can be synthesised in a number of ways, ranging from thermal degradation of porphyrins (Falk, 1964) and plant alkaloids (Cordell, 1981). Schimmelmann et al. (1998) demonstrated the uptake of ammonia into kerogen during hydrous pyrolysis, so incorporation of ammonia during sedimentary diagenesis and consolidation in heterocyclic molecules could also contribute to the formation of pyridinic and pyrrolic structures.

Hydropyrolysis refers to pyrolysis assisted by high hydrogen gas pressures (>10 MPa). Love et al. (1995) demonstrated the unique ability of hydropyrolysis to maximise the yields of covalently bound alkane biomarkers without adversely affecting their stereochemistry with product distributions of triterpanes dominated by hopanes up to C_{35} with the biologically inherited, but thermodynamically unstable, 17β(H), 21β(H) configuration. The technique has since demonstrated its ability to release several nitrogen compound types from kerogens isolated from immature sediments of the Kimmeridge Clay Formation (Bennett and Love, 2000).

In this paper, we report a detailed investigation of the nitrogen compounds released by hydropyrolysis of the macromolecular material of algae, bacteria, archaea and Recent sediments (Priest Pot, UK; Lake Pollen, Norway). The aim of the study was to investigate the types and distributions of nitrogen compounds released during hydropyrolysis of biomass and Recent sediments in order to shed light on the origin of nitrogen compounds in petroleum.

2. Methods

2.1. Samples

General sample information for the algae, bacteria and archaea is given in Table 1. The algae, bacteria and archaea sample material was made available for this study following a wider investigation of lipid biomarkers from microbial cultures using hydropyrolysis (Love et al., 2004). For algae, bacteria and archaea samples, freeze dried biomass was extensively and sequentially extracted ultrasonically using dichloromethane (DCM)/methanol (1:1, vol:vol), DCM / methanol (3:1, vol:vol), DCM, acetone and finally petroleum ether. For the *Phormidium luridum* sample, exhaustive thermal extraction with chloroform/methanol (2:1, vol:vol) was performed using a Soxtherm apparatus (Soxtherm automatic, Gerhardt).

The sediment samples were obtained from Priest Pot (UK) and Lake Pollen (Norway). Priest Pot is a small highly productive hyper-eutrophic lake in the English Lake District. The sediments are permanently anoxic, although the overlying bottom waters are oxygenated during the winter when the lake is no longer stratified (Farrimond et al., 2000, and references therein). Samples from Priest Pot were obtained from core depths of 1, 8, 16, 19 and 22 cm. Lake Pollen is a small lake lying 20 km south of Oslo. It was once part of the Oslofjord but became isolated in the late 18th century due to isostatic uplift following the removal of glacial ice sheets. Presently, the lake contains remnant seawater below a seasonally developed chemocline. The bottom water remains anoxic throughout the year, despite overturn of the water

Table 1
List of cultures used in hydropyrolysis experiments

Name	Strain number/ supplier	Taxonomic group
Marine algae		
Emiliana huxleyi	920/2	Haptophyceae
Porphyridium purpureum	1308/3	Bangiophyceae
Fresh water algae		
Scenedesmus quadricauda	276/21	Chlorophyceae
Cryptomonas sp.	979/26	Crytophyceae
Tribonema aequale	880/1	Xanthophyceae
Bacteria		
Phormidium luridum	MIT	Cyanophyceae
Phormidium RCO	Ames	Cyanophyceae
Chloroflexus sp.	Ames	Chloroflexaceae
Chlorobium tepidum	Ames	Chlorobiaceae
Chlorobium thiosulfatophilum	MIT	Chlorobiaceae
Archaea		
Halobacterium saccharovorum	Ames	Halobiaceae

Key to sample suppliers: samples listed under strain number = Culture Collection of Algae and Protozoa for the United Kingdom, samples supplied by Dr. John Day; Ames, NASA Ames Research Centre, samples supplied by Dr. Linda Jahnke; MIT, Massachusetts Institute of Technology, samples supplied by Dr. Roger Summons.

column, and consequent disruption of the stratification, in autumn/winter. A sediment core (46 cm long) was collected from the deepest part of the lake (June, 1992) using a Jenkin corer (Farrimond et al., 2000, and references therein). The geochemical changes associated with transformation from fjord to lake have been observed in the hopanoid compounds at a depth of 25 and 30 cm (Innes et al., 1998). The samples obtained for this study were taken from both the fjord and lake phase of the depositional history at depths of 3.5, 12.7, 20.7, 30 and 39 cm.

A bituminous mud sample from the Kimmeridge Clay Formation (TOC 11.6) was pre-extracted via soxhlet using azeotropic DCM: methanol (93:7, vol:vol). The solvent extracted sediment residue was subjected to hydropyrolysis. The nitrogen compound product distributions were characterised previously in Bennett and Love (2000). The hydropyrolysate was utilised in this study to perform compound assignments based on co-chromatography investigations with recently acquired authentic standards (Table 2).

2.2. Catalytic hydropyrolysis

The sediment samples were pre-extracted via soxhlet using azeotrope DCM:methanol (93:7 vol:vol). The solvent-extracted sediment residues and samples of pre-extracted algae, bacteria and archaea were impregnated with an aqueous solution of ammonium dioxydithiomolybdate [$(NH_4)_2MoO_2S_2$] to give a nominal loading of molybdenum of 1 wt%. The apparatus and basic procedures used for temperature-programmed hydropyrolysis have been described in detail elsewhere (Love et al., 1995). Briefly, samples were mixed with acid-washed sand (1:5 w/w) and heated in a stainless steel reactor tube from 150 to 520 °C at 8 °C min^{-1} using a hydrogen pressure of 15.0 MPa. A hydrogen flow of 10 l min^{-1}, measured at ambient temperature and pressure, through the reactor ensured that the overall conversion was not limited by mass transfer in the sample bed and that volatiles escaped quickly. The tar product (hydropyrolysate) was collected in a trap cooled with dry-ice and recovered in DCM/methanol (93:7 vol:vol) for subsequent fractionation.

2.3. Recovery of nitrogen compounds by solid phase extraction

The organic nitrogen compounds were isolated from the hydropyrolysates using the solid phase extraction (SPE) method described in Bennett et al. (2002). Briefly, following complete removal of DCM and methanol, the

Table 2
List of authentic standards utilised for peak assignments following co-chromatography experiments

Number	Compound	Number	Compound	Number	Compound
1	Indole (I)	11	2-Methylcarbazole	21	7-Methylquinoline
2	7-Methylindole	12	4-Methylcarbazole	22	3-Methylquinoline
3	3-Methylindole	13	Benzo[*a*]carbazole (IV)	23	4-Methylquinoline
4	2- + 5- + 6-Methylindoles	14	Benzo[*b*]carbazole (V)	24	2,5-Dimethylquinoline
5	4-Methylindole	15	Benzo[*c*]carbazole (VI)	25	2,7-Dimethylquinoline
6	2,5-Dimethylindole	16	Quinoline (VII)	26	2,4-Dimethylquinoline
7	2,3-Dimethylindole	17	Iso-quinoline (VIII)	27	Benzo[*h*]quinoline (IX)
8	Carbazole (III)	18	2-Methylquinoline	28	Acridine (X)
9	1-Methylcarbazole	19	8-Methylquinoline	29	Phenanthridine (XI)
10	3-Methylcarbazole	20	6-Methylquinoline	30	Benzo[5,6]quinoline (XII)

hydropyrolysate residue was dissolved in *n*-hexane (agitated via sonication) and then transferred to a C_{18} non-endcapped SPE cartridge (Jones Chromatography, UK). A deuteriated (D8) carbazole standard was also added to the SPE cartridge to allow quantification of nitrogen compounds. Firstly, the aliphatic and aromatic hydrocarbon fraction was eluted with *n*-hexane (5 ml). The polar non-hydrocarbon fraction containing the organic nitrogen compounds was recovered in DCM (5 ml) and then the solvent reduced to minimum volume under nitrogen gas prior to gas chromatography–mass spectrometry (GC–MS) analysis. A second standard (*N*-phenylcarbazole) was added to assess the recovery of the D8 carbazole internal standard.

2.4. Gas chromatography–mass spectrometry

The organic nitrogen compounds were analysed on a fused silica capillary column with the following coating, ZB-35 (65%/35%, methyl/phenyl silicone). The column dimension was 30 m × 0.32 mm i.d. × 0.25 μm film thickness (Hewlett–Packard)). The GC oven temperature programme was 40 °C held for 2 min then 4 °C min^{-1} to 300 °C and held at the final temperature for 20 min.

Mass spectral characterisation of the organic nitrogen compounds was carried out using combined GC–MS on a Hewlett–Packard 5890 GC (using splitless injection) interfaced to a HP 5970B quadrupole mass selective detector (electron input energy 70 eV, source temperature, 250 °C).

Compound identification was based on relative retention times, comparison of spectra with published spectra and, where standard compounds were available, by co-chromatography. The co-chromatography experiments were carried out on three different GC phases: ZB-35; HP-5 (95%/5%, methyl/phenyl silicone) and ZB-1701 (86%/14%, methyl/cyanopropylphenyl silicone).

A standard stock solution of deuteriated (D8) carbazole was prepared in hexane:toluene (9:1, vol:vol) and *N*-phenylcarbazole was prepared in DCM. Peak area integration during GC–MS analysis used MASSLAB software. The relative response factor (RRF) between D8-carbazole and related compounds was assumed to be 1.

3. Results and discussion

3.1. Hydropyrolysis of algae, bacteria, archaea and sedimentary organic matter

Upon hydropyrolysis, the algae, bacteria and archaea samples released significant quantities of pyrrolic and pyridinic nitrogen compounds. The nitrogen compounds were assigned exclusively to the pyrolysate products, with no pyrrolic or pyridinic nitrogen compounds being detected in the solvent extracts of the algae, bacteria and archaea. Thus, hydropyrolysis results in the breakdown of the insoluble macromolecular organic matter of the cell constituents, producing solvent extractable pyrolysates enriched in nitrogen compounds. Hydropyrolysis of sedimentary organic matter isolated from Priest Pot and Lake Pollen also resulted in the production of nitrogen compounds. Bennett and Love (2000) previously characterised nitrogen compounds released during hydropyrolysis of "immature" kerogen isolated from a bituminous mud from the Kimmeridge Clay Formation. A suite of standards (see Fig. 1 and Table 2) has since been acquired allowing further characterisation of the nitrogen compounds generated during hydropyrolysis.

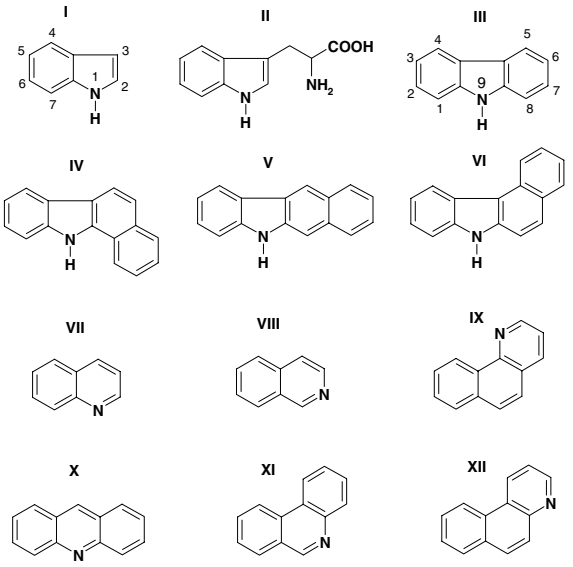

Fig. 1. Structures of nitrogen compounds.

3.2. Indoles

The partial mass chromatogram of the molecular ions, representing the distribution of C_0–C_2 indoles in the hydropyrolysate of *Porphyridium purpureum*, is shown in Fig. 2(a). The assignment of C_0–C_2 indoles was performed by co-chromatography studies employing authentic standards. The most abundant compound in the hydropyrolysate of *P. purpureum* is represented by indole (I) followed by methylindoles and then the C_2 indoles. The 3-methylindole is the most abundant methylindole. Amongst the C_2 indoles, co-chromatography indicates 2,5-dimethylindole is apparently the most abundant isomer, as suggested by its co-elution with the authentic standard on HP-5, ZB-1701 and ZB-35 phases. Comparison of the mass spectrum of the authentic standard [Fig. 3(a)] and the mass spectrum [Fig. 3(b)] obtained from the corresponding elution interval in

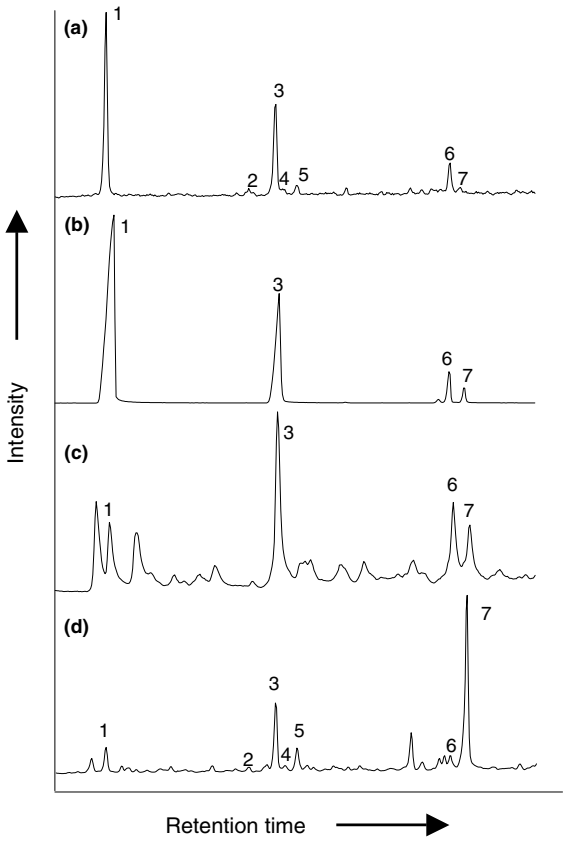

Fig. 2. Partial mass chromatograms of summed ions representing indole (*m*/*z* 117), methylindoles (*m*/*z* 131) and C_2 indoles (*m*/*z* 145) in C_{18} non-endcapped solid phase extraction isolates from: (a) hydropyrolysate of *P. purpureum*, (b) anhydrous pyrolysate of tryptophan (c) hydropyrolysate of Priest Pot sediments and (d) hydropyrolysate generated from the Kimmeridge Clay Formation sample. See Table 2 for compound assignments.

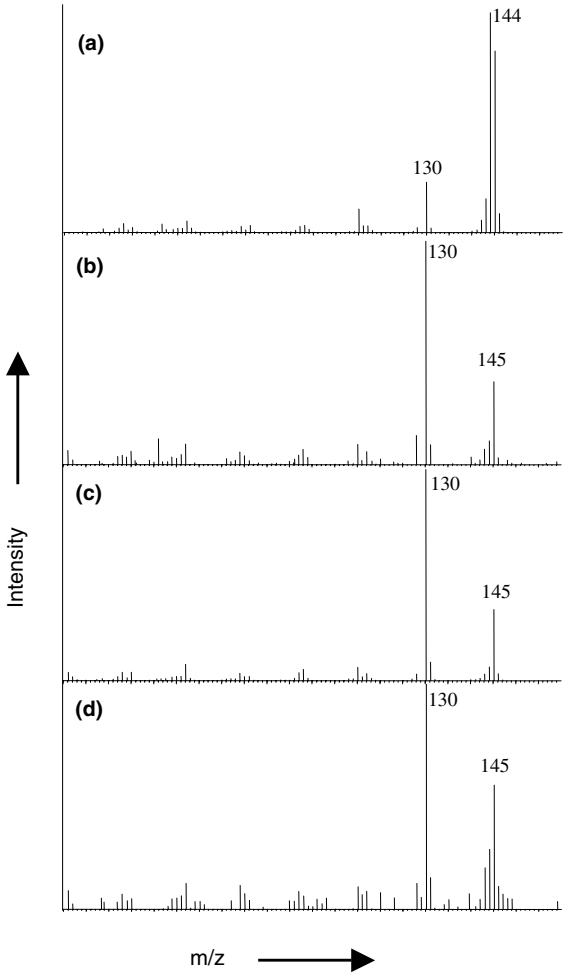

Fig. 3. Mass spectra of: (a) 2,5-dimethylindole authentic standard, (b) peak 6 in Fig. 2(a), (c) peak 6 in Fig. 2(b), and (d) peak 6 in Fig. 2(c).

Fig. 2(a; labelled as peak 6) suggests co-elution with a C_2 indole isomer. The mass spectrum of 2,5-dimethylindole standard [Fig. 3(a)] is dominated by a major ion at *m*/*z* 144 (loss of 1 mass unit from the molecular ion) and molecular ion at *m*/*z* 145 and a significant contribution at *m*/*z* 130, due to the loss of a methyl group (M-15). The mass spectrum at the retention time corresponding to 2,5-dimethylindole in the hydropyrolysate of *P. purpureum* is shown in Fig. 3(b). It is dominated by the ion at *m*/*z* 130, with a significant contribution due to the molecular ion at *m*/*z* 145. The base peak at *m*/*z* 130 indicates a facile fragmentation in the C_2 indole, suggesting an ethyl side chain. No authentic 3-ethylindole standard was available to confirm identification; therefore, we extended our investigations on the assignment of indoles through a comparison of the product distributions generated during a heating experiment with tryptophan.

Tryptophan (II) is a natural product amino acid consisting of an indole nucleus with a side chain containing a functional moiety of a carboxylic acid and amine group. Approximately 5 mg of tryptophan were loaded into a glass tube and then flushed with nitrogen gas and the tube was sealed in vacuo and subsequently heated at 300 °C for 16 h (anhydrous pyrolysis conditions). Following termination of the experiment, the glass tube was cooled in liquid nitrogen and broken to release the pyrolysis products which were recovered in DCM. They were analysed directly by GC–MS and the resulting mass chromatogram is shown in Fig. 2(b). The major product is represented by indole, followed by 3-methylindole and C_2 indoles. Other products in the anhydrous pyrolysate (not shown) included indole-3-acetaldehyde and ethanone 1-(1H-indol-3-yl). The presence of 2,3-dimethylindole suggests there was a small amount of rearrangement during the anhydrous

pyrolysis experiment. The mass spectrum of the C_2 indole following degradation of tryptophan is shown in Fig. 3(c) and is compared with the spectrum obtained from the *P. purpureum* in, Fig. 3(b). The reaction product distribution resulting from anhydrous pyrolysis of tryptophan is the result of stepwise degradation (tryptophan to 3-ethylindole to 3-methylindole to indole) and therefore the unknown C_2 indole (peak 6 in Fig. 2) is most likely to be predominantly 3-ethylindole.

Indole was present in all the hydropyrolysates generated from algae, bacteria and archaea samples. The alkylated indoles were abundant components only in *P. purpureum*, *Tribonema aequale* and *Cryptomonas* sp. whereas in the hydropyrolysates of the other biomass samples (Table 1) only the parent indole was identified.

In the hydropyrolysates of sediment samples, indoles represent major products. Fig. 2(c) shows partial mass chromatogram representing C_0–C_2 indoles in a hydropyrolysate generated from Priest Pot solvent-extracted sediments. The presence of indole, 3-methylindole, 3-ethylindole and 2,3-dimethylindole was assigned from their mass spectra. The mass chromatogram is inherently more complex due to ions arising from phenolic compounds which may interfere with the assignment of indole components. The major compounds are typical of those encountered in the hydropyrolsates generated from biomass. A similar GC distribution of C_0–C_2 indoles was found in the hydropyrolysates from Lake Pollen sediments (not shown). Fig. 2(d) shows the partial mass chromatogram representing C_0–C_2 indoles in a hydropyrolysate generated from a bituminous mud from the Kimmeridge Clay Formation. The relative abundance amongst the C_0–C_2 indoles follows the order $C_2 > C_1 >$ indole, with 2,3-dimethylindole representing the dominant compound. The relative distribution of the carbon number homologues is the reverse of the order of biomass samples, with the relative abundance dominated in the order C_2 indoles > methylindoles > indole. Amongst the C_2 indoles, 2,3-dimethylindole is the most abundant isomer, the mass spectrum in Fig. 3(d) also suggests the presence of 3-ethylindole. The presence of 2,3-dimethylindole is more strongly represented in hydropyrolsates generated from sediment samples compared to that in the hydropyrolysates from biomass samples. Its presence in tryptophan degradation products arises from secondary rearrangement occurring under the closed system pyrolysis conditions. During hydropyrolysis secondary reactions such as structural rearrangements are minimalised, allowing generation of products most likely to be representative of their inherited state prior to release from the bound macromolecular phase. Thus biomass samples yield predominantly 3-ethylindole, 3-methylindole and indole which may arise from the stepwise degradation of tryptophan. The sediment samples are inherently more complex, displaying a range of isomers. The predominance of 2,3-dimethylindole may suggest a direct inheritance from a precursor (e.g., alkaloids). Alternatively, 2,3-dimethylindole may result from processes occurring during early diagenesis such as structural rearrangements within tryptophan-derived molecules.

The presence of indole, 3-methylindole, 2,3-dimethylindole and 3-ethylindole, could be ascribed to pyrolysis products of protein because these four molecules are found in the pyrolysate fingerprint of tryptophan (see also Tsuge and Matsubara, 1985; Chiavari and Galletti, 1992). In the remaining algae, bacteria and archaea only the parent indole was identified. The presence of indole alone without any of its higher homologues suggests an origin that cannot be ascribed solely to a tryptophan moiety. This implies that indole may have more than one precursor. In previous studies of suspended organic matter from the Rhone delta, sediment trap material from the north-western Mediterranean sea and recent sediment of the Danube delta (Sicre et al., 1994; Peulve et al., 1996; Çoban-Yildiz et al., 2000; Garcette-Lepecq et al., 2000), the conclusion reached was that indole and all its alkylated isomers were related to protein products and/or to melanoidin-type structures. This may be true for sediments where the full range of indole-alkylated isomers is present but it seems very unlikely that a melanoidin structure would give just indole as a pyrolysate product. Therefore other precursors may be important such, as the indole alkaloids which are found in plant material, algae and bacteria (Zeng et al., 1999). Van Binst et al. (1966) performed numerous studies on the pyrolysis of indole alkaloids and have shown that indole alkaloids decompose into indole, so alkaloids may also serve as precursors of indole.

3.3. Alkylcarbazoles

The partial mass chromatograms representing the distribution of C_0–C_2 carbazoles isolated from the hydropyrolysate of the alga *P. purpureum* are shown in Fig. 4. Carbazole (III) and methylcarbazoles were identified in all hydropyrolysates generated from algae, bacteria and archaea, with the parent molecule carbazole representing the dominant compound. The C_2 carbazoles were identified but were only found in low levels in most biomass samples, with appreciable quantities found in *P. purpureum* (Fig. 4(c)).

Fig. 5 shows the distributions of C_0–C_2 carbazoles in a hydropyrolysate from a bituminous mud from the Kimmeridge Clay Formation. The parent molecule carbazole represented the dominant compound with appreciable quantities of methylcarbazoles and C_2 carbazoles. The dominance of carbazole relative to methylcarbazole and C_2 carbazoles was also confirmed in the hydropyrolysates from Priest Pot and Lake Pollen sediments. The concentration data for the C_0–C_2 carbazoles isolated from hydropyrolysates of Priest Pot and Lake Pollen

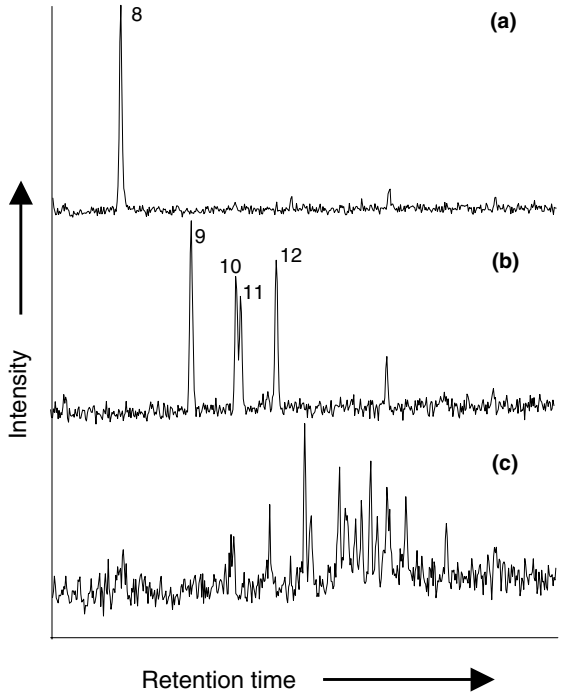

Fig. 4. Partial mass chromatograms representing: (a) carbazole (*m*/*z* 167), (b) methylcarbazoles (*m*/*z* 181) and (c) C_2 carbazoles (*m*/*z* 195) in C_{18} non-endcapped solid phase extraction isolates from the hydropyrolysate generated from the alga *P. purpureum*. See Table 2 for compound assignments.

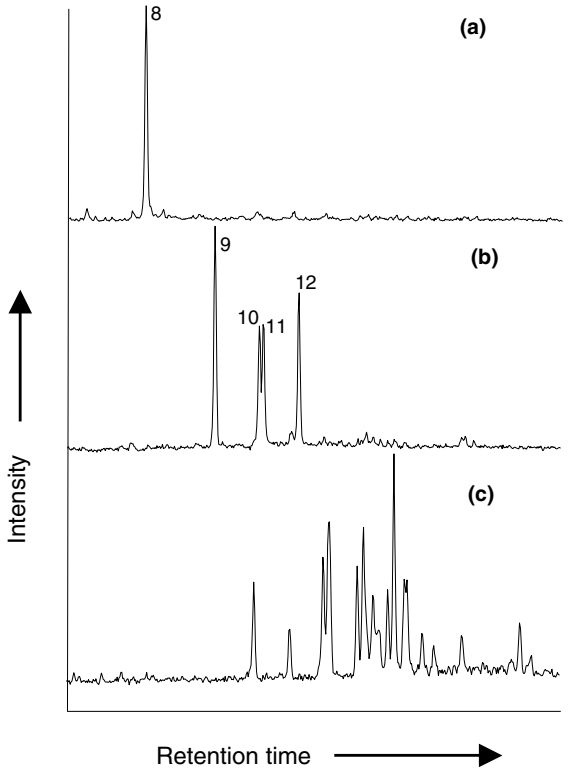

Fig. 5. Partial mass chromatograms representing: (a) carbazole (*m*/*z* 167), (b) methylcarbazoles (*m*/*z* 181) and (c) C_2 carbazoles (*m*/*z* 195) in C_{18} non-endcapped solid phase extraction isolate from the hydropyrolysate generated from solvent extracted Kimmeridge Clay Formation sample. See Table 2 for compound assignments.

are shown, respectively, in Tables 3 and 4. In the Priest Pot hydropyrolysates, high yields of nitrogen compounds were found, while in Lake Pollen the carbazoles were present at approximately half the content of that in Priest Pot.

Carbazoles are well known in petroleum geochemistry (Dorbon et al., 1984a, b; Li et al., 1995; Larter et al., 1996; Clegg et al., 1997; Bennett and Love, 2000), while in soil science and in the study of recent sediments, the presence of carbazole and higher homologues has never been reported (Sicre et al., 1994; Peulve et al., 1996; Schulten et al., 1997; Garcette-Lepecq et al., 2000). This is not surprising since hydropyrolysis is able to release compounds from the bound phase, which is not accessible using conventional solvent extraction techniques.

Initially it was proposed that the source of alkylcarbazoles and alkylbenzocarbazoles in petroleum was from alkaloids (Snyder, 1965), although this idea was ruled out since alkaloids were only found in plants. Recently, due to the increase of interest in the properties of alkaloids in medicinal chemistry, numerous alkaloids have been discovered in nearly every class of algae and bacteria (Zeng et al., 1999). Discoveries of marine alkaloids (Cardellina et al., 1979) and the fact that in this study, significant amounts (up to 36.5 ppm) of carbazoles were measured in the hydropyrolysates generated from recent sediments, algae, bacteria and archaea where condensation/secondary rearrangement reactions are limited (cf. Love et al., 1995), suggests that alkaloids may play a more important role as carbazole precursors than originally thought (Snyder, 1965). The presence of carbazoles in biomass hydropyrolysates suggests that carbazoles or precursor entities are present in the bound state and only released as solvent extractable products following hydropyrolysis.

3.4. Benzocarbazoles

The hydropyrolysates generated from algae, bacteria and archaea samples yielded no benzocarbazoles, while those generated from Lake Pollen and Priest Pot [Fig. 6(a)] sediments, GC–MS revealed the presence of benzo[*a*]carbazole (IV), benzo[*b*]carbazole (V) and benzo[*c*]carbazole (VI). The GC–MS distribution of benzocarbazole isomers isolated from a typical non-degraded North Sea oil is shown for comparative

Table 3
Concentrations (μg/g TSE) of alkylcarbazoles and benzocarbazole isomers in hydropyrolysates generated from solvent extracted Priest Pot sediments

Depth (cm)	Carbazole	Methylcarbazoles				Benzocarbazoles			
		1-MC	3-MC	2-MC	4-MC	[*a*]	[*b*]	[*c*]	[*a*]/([*a*] + [*c*])
1	36.5	20.0	8.1	1.1	14.7	4.8	1.9	4.7	0.51
8	26.4	16.0	6.7	0.7	13.2	2.7	1.3	2.7	0.50
16	19.8	14.9	10.8	1.1	7.6	2.3	1.0	2.5	0.48
19	23.4	20.6	5.4	0.7	12.0	2.4	1.2	2.6	0.48
22	25.2	17.5	5.7	1.0	10.1	2.4	0.9	2.5	0.49

Table 4
Concentrations (μg/g TSE) of alkylcarbazoles and benzocarbazole isomers in hydropyrolysates generated from solvent extracted Lake Pollen sediments

Depth (cm)	Carbazole	4-MC	Benzocarbazoles				
			[*a*]	[*b*]	[*c*]	[*a*]/([*a*] + [*c*])	[*b*]/([*b*] + [*c*])
3.5	13.6	5.2	0.37	0.14	0.35	0.50	0.29
12.7	10.3	3.7	0.29	0.09	0.28	0.51	0.24
20.7	9.5	4.8	0.35	0.06	0.18	0.66	0.25
30	9.7	2.9	0.29	0.04	0.13	0.69	0.24
39	10.5	3.9	0.17	0.01	0.07	0.71	0.13

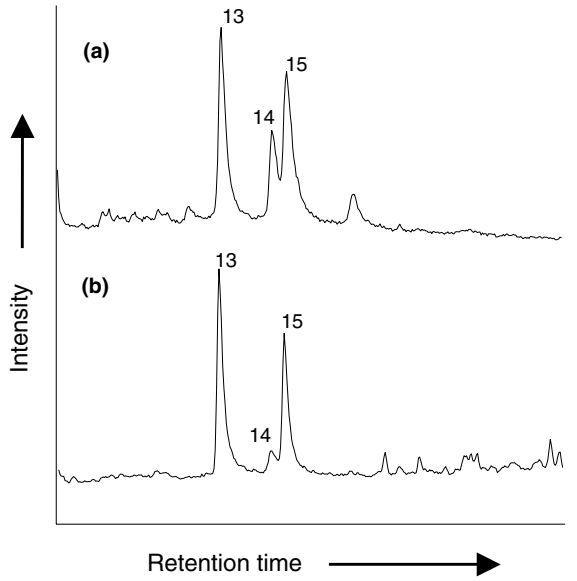

Fig. 6. Partial mass chromatograms representing benzocarbazoles in C_{18} non-endcapped solid phase extraction isolates from: (a) hydropyrolysate generated from a Kimmeridge Clay Formation sample and (b) North Sea oil.

purposes [Fig. 6(b)]. The concentration data for benzocarbazole isomers in Priest Pot and Lake Pollen hydropyrolysates are shown in Tables 3 and 4, respectively. The concentrations of benzocarbazole in Lake Pollen hydropyrolysates are an order of magnitude lower than in Priest Pot and those reported for Kimmeridge Clay Formation (Bennett and Love, 2000). In the Priest Pot samples a similar trend was observed in benzocarbazole [*a*]/([*a*] + [*c*]) ratio throughout the depth interval studied, suggesting a common but consistent organic matter contribution to the samples. Fig. 7 shows the behaviour of the benzocarbazole [*a*]/([*a*] + [*c*]) parameter in the hydropyrolysates generated from Lake Pollen sediments versus depth. In Lake Pollen, there is a strong deflection in the benzocarbazole [*a*]/([*a*] + [*c*]) from 0.5 to 0.7 with increasing depth. The change in the benzocarbazole ratio [*a*]/([*a*] + [*c*]) appears to coincide with a major environmental change recognised in the Lake Pollen sediments. Lake Pollen was once part of a fjord system before it became a lake. Previous work has shown that the environmental changes from fjord to lake can be identified using hopanoid compounds in the core between 15 and 25 cm (Innes et al., 1998). This coincidence of the changes in the benzocarbazole [*a*]/([*a*] + [*c*]) ratio with the development of Lake Pollen from a fjord are described schematically in Fig. 7. In general the levels of benzo[*a*]carbazole isomer are similar in most samples (Table 4), suggesting a consistent contribution of benzo[*a*]carbazole throughout the transition period. Whereas the concentration data (Table 4) reveal that the benzo[*c*]carbazole (and benzo[*b*]carbazole) decrease with increasing depth, implying the contributors for these compounds during the fjord phase were limited and increased during development of the lake phase.

In Lake Pollen sediments it appears that the benzocarbazole data concur with changes documented historically and with geochemical parameters based on

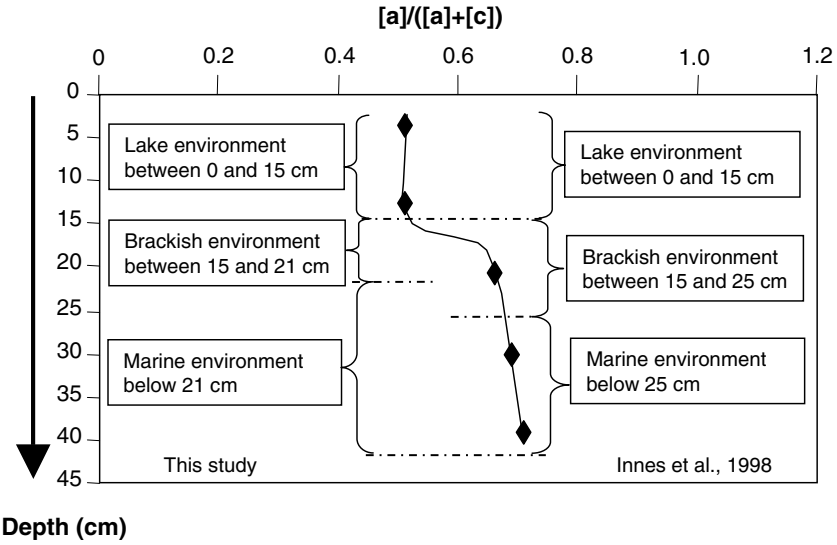

Fig. 7. Delimitation of the different environmental changes during the development of Lake Pollen, on the left the results suggested by the changes in [*a*]/([*a*] + [*c*]) data. On the right, the results obtained by monitoring hopanoids (Innes et al., 1998). Note the discrepancy in the brackish to marine transition is due to the sample at 25 cm (only 20.7 and 30 cm) was not available for this study.

the hopanoids. Thus, in this case, benzocarbazole distributions reflect a major environmental change during the transition from fjord to lake. Clegg et al. (1997) were able to distinguish changes in nitrogen compound types and distributions in two lithofacies of the Middle Devonian carbonates in the Western Canada sedimentary basin. Clegg et al. (1997) showed that benzo[*a*]carbazole was produced at a greater rate than benzo[*c*]carbazole and suggested that there was a specific precursor for benzo[*a*]carbazole. Bakr and Wilkes (2002) reported that facies and depositional environment of the relevant source rocks were the major factors influencing benzocarbazole distributions in oil samples from the Gulf of Suez, Egypt. In contrast, Li et al. (1995), analysing oils from many different environments of deposition, showed no significant differences in nitrogen species, suggesting a common mode of origin, possibly from consistent facies independent precursor, such as proteins or their nitrogen cycle products, as a universal source of nitrogen (Li et al., 1995). Silliman et al. (2002) revealed strong biomarker changes in parallel with facies changes but were unable to detect facies related changes in the pyrrolic nitrogen species of the Permian Phosphoria derived oils.

The hydropyrolysates generated from algae, bacteria and archaea, representing potential contributors to sedimentary organic matter, did not show any detectable benzocarbazoles, suggesting direct inheritance from biomass, similar to that indicated for carbazoles and indoles, is unlikely. However, based on the few samples we have analysed, at this time a source of benzocarbazoles from other biomass samples cannot be ruled out. The presence of benzocarbazoles in the sediments from shallow depths (few cm) in Priest Pot and Lake Pollen suggests that a mechanism leading to benzocarbazole (or benzocarbazole precursors) must take place during early diagenesis.

3.5. Nitrogen bases

Partial mass chromatograms (*m*/*z* 129, 143, 157) showing the distributions of C_0–C_2 quinolines in a hydropyrolysate generated from a bituminous mud from the Kimmeridge Clay Formation are displayed in Fig. 8. The identification of C_0–C_2 quinolines was based on comparison of mass spectra and co-chromatography with authentic standards. Isoquinoline was also identified in the hydropyrolysate [Fig. 8(a)]. The identification of quinoline-related compounds in hydropyrolysates from Lake Pollen and Priest Pot extracted sediments was difficult to confirm due to co-elution with phenolic compounds. A series of quinolines was identified in all algae, bacteria and archaea samples, but mass spectral assignments were complex due to interference with phenolic compounds. The partial mass chromatograms of C_0–C_2 quinolines in the hydropyrolysate generated from *Scenedesmus quadricauda* is shown in Fig. 9. The dominance of the parent quinoline (VII) was common to most algae, bacteria and archaea hydropyrolysates, although in some cases equal abundances of quinoline and alkylquinolines were observed.

The GC–MS investigation of the distribution of benzoquinoline isomers in a hydropyrolysate from Kimmeridge Clay Formation are shown in Fig. 10(a)–(c). The

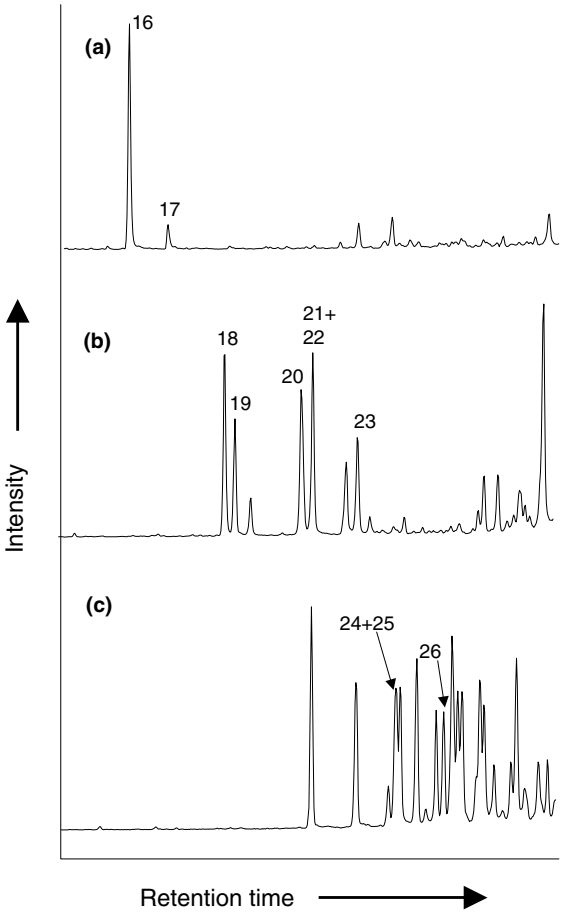

Fig. 8. Partial mass chromatograms representing: (a) quinoline (*m*/*z* 129), (b) methylquinolines (*m*/*z* 143) and (c) C_2 quinolines in C_{18} non-endcapped solid phase extraction isolate from the hydropyrolysate generated from the solvent extracted Kimmeridge Clay Formation sample. See Table 2 for compound assignments.

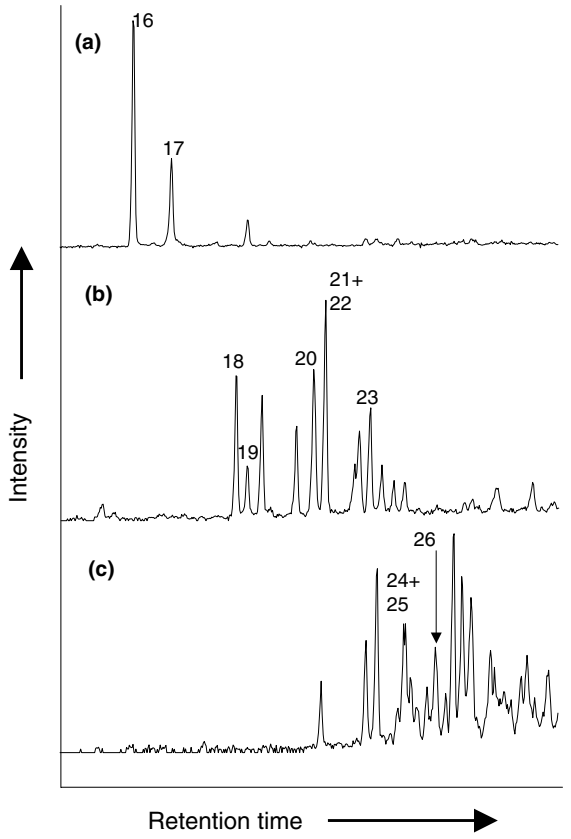

Fig. 9. Partial mass chromatograms representing: (a) quinoline (*m*/*z* 129), (b) methylquinolines (*m*/*z* 143) and (c) C_2 quinolines in C_{18} non-endcapped solid phase extraction isolate from the hydropyrolysate generated from freshwater alga *S. quadricauda*. See Table 2 for compound assignments.

partial mass chromatogram representing benzoquinolines displays three major peaks [Fig. 10(a)]. Bennett and Love (2000) assigned benzo[*h*]quinoline (IX) and acridine (X) but noted the co-elution of phenanthridine (XI) and benzo[5,6]quinoline (XII). Further chromatography experiments were conducted using two additional GC phases, ZB-1701 and ZB-35. Fig. 10(b) shows the presence of four peaks displaying partial separation of benzo[*f*]quinoline and phenanthridine on the ZB-1701 phase. However, using ZB-35 the *m*/*z* 179 mass chromatogram displays five peaks with complete separation of benzo[*f*]quinoline and phenanthridine [Fig. 10(c)]. The benzoquinolines were found in hydropyrolysates generated from *E. huxleyi*, *S. quadricauda* [Fig. 8(d)] and as trace products in other algae and bacteria hydropyrolysates, although they were not found in the *Halobacterium saccharovorum* hydropyrolysate.

Schmitter et al. (1982, 1983, 1984) and Dorbon et al. (1984a,b), reported that the distributions of azaarenes and carbazoles show similar distributional patterns in crude oils of different origins, suggesting the similarity reflected their source in restricted precursors and common mechanism of formation. Interestingly, the ratio of basic nitrogen to total nitrogen in crude oils is approximately 30% regardless of the source and therefore implies a common source input. Little is known about the origin of quinolines and they are rarely reported in soil organic matter studies. Possible precursors include alkaloids since they contain the nitrogen heteroatom and are widely reported in land plants, and recently in algae (Zeng et al., 1999). For example, Van Binst et al. (1966) identified quinoline and its alkylated isomers in decomposition products from the pyrolysis of indole alkaloids. A possible alternative pathway for organic nitrogen compounds is cyclisation of amine-containing compounds following incorporation of pro-

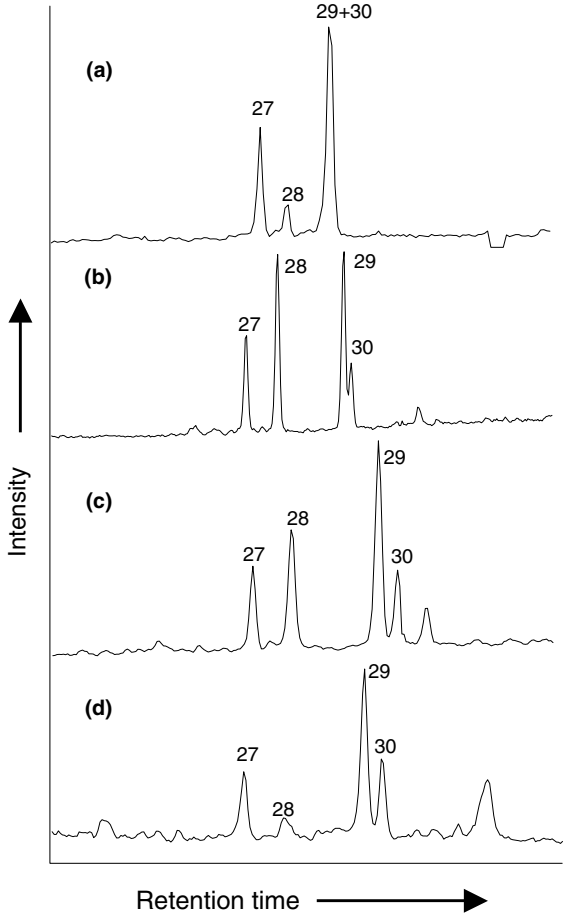

Fig. 10. Partial mass chromatograms representing the assignment of benzoquinolines (*m*/*z* 179) in the hydropyrolysate generated from solvent extracted Kimmeridge Clay Formation following GC–MS analysis on: (a) HP-5, (b) ZB-1701, (c) ZB-35 and the benzoquinolines present in the hydropyrolysate generated from fresh water alga *S. quadricauda* using ZB-35. See Table 2 for compound assignments.

tein rich material into humic acids during diagenesis. The presence of basic nitrogen compounds in the hydropyrolysates of biomass samples suggests that structures able to produce quinolines and benzoquinolines must be present in the bound state or formed during the release process. The latter processes are thought to be restricted under the hydropyrolysis conditions since compounds that are released from the macromolecular phase are rapidly swept away from the reaction zone and are quenched, thus minimising structural modifications. The conservative nature of the hydropyrolysis technique has been demonstrated for hydrocarbon biomarkers; hence, compound structures identified in hydropyrolysates are thought likely to be representative of the components in the bound phase.

4. Conclusions

Hydropyrolysis of algae, bacteria, archaea and sediments from Priest Pot and Lake Pollen yielded significant quantities of aromatic nitrogen compounds. A suite of indoles, carbazoles, quinolines and benzoquinolines was identified by comparison with authentic standards. Benzoquinoline isomers, phenanthridine and benzo[5,6]quinoline co-eluted under the GC conditions employed using the HP-5 GC phase, but showed complete separation on the ZB-35 phase.

Benzocarbazoles were absent from the biomass samples, but were present in the hydropyrolsates generated from Lake Pollen and Priest Pot sediments. In lake Pollen, changes in the benzocarbazole [*a*]/([*a*] + [*c*]) ratio corresponded to a marked shift in environmental conditions from fjord to an isolated lake, suggesting hydropyrolysis of sediments may liberate information in the form of product distributions that are sensitive to changes in depositional environment.

The generation of aromatic nitrogen compounds during hydropyrolysis of biomass suggests that nitrogen compounds and/or their precursor entities are present in the bound phase of source material contributing to sedimentary organic matter. The presence of carbazole and methylcarbazole in the hydropyrolysates, which are common constituents of crude oils, suggests an early origin for these naturally occurring petroleum nitrogen species. The lack of benzocarbazoles in the hydropyrolysates generated from biomass, but their presence in recent sediment hydropyrolysates suggests that an early diagenetic mechanism may be responsible for the origin of benzocarbazoles, although due to the size of the sample suite studied, a direct source from biomass could not be ruled out. The application of hydropyrolysis as a structurally conservative pyrolysis technique was previously established for the hydrocarbon biomarkers where product distributions yielded biologically inherited structures. However, the conservative nature of hydroypyrolysis has yet to be demonstrated for the aromatic nitrogen compounds. Further work is required to establish the possible effect of structural rearrangements/secondary reactions associated with the release of aromatic nitrogen compounds during hydropyrolysis to verify employing the technique for investigating the origin of nitrogen compounds in the geosphere.

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