Thermal release of nitrogen organics from natural organic matter (NOM) using micro scale sealed vessel (MSSV) pyrolysis

Lyndon Berwick, Paul Greenwood, Robert Kagi, Jean-Philippe Croué

PII: S0146-6380(07)00065-4
DOI: 10.1016/j.orggeochem.2007.02.015
Reference: OG 1993

To appear in: *Organic Geochemistry*

Received Date: 20 June 2006
Revised Date: 1 October 2006
Accepted Date: 22 February 2007

Please cite this article as: Berwick, L., Greenwood, P., Kagi, R., Croué, J-P., Thermal release of nitrogen organics from natural organic matter (NOM) using micro scale sealed vessel (MSSV) pyrolysis, *Organic Geochemistry* (2007), doi: 10.1016/j.orggeochem.2007.02.015

Thermal release of nitrogen organics from natural organic matter (NOM) using micro scale sealed vessel (MSSV) pyrolysis

Lyndon Berwick,[a*] Paul Greenwood,[a,b] Robert Kagi,[a] Jean-Philippe Croué [c]

[a] *CRC for Water Quality and Treatment and Centre for Applied Organic Geochemistry (CAOG), Department of Applied Chemistry, Curtin University of Technology, Perth, WA 6845, Australia*
[b] *Centre for Land Rehabilitation, University of WA, Stirling Hwy, Crawley, 6009, Australia*
[c] *Laboratoire de Chimie de l'Eau et de l'Environnement, ESA6008, ESIP, Université de Poitiers, 40, avenue du Recteur Pineau, 86022 Poitiers Cedex, France*

- Corresponding author. Tel: +61 8 9266-7534.
- *E-mail address*: L.Berwick@exchange.curtin.edu.au.

Keywords: Natural Organic Matter, dissolved organic matter, MSSV pyrolysis, analytical pyrolysis, thermal maturation, nitrogen organics, Maillard reaction

Abstract

Characterisation of recent organic matter such as aquatic natural organic matter (NOM) can be aided by the artificial maturation provided by closed system, micro scale sealed vessel (MSSV) pyrolysis. Gas chromatography-mass spectrometry (GC-MS) analysis of the products released via MSSV pyrolysis of several NOM fractions showed complex and varied product distributions that included a range of nitrogen-containing organic products (N organics) such as pyrroles, pyridines, pyrazines, indoles and carbazoles. N organics were found in highest abundance in the products from the transphilic and colloid fractions of NOM. A much larger number and higher abundance of N organics were detected with MSSV pyrolysis than with flash pyrolysis of the same samples. To better understand the sources of N organic products detected with MSSV pyrolysis of NOM, the distinctive N pyrolysate distributions from several likely precursors (i.e. peptide, amino sugar, porphyrin and a cultured bacterium) of dissolved organic nitrogen are reported. A number of qualitative distinctions between these precursors was evident, such as high abundances of C_{1-3} pyrroles from the amino sugar and C_{4-5} pyrroles from the porphyrin. The thermal profile of the N organic products from the pentaglycine and porphyrin standards was established by analysing these samples with several different MSSV

temperatures. The abundance of the N organics in most pyrolysates increased with temperature, but the relatively constant ratio of particular N organic product abundances (e.g. ethyl dimethyl pyrrole / diethyl methyl pyrrole) suggests these may be useful for source distinction across a broad range of thermal analytical conditions.

1. Introduction

Micro scale sealed vessel (MSSV) pyrolysis can complement the analytical characterisation afforded by more traditional pyrolysis techniques (e.g. flash pyrolysis). Performed in a closed system using lower temperatures (typically 250 – 350˚C) over longer time periods (e.g. days) than the high temperatures (> 500˚C) and ballistic heating rates associated with flash pyrolysis, MSSV pyrolysis can provide additional speciation information useful for establishing the structures of and source inputs to recent organic material. The method has been widely applied to study the kinetics of petroleum generation from source organics by simulating the natural thermal alteration (i.e. pseudo-maturation) of sedimentary hydrocarbons over millions of years (e.g. Horsfield et al., 1989; Schenk and Horsfield, 1993; Dieckmann et al., 1998; 2000); however, characterisation-based studies of more recent organic material (e.g. organic matter present in source waters prior to deposition in sediments) have received relatively little attention.

The recent detection of hopanes (pentacyclic triterpenoids) in the MSSV pyrolysis products of aquatic natural organic matter (NOM) represents an example of the thermal release of hydrocarbon biomarkers from functionalized and macromolecularly bound precursors (i.e. amphilic polyhydroxy hopanoids of prokaryotic cells; Greenwood et al., 2006). Thermal maturation of extant or recent organic matter (OM) may facilitate the thermal defunctionalisation of a wide variety of biochemicals. The MSSV pyrolysates of some NOM samples analysed by Greenwood et al. (2006) included higher plant biomarkers such as retene and cadalene, but the majority of the MSSV products were of lower molecular weight (MW). Unlike the clear hopane-bacterial, product-source relationship, the predominant lower MW products provide minimal diagnostic information due to largely undefined MSSV behaviour and potential derivation of compounds from multiple sources.

Further study is now being undertaken to investigate more comprehensively the sources and mechanisms of formation of several low MW products detected from the MSSV pyrolysis GC-MS analysis of NOM. Here, we focus on the generation of nitrogen-containing organic (N

organic) products, which have proved very receptive to the thermal conditions of the MSSV technique. The relatively moderate thermal conditions employed with MSSV can help reduce much of the biologically inherited structural functionality of NOM, allowing characterisation of an increased proportion of hydrocarbon products using GC-MS. Some of these products may preserve a structural link to their biological origin.

In addition to a better mechanistic understanding of the thermal processes operating during MSSV pyrolysis, this study aims to identify N organic structural moieties in NOM, which may include precursors of N-containing disinfection byproducts (N-DBPs). N-DBPs in treated waters and their potential source precursors have attracted considerable recent attention, as some (e.g. N-nitrosodimethylamine (NDMA)) may pose a greater health risk than currently regulated DBPs such as trihalomethanes and halo acetic acids (Shang et al., 2000; Najm and Trussel, 2001; Westerhoff and Mash, 2002). DBPs of potential toxicological significance continue to be a major challenge to water utilities, regulators and policy makers in the provision of safe potable water. A better understanding of the composition, structure and origin of nitrogen moieties in NOM, and their behaviour during water treatment processes, is crucial for the efficient management and improved quality of potable water resources.

Potable water supplies receive input of N-containing organic components from a number of sources. Proteins, amino sugars, porphyrins, nucleic acids and alkaloids derive from many vascular plants, and algal and microbial sources. Freshwater resources may also be significantly impacted by N compounds from agricultural chemicals (e.g. fertilizers) and wastewater inputs.

The precise structure and origin of organic N compounds in natural systems such as soils, sediments and aquatic environments remains poorly understood (Schulten et al., 1997; Schulten and Schnitzer, 1998), partly due to analytical challenges imposed by their low abundance (Westerhoff and Mash, 2002) and high structural polarity. Solid state NMR studies (^{13}C and ^{15}N) have shown most of the N incorporated during humification in soil and sedimentary organic matter (OM) to be in the amide form (Knicker et al., 1995; 1996; 1997). Chemical data and pyrolysis MS data have indicated that N heterocyclics (e.g. pyrroles, pyridines and pyrrolidines) are also significant contributors to humic substances, dissolved organic matter and recent sediments (Ikan et al., 1992; Patience et al., 1992; Schulten and Gleixner, 1999) and may also be

produced in high abundance through charring or thermal alteration of plant materials and peat (Knicker et al., 1996; Knicker and Skjemstad, 2000; Almendros et al., 2003).

This study attempts to correlate specific biological sources with N organic products produced by MSSV pyrolysis of a range of NOM samples, including those rich in N or containing particular N functional groups. The effect of MSSV pyrolysis temperature on selected samples was also investigated for insight into the formation mechanisms of N organic pyrolysis products. Flash pyrolysis was also conducted for data comparison.

2. Experimental

2.1. Samples

XAD resin fractions (hydrophobic and transphilic) and colloid fractions of NOM samples were obtained from different freshwater environments. Model compounds and nitrogen-containing standards including a peptide (pentaglycine), an amino sugar (N-acetyl-D-glucosamine), an amino acid (D-tyrosine), a sugar (D-glucose), a porphyrin (2,3,7,8,12,13,17,18-octaethyl-21*H*,23*H*-porphine) and a cultured bacterium (*Frateuria aurantia*) were also analysed to investigate the MSSV behaviour of potential N-organic precursors of NOM. A mixture of D-glucose and D-tyrosine was analysed to specifically investigate the occurrence of Maillard processes during MSSV pyrolysis. The pentaglycine and porphyrin samples were analysed at several MSSV temperatures over the range 250-350˚C to investigate the formation mechanisms and thermal integrity of the N-organic pyrolysates detected from these samples.

2.1.1. Hydrophobic and Transphilic fractions of Gartempe River NOM

Details about the collection and isolation of Gartempe River NOM can be found in Templier et al. (2005a). In brief, the raw water was successively filtered through a 10 mm Polygard CR and a 0.45 mm milligard cartridge filter and passed through a sodium exchanger unit. It was then concentrated by reverse osmosis, acidified to pH 2 and pumped through two superimposed XAD-8 and XAD-4 resins to yield hydrophobic (HPO) and transphilic (TPI) acid fractions, respectively, as termed by Croué et al. (1999). The HPO fraction accounted for 53% of the DOC and the TPI fraction for 16%.

2.1.2. Colloid fraction of St. Julien River and Brittany River NOM

Details about the collection of St. Julien River and Brittany River waters and isolation of their colloid fraction can be found elsewhere (Croué et al., 2006).

2.1.3. Cultured Frateuria aurantia

Frateuria aurantia DSM 6220^T (DSM = German National Culture Collection, Braunschweig, Germany), cultured in our previous study focused on hopanoid biomarkers in NOM (Greenwood et al., 2006), was also used here to represent a biological sample rich in protein.

2.1.4. Model compounds

Pentaglycine (peptide), N-acetyl-D-glucosamine (amino sugar), D-tyrosine (amino acid), D-glucose (sugar) and a standard porphyrin (2,3,7,8,12,13,17,18-octaethyl-21*H*, 23*H*-porphine) were commercially sourced from Sigma-Aldrich and analysed without further purification.

2.2 MSSV Pyrolysis GC-MS

A small amount of sample (<0.1 – 2 mg) was loaded into the middle of 5 cm x 5 mm i.d. glass tubes. Glass beads were used to fill the void above and below the sample. The tubes were flame sealed, with care taken to avoid direct heating of the sample, and placed in an oven for 72 h at selected isothermal temperatures. A temperature of 300˚C, previously identified as the optimal temperature for the generation of hopanes indicative of bacterial structures in NOM (Greenwood et al., 2006), was used to for all samples. Pentaglycine and 2,3,7,8,12,13,17,18-octaethyl-21*H*, 23*H*-porphine were studied at several additional temperatures over the range 250-350˚C.

The sealed vessel with matured sample was then loaded into an MSSV injector (300˚C) installed on top of a GC oven. The tube was cracked with a plunger and the volatile products released and transferred to the GC column with carrier gas. The products were initially cryo-trapped for 1 minute at the start of the column using liquid nitrogen, after which the GC-MS analysis was started.

GC-MS analysis was performed with a Hewlett Packard (HP) 5890 Series II GC interfaced to an Autospec (UltimaQ) double-focusing mass spectrometer. Due to the limited availability of this instrument, several analyses were performed with a HP 5890 GC interfaced to 5971 mass selective detector (MSD). A 30 m x 0.25 mm i.d. x 1 μm film ZB5-MS column was used with helium carrier gas (10 psi) on both instruments. Samples were run with a split of between 20 – 60 ml/min. The GC oven was initially held isothermal at 40˚C for 2 minutes, then increased at 4˚C/min to a final temperature of 310˚C and held for 15 minutes. Analysis of tyrosine, glucose and their 1:1 mixture was conducted with a relatively polar Agilent Innowax column (60 m x 0.25 mm i.d. x 0.25 μm film), necessitating a final GC oven temperature of 265˚C, which was held for 30 minutes. Full scan analyses were performed from 50-550 Da at ~ 3 scan/s. Standard mass spectral conditions were typically applied (e.g. electron energy of 70 eV, transfer line 310˚C). Other Autospec parameters included a filament current of 200 μA, a source temperature of 290˚C, an accelerating potential of 8 kV, an electron multiplier of 200 V and a mass resolution of 1000. Tentative peak identifications were based on retention times, mass spectral comparisons with library spectra (Wiley 275), and published data.

2.3 Flash pyrolysis GC-MS

Flash pyrolysis (0.5 – 1 mg sample) was performed at ~550˚C for 10 s using a Chemical Data Systems 160 pyroprobe with the pyrolysis chamber held at 250˚C. A HP 5890 Series II chromatograph coupled to a 5971 mass selective detector (MSD) was used. A 30 m x 0.25 mm i.d. x 1 μm film ZB-5MS GC column was used with He carrier gas (9 psi) in split mode. Samples were run with a split of between 20 – 50 ml/min. The GC oven temperature programme was: 40˚C isothermal (2 min), heated at 4˚C/min to 310˚C and held at 310˚C for 15 min. Full scan mass spectra were acquired (*m/z* 50 – 550, ~ 4 scan/s) with other parameters relatively standard (e.g. electron energy 70 eV, transfer line 310˚C).

3. Results and discussion

3.1 Distributions of nitrogen organics in MSSV pyrolysates from NOM fractions

MSSV pyrolysis of the NOM fractions showed complex and diverse distributions of N products. The total ion chromatograms (TICs) of the Gartempe River hydrophobic and

transphilic fractions and the Brittany River colloids are shown in Fig. 1. The major N products include alkylated pyrroles, pyridines, pyrazines, indoles and carbazoles. The parent structures of these compounds are shown in Fig. 2. The NOM fractions exhibited different N organic product profiles, reflecting variation in the nature and abundance of their contributory precursors.

The MSSV pyrolysate of the hydrophobic acid fraction of Gartempe River NOM (Fig. 1a) was dominated by C_0-C_3 alkyl phenols and showed very few N-containing pyrolysate products. In contrast, the transphilic fraction (Fig. 1b) and Brittany colloid data (Fig. 1c) showed diverse and abundant distributions of N-products. Previous analysis (Templier et al., 2005a) of the same two Gartempe fractions using Curie-point pyrolysis GC-MS and solid state ^{13}C-NMR spectroscopy also revealed a large proportion of phenolic products in the HPO fraction, consistent with lignin input. The Templier et al. (2005a) study also showed a much higher proportion of N pyrolysis products from the transphilic fraction, despite the not too dissimilar N content of each fraction (HPO - 1.9% N, TPI - 2.6% N; Templier et al., 2005b). ^{15}N-NMR analysis showed that amide was the only detectable form of N in the hydrophobic fraction (Templier et al., 2005b), which suggests that amide moieties may be relatively recalcitrant to thermal degradation and do not generate high yields of N-containing pyrolysis products.

The thermal resistance of particular amide forms in biomacromolecules and plant material has been previously established using ^{15}N-NMR and pyrolysis techniques (Derenne et al., 1993; Knicker et al., 1996). Interestingly, ^{13}C- and ^{15}N-NMR based studies have shown that most of the N incorporated during humification in soil and sedimentary OM represent amide forms derived predominantly from proteinaceous components (Knicker et al., 1996; 1997). During diagenesis, peptides and proteins normally degrade rapidly or are mineralized by microbial and/or enzymatic degradation; however, part of the N can be incorporated into a more stable fraction (Knicker and Skjemstad, 2000). For example, intact proteinaceous material has been identified in humic acids (Zang et al., 2000), sediments (Knicker et al., 1996) and fossil remains (Poinar and Stankiewicz, 1999). The preservation of proteins in humic acids has been linked to encapsulation into non-extractable phases of the humic structure (Knicker and Hatcher, 1997; Zang et al., 2000). The seemingly intractable response of these structural precursors to wet chemical and fast pyrolysis techniques may lead to under representation of this quantitatively significant component of the N content of NOM.

The larger proportion of N organic products detected from the transphilic fraction may be attributed to the more significant pyrrole, substituted pyrrole and amine precursors in this sample identified using ^{15}N-NMR analysis (Templier et al., 2005b). The ability of amino acid and peptide functional groups, major environmental N organic precursors of NOM, to form hydrogen bonds with surrounding water molecules contributes to the hydrophilic character of NOM (Westerhoff and Mash, 2002). Hence, nitrogen is concentrated in the more polar (i.e. hydrophilic or transphilic) fractions of NOM, with the hydrophobic acid fractions being relatively lean in N organic content (Croué et al., 2003).

The colloidal fraction of NOM also typically contains high organic N concentrations (Sigleo et al., 1982; Rostad et al., 1997; Croué et al., 2006). Heterocyclic nitrogen structures dominate the MSSV profile for both the transphilic and colloidal fractions. The samples can be distinguished by a higher abundance of pyridine derivatives from the transphilic fraction and a much greater proportion of alkylated pyrrole products from the colloidal fraction. The broad distribution of pyrrole products observed from MSSV pyrolysis of the Brittany (Fig. 1c) and St. Julien River colloids (Fig. 3a, 4a) may reflect a significant contribution from decomposed cellular material derived from microorganisms (e.g. N-acetyl amino sugars), which has been shown to concentrate in colloidal fractions (Rostad et al., 1997; Leenheer et al., 2001; Croué et al., 2006), rather than from soil or plant OM.

Whilst the relative proportions of N organics detected in the MSSV pyrolysates from the Gartempe fractions was consistent with previous Curie-point pyrolysis data (i.e. transphilic > hydrophobic; Templier et al., 2005a), the amount of N organic products from each fraction was much greater with the MSSV method. Likewise, our flash pyrolysis GC-MS analysis of the same fractions showed significantly fewer N organic compounds in the pyrolysate than for MSSV pyrolysis.

The N organic product distributions detected using MSSV and flash pyrolysis GC-MS of St. Julien colloids are shown in Fig. 3 with the major (tentatively identified) products listed in Table 1. The summed ion chromatograms shown in Fig. 4 were used to selectively reveal specific classes of N products, including alkyl pyrroles (*m/z* 80+94+108+109+122+123), alkyl pyridines (*m/z* 93, 106, 107, 120, 121), alkyl indoles (*m/z* 130, 144, 158), alkyl carbazoles (*m/z* 167, 180, 181, 195) and acetamide derivatives (*m/z* 125+150+167). The number and abundance of N organic (and other) products from MSSV pyrolysis was much larger than for flash

pyrolysis. In particular, the C_1-C_5 alkyl pyrroles, C_0-C_3 alkyl pyridines and C_0-C_1 pyridinamines (Fig. 4a) were detected in much higher abundance with MSSV pyrolysis (cf. Fig. 4e), indicating the release of additional structural fragments of this type with the more moderate thermal conditions of the MSSV experiment. Furthermore, a number of indole (***50-53, 57-63, 70-71, 73***; Fig. 4b) and carbazole (***65-68, 72, 74-75***; Fig. 4c) derivatives identified in MSSV pyrolysates, were either not detected or present in only minor abundances in the flash pyrolysis case (Fig. 4f and 4g, respectively).

The high N organic content of the St. Julien colloid fraction is likely to be derived from proteinaceous material and bacterial cell wall residues (Leenheer et al., 2001; Croué et al., 2006), although a broad range of precursor sources may contribute to the N organic moiety of NOM and the N compounds detected in the pyrolysates. For example, indole, 3-methyl indole and 3-ethyl indole are well known pyrolysis products of the amino acid tryptophan (Chiavari and Galletti, 1992) but have also been correlated with indole alkaloids (Bennett et al., 2004) present in plant material, algae and bacteria (Zeng et al., 1999). Carbazole and alkyl carbazoles were also reported to be significant hydropyrolysis products of these indole alkaloids present in sedimentary OM (Bennett et al., 2004). It was hypothesized that carbazoles or their precursor entities are present in the bound state and only released as solvent extractable products following hydropyrolysis (Bennett et al., 2004). The moderate MSSV thermal conditions may similarly release these structural entities from bound molecular precursors in NOM (Greenwood et al., 2006). The MSSV technique may be simulating the natural diagenetic/catagenetic transformation of recent organic N inputs into the more stable and recalcitrant forms preserved in sediments. Heterocyclic constituents such as pyrroles and indoles have been identified as being enriched in fossil algal sediments (Knicker et al., 1996).

Alkyl pyrroles have been detected in previous pyrolytic studies of amino acids such as proline, hydroxyproline, glutamine and asparigine (Chiavari and Galletti, 1992), while pyridine and alkyl pyridines may be produced by the pyrolysis of polyalanine, polyglycine (Baziuk and Douda, 2000) or chitin (Stankiewicz et al., 1996). Similar heterocyclic N organics have been reported as pyrolysis products of the 'unknown', macromolecularly-bound fraction of soil N, which is intractable to wet chemical or other spectroscopic methods (Schulten et al., 1997). C_1-C_6 alkylated pyrroles have also been identified as major constituents of the flash pyrolysate of

some kerogens and were attributed to tetrapyrrole structures in the chlorophyll of plants (Sinninghe-Damsté et al., 1992).

Some of the heterocyclic N products identified by MSSV may be formed through secondary reaction processes occurring during the off-line pyrolysis. Increased concentrations of indoles and pyrroles from thermally oxidized peat samples were observed with increasing temperature (Almendros et al., 2003). The progressive formation of heterocyclic N structures was ascribed to a relative enrichment combined with the thermal degradation of other labile structures, as well as newly synthesized structures (Almendros et al., 2003). Heterocyclic aromatics (e.g. pyrroles, indoles and carbazoles) may, for example, form via an auto-condensation reaction between liberated NH_3 and aromatic components (Knicker et al., 1996) or through Maillard reactions between reducing sugars and amino acids in the sample.

N-heterocyclics (e.g. alkyl pyrazines) are major byproducts of Maillard reactions and their recent identification in buried plant remains helped confirm the occurrence of Maillard processes during sedimentary diagenesis (Evershed et al., 1997). The colloid fraction of NOM comprises significant amounts of both carbohydrate and protein input and Maillard reactions may be promoted by the elevated temperature of the MSSV experiments. Other volatile products typical of Maillard processes such as alkyl poly-sulfides and alkyl furans were also present in the MSSV products.

In addition to volatile byproducts, the Maillard reaction also results in the formation of less volatile higher MW heteropolymeric products called melanoidins. This process has been widely proposed as a potential pathway for the formation of humic substances in the natural environment (Yamamoto and Ishiwatari, 1992). ^{15}N-NMR analysis of synthetic melanoidins showed secondary amide, pyrrolic N and pyridinic N signals (Benzing-Purdie et al., 1983). Thermal degradation products of model melanoidins formed via Maillard synthesis reactions (Coleman and Chung, 2002; Tehrani et al., 2002; Adams et al., 2003) have included a number of the lower MW N heterocyclic products (e.g. pyrroles, pyridines, pyrazines) detected in the present MSSV study of NOM. Thus, some of the dominant N heterocyclic MSSV products from the transphilic and colloidal NOM fractions may also derive from similar polymeric melanoidin structures indigenous to the source material (Ikan et al., 1992).

The flash pyrolysis data did include several N pyrolysate products not detected with MSSV pyrolysis. These include high abundances of acetamide derivatives (Fig. 4h; e.g.

acetamido furan ***44***, dihydroxy phenyl acetamides ***54, 55***, amino phenyl acetamide ***56*** and acetamido acetyl furan ***64***) and lower abundances of oxygenated N heterocycles (e.g., methyl hydantoin ***24***, pyrrole-2-carboxaldehyde ***25***, 2,5-pyrrolidinedione ***37*** and 2,5-diketopiperazine ***69***) and aromatic nitriles (e.g., benzeneacetonitrile ***38***, benzenediacetonitrile ***43*** and benzenepropanenitrile ***46***). The acetamide derivatives probably derive from N-acetyl amino sugars in bacterial peptidoglycans, which have been shown through spectral characterisation to dominate colloidal NOM fractions (Leenheer et al., 2001); 2,5-pyrrolidinedione and 2,5-diketopiperazine have been widely reported as pyrolysis products of peptides and amino acids (Chiavari and Galletti, 1992; Voorhees et al., 1994; Basiuk and Douda, 2000; Sharma et al., 2005). The variations in product abundances and distributions, likely due to the different mechanisms of product formation associated with each pyrolysis method, provide complementary information.

3.2 Diagnostic value of N organic pyrolysate products detected using MSSV pyrolysis

There are a number of potential sources of the N organic products identified using MSSV pyrolysis of the NOM fractions. For example, the alkylated pyrroles detected in high abundance from the colloid fraction could derive from any of protein, amino acid or porphyrin precursors. MSSV pyrolysates may also be produced by secondary reactions, which can occur during the thermal treatment of the closed tubes. To investigate the MSSV behaviour of potential N organic precursors of NOM, and improve the diagnostic value of MSSV pyrolysates, peptide, porphyrin, amino acid, amino sugar and cultured bacterium samples have been analysed using MSSV pyrolysis GC-MS.

3.3 MSSV pyrolysis of model compounds

Fig. 5 shows the TIC chromatograms obtained from MSSV pyrolysis of pentaglycine (peptide), N-acetyl-D-glucosamine (amino acid), 2,3,7,8,12,13,17,18-octaethyl-21*H*,23*H*-porphine (a porphyrin) and *Frateuria aurantia* (cultured bacterium). The major N products tentatively identified on the basis of their mass spectra, are given in Table 2. MSSV pyrolysis of pentaglycine (Fig. 5a) and the bacterium (Fig. 5d) afforded more abundant N product distributions.

Pentaglycine MSSV pyrolysates (Fig. 5a) were distinguished by dominant peaks for methyl pyrrolidinedione (***26***), trimethyl pyrazole (***34***) and methyl nitro-imidazole (***29***). Many other products were also identified in significant abundance, including acetamide (***3***), N-methyl acetamide (***4***), dimethyl pyrazine (***8***) and C_1-C_3 alkyl pyrroles (***6-7, 9-12, 14, 16, 21***). Conversion of amide N to heterocyclic structures like pyrroles and imidazoles has been shown via ^{15}N-NMR analysis of thermally treated biomass (Knicker et al., 1996). MSSV data from the amino sugar, N-acetyl-D-glucosamine (Fig. 5c), showed pyridine (***1***), pyrrole (***2***), methyl pyridine (**5**), dimethyl aniline (***30***) and C_1-C_4 alkyl pyrroles, with very few other N products detected. A characteristic series of *n*-aldehydes was also identified. MSSV pyrolysis of the porphyrin standard (Fig. 5b) also afforded alkyl pyrroles, with a high predominance of the C_4-C_5 alkyl moieties (***25, 27-28, 31-33***), but these were almost the only N products detected. The cultured bacterium, which represents a more complex sample matrix, exhibited a markedly different MSSV pyrolysis profile. It was characterized by dominant C_{14}, C_{16} and C_{18} long chain *n*-alkyl nitriles (***46-48***) and the occurrence of indole (***17***), methyl indole (***24***), ethyl indole (***38***), acetyl indole (***39***) and benzenepropanenitrile (***15***).

A number of the N products generated from the model compounds were also detected in the products from the NOM fractions. For example, alkylated pyrroles, abundant products of the peptide, amino sugar and porphyrin standards, were also significant products of the colloid fractions (Figs. 1c, 3a and 4a). The standards investigated do reflect varied isomeric distributions, which suggest these molecular signatures may aid source distinction in complex environmental samples such as NOM. For example, C_4 and C_5 pyrroles dominated the data for the tetrapyrrole ring structure in the porphyrin (Fig. 5b), whereas C_2 and C_3 structures dominated the alkyl pyrrole profile from both pentaglycine (Fig. 5a) and N-acetyl-D-glucosamine (Fig. 5c), with the abundance of these products much greater for the latter sample.

The detection of indole (***17***), methyl indole (***24***), ethyl indole (***38***), acetyl indole (***39***) and indolyl acetone (***41***) in the bacterium products is probably due to the presence of tryptophan-containing peptides. This is further supported by the tentative identification of N-acetyl (***45***) and methyl ester (***40***) derivatives of tryptophan. A number of indole derivatives were also identified in the MSSV pyrolysate of colloid NOM. The long chain alkyl nitriles (***46-48***) tentatively identified in the bacterium pyrolysates most likely originate from the dehydration of amides formed either as primary pyrolysis products or as secondary products by reaction of fatty acids

with NH_3 (Simoneit et al., 2003). N-alkyl nitriles have previously been identified in pyrolysates of non-hydrolysable biomacromolecules from the outer cell walls of green algae (Derenne et al., 1993). Extraction of the lipid component of the bacterial culture may facilitate an improved characterisation of the different N forms and corresponding pyrolysis products.

It should be noted that the standard organic N samples studied here represent only basic structural units of potential NOM precursors, so interpretations based on these data should be considered with caution. NOM comprises a complex macromolecular matrix containing various classes of organic compounds and the pyrolysis products identified from small sub-units may not be representative of the parent macromolecule. More comprehensive investigation is required to unequivocally establish the source of the MSSV pyrolysis products reported here. Notwithstanding, the MSSV approach holds promise for the improved characterisation of the N organic moiety of NOM.

3.4 Tyrosine/Glucose (1:1) mixture

MSSV pyrolysis GC-MS data for the amino acid and carbohydrate representatives (tyrosine and glucose) and their 1:1 mixture are shown in Fig. 6. MSSV pyrolysates from the amino acid/carbohydrate mixture (Fig. 6b) included a combination of the products observed from the individual analysis of these samples. Tyrosine (Fig. 6a) showed major phenol and alkyl phenol pyrolysates, with minor quantities of benzofuran, methyl benzofuran and methyl pyridine. A high relative abundance of alkyl phenols was confirmed via separate flash pyrolysis GC-MS of tyrosine (data not shown). MSSV pyrolysis of glucose (Fig. 6c) yielded very few products, with furan, methyl furan, ethyl furan, dimethyl furan and ethyl methyl furan comprising the majority of the TIC chromatogram. This suggests that monosaccharides like glucose fragment to low molecular weight gases when subject to MSSV treatment.

Fig. 6b shows that pyrolysis signatures of each precursor are identifiable in the MSSV chromatogram from the combination of tyrosine and glucose. Although similar molar quantities of each compound were used, the phenolic products from tyrosine dominated. Typical Maillard reaction products such as pyrazines, pyridines and oxazoles (Tehrani et al., 2002) were not detected, suggesting that these reactions are not favoured with the closed system MSSV thermal conditions (300˚C/72 h). The thermal behaviour of other starting materials must be studied to investigate this issue more robustly.

3.5 Effect of MSSV temperature on N organic distributions in MSSV pyrolysates

The porphyrin and peptide (pentaglycine) standards were each studied over a range of off-line MSSV temperatures to monitor the thermal behaviour of N organic products. The summed selected ion chromatograms (80+94+108+109+122+123 *Da*) and total ion chromatograms in Fig. 7 show the distribution of alkyl pyrroles identified at three separate off-line pyrolysis temperatures (260, 300, 340˚C). A range of different N products was observed for the peptide sample, including many of the same heterocyclic products detected in the NOM fractions, so a selected ion chromatogram was used to highlight the distribution of one particular group, the alkyl pyrroles. In contrast, the porphyrin afforded pyrroles exclusively over this retention time window, so the total ion chromatograms are shown. The two samples show quite distinctive alkyl pyrrole distributions and a list of tentatively assigned products is given in Table 3.

The abundance of the alkyl pyrroles obtained from the porphyrin standard increased with pyrolysis temperature. A very limited number of products was detected at 260˚C (Fig. 7d), suggesting that these thermal conditions are too mild to thermally release abundant alkyl pyrroles from the precursor structure. The dominant products at 260˚C were isomers of diethyl methyl pyrrole (***28***) and ethyl dimethyl pyrrole (***23***). At 300˚C (Fig. 7e) there was an increase in the abundance of all pyrrole products but the overall distribution remained similar, with the same C_4 and C_5 isomers dominating. At 340˚C (Fig. 7f), however, the abundance and range of products increased dramatically, likely due to secondary reactions, including isomeric rearrangement. There was also a noticeable increase in the abundance of the C_2 and C_3 alkyl pyrroles, although the C_4 and C_5 analogues were still most abundant.

The alkylated pyrrole distributions from pentaglycine were far more stable to temperature. Trimethyl pyrrole (***11***) was the most abundant product over the entire temperature range. There was a notable increase in the abundance of ethyl methyl pyrrole (***10***) and a slight increase in the abundance of the higher MW products (***18, 20, 21, 28***) from 260˚C (Fig. 7a) to 300˚C (Fig. 7b). However, little change in isomeric distribution of alkylated pyrroles was evident at the highest temperature of 340˚C (Fig. 7c). Preservation of the isomeric integrity of the pyrrole distribution points to a selective rearrangement of the peptide precursor to specific alkylated pyrroles. Although there was an increased abundance of all pyrrole products at higher

temperatures, thermal parameters do not greatly influence their isomeric configuration. The thermal profiles of several pentaglycine products do differ. A number of amide compounds (e.g. acetamide, methyl acetamide, propanamide and methyl propanamide) and 2,5-diketopiperazine, an oxygenated N-heterocyclic product diagnostic of peptides (Chiavari and Galletti, 1992; Poinar and Stankiewicz, 1999; Douda and Basiuk, 2000), were all detected at 260˚C, but not 340˚C, indicating thermal degradation or molecular rearrangement at the higher temperature.

Although product distributions showed some variation with thermal conditions, many consistent qualitative features allowed distinction of the two precursors irrespective of temperature. For example, at all MSSV temperatures, 2,5-dimethyl pyrrole (***4***) and ethyl pyrrole (***8***) were the dominant C_2 pyrrole isomers from pentaglycine and the porphyrin, respectively. Specific C_4 (ethyl methyl pyrrole ***18***) and C_5 (ethyl dimethyl pyrrole ***28***) isomers were detected in the products from both precursors, but their ratio (***18***/***28***) distinguished the two samples at all temperatures (pentaglycine >1; porphyrin <1). Interestingly, trimethyl pyrroles (***11, 14***) and tetramethyl pyrrole (**21**) were consistently dominant products of pentaglycine at all three pyrolysis temperatures. Despite the extensive molecular rearrangement reflected in the porphyrin products at higher temperature, none of these methylated C_3 and C_4 pyrroles were detected in any of the porphyrin experiments.

4. Conclusions

MSSV pyrolysis GC-MS analysis has proved effective at releasing polar N functionalities in immature biomacromolecules present in aquatic NOM. The relatively moderate temperatures afforded a much higher proportion of GC-MS detectable, N organic pyrolysates from several NOM fractions than flash pyrolysis. A broad distribution of alkylated pyrroles, pyridines, pyrazines, indoles and carbazoles were detected in the products from the transphilic and colloid fractions, but few N organic products were detected for the HPO fraction studied.

Corresponding MSSV analysis of a suite of N-containing standards afforded many of the same products as for the NOM samples. Qualitative and quantitative differences between MSSV data from the standards were observed and will be useful for distinguishing their precursory contribution in environmental samples such as NOM. For example, high abundances of pyrrole

products from the transphilic and colloidal fractions, as well as the peptide and amino sugar standards, suggests significant peptide or amino sugar input to the NOM of the water used.

Several heterocyclic N products from the colloid and transphilic NOM fractions could be formed by Maillard reactions occurring naturally in freshwater aquatic environments. No evidence to suggest these products may be artifacts of the MSSV pyrolysis process was evident from the analysis of a representative mixture of an amino acid (D-tyrosine) and a carbohydrate (D-glucose).

Further analyses of potential N organic precursors need to be conducted to provide more reliable and definitive precursor distinction. More research is also needed to accurately distinguish the primary structural or thermally induced origins of many of the N organic products. N organic distributions from the separate analysis of the peptide (pentaglycine) and porphyrin standards at different MSSV temperatures were consistently different from each other, allowing clear distinction and demonstrating the utility of the MSSV approach for the highly sensitive characterization of N organics in recent organic samples over a broad range of thermal conditions.

Acknowledgements

Thanks go to Mr. Geoff Chidlow for assistance with GC-MS maintenance and analyses and Associate Professor Kliti Grice for provision of the porphyrin sample. The scholarship support for L.B. provided by Curtin University of Technology and the CRC for Water Quality and Treatment is gratefully acknowledged. Thanks also go to T. Ruble and J. van Leeuwin for constructive suggestions.

Guest Associate Editor – **Linda Stalker**

References

Adams, A., Tehrani, K.A., Kersiene, M., Venskutonis, R., De Kimpe, N., 2003. Characterisation of model melanoidins by the thermal degradation profile. Journal of Agricultural and Food Chemistry 51, 4338-4343.

Almendros, G., Knicker, H., Gonzalez-Vila, F.J., 2003. Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ^{13}C and ^{15}N-NMR spectroscopy. Organic Geochemistry 34, 1559-1568.

Basiuk, V.A., Douda, J., 2000. Pyrolysis of poly-glycine and poly-L-alanine: Analysis of less volatile products by gas chromatography/Fourier transform infrared spectroscopy/mass spectrometry. Journal of Analytical and Applied Pyrolysis 55, 235-246.

Bennett, B., Lager, A., Russell, C.A., Love, G.D., Larter, S.R., 2004. Hydropyrolysis of algae, bacteria, archaea and lake sediments: Insights into the origin of nitrogen compounds in petroleum. Organic Geochemistry 35, 1427-1439.

Benzing-Purdie, L.M., Ripmeester, J.A., Preston, C.M., 1983. Elucidation of the nitrogen forms in melanoidins and humic acids by nitrogen-15 cross-polarization-magic angle spinning nuclear magnetic resonance. Journal of Agricultural and Food Chemistry 31, 913-915.

Chiavari, G., Galletti, G.C., 1992. Pyrolysis-gas chromatography/mass spectrometry of amino acids. Journal of Analytical and Applied Pyrolysis 24, 123-137.

Coleman, W.M., Chung, H.L., 2002. Pyrolysis GC-MS analysis of Amadori compounds derived from selected amino acids with glucose and rhamnose. Journal of Analytical and Applied Pyrolysis 63, 349-366.

Croué, J-P., Benedetti, M.F., Viollaeu, D., Leenheer, J.A., 2003. Characterisation and copper binding of humic and nonhumic organic matter isolated from the South Platte River: Evidence for the presence of nitrogenous binding site. Environmental Science and Technology 37, 328-336.

Croué, J-P., Debroux, J.F., Aiken, G.R., Leenheer, J.A., Amy, G.L, 1999. Natural organic matter: Structural characteristics and reactive properties. In: Singer, P.C. (Ed.), Formation and Control of Disinfection By-products in Drinking Water. American Water Works Association.

Croué, J-P., Gallard, H., Ambonguilat, S., Greenwood, P., Berwick, L., Boyd, L., Grice, K., 2006. Characterisation of colloids isolated from surface waters. Proceedings of Combined Australian Organic Geochemists/Natural Organic Matter Users Conference, Perth, Western Australia, 12-15th February 2006, pp. 25-26.

Derenne, S., Largeau, C., Taulelle, F., 1993. Occurrence of non-hydrolysable amides in the macromolecular constituent of *Scendesmus quadricauda* cell wall as revealed by ^{15}N-NMR: Origin of n-alkylnitriles in pyrolysates of ultralaminae-containing kerogens. Geochimica et Cosmochimica Acta 57, 851-857.

Dieckmann, V., Schenk, H.J., Horsfield, B., Welte, D.H., 1998. Kinetics of petroleum generation and cracking by programmed-temperature closed-system pyrolysis of Toarcian Shales. Fuel 77, 23-31.

Dieckmann, V., Schenk, H.J., Horsfield, B., 2000. Assessing the overlap of primary and secondary reactions by closed- versus open-system pyrolysis of marine kerogens. Journal of Analytical and Applied Pyrolysis 56, 33-46.

Douda, J., Basiuk, V.A., 2000. Pyrolysis of amino acids: Recovery of starting materials and yields of condensation products. Journal of Analytical and Applied Pyrolysis 56, 113-121.

Evershed, R.P., Bland, H.A., van Bergen, P.F., Carter, J.F., Horton, M.C., Rowley-Conway, P.A., 1997. Volatile compounds in archaeological plant remains and the Maillard reaction during decay of organic matter. Science 278, 432-433.

Greenwood, P.F., Leenheer, J.A., McIntyre, C., Berwick, L., Franzmann, P., 2006. Bacterial biomarkers thermally released from dissolved organic matter. Organic Geochemistry 37, 597-609.

Horsfield, B., Disko, U., Leistner, F., 1989. The micro-scale simulation of maturation: Outline of a new technique and its potential applications. Geologische Rundschau 78, 361-374.

Ikan, R., Ioselis, P., Rubinsztain, Y., Aizenshtat, Z., Miloslavsky, I., Yariv, S., Pugmire, R., Anderson, L.L., Woolfenden, W.R., Kaplan, I.R., Dorsey, T., Peters, K.E., Boon, J.J., de Leeuw, J.W., Ishiwatari, R., Morinaga, S., Yamamoto, S., Macihara, T., Muller-Vonmoos, M., Rub, A., 1992. Chemical, isotopic, spectroscopic and geochemical aspects of natural and synthetic humic substances. The Science of the Total Environment 117/118, 1-12.

Knicker, H., Almendros, G., Gonzalez-Vila, F.J., Ludemann, H.D., Martin, F., 1995. ^{13}C and ^{15}N-NMR analysis of some fungal melanins in comparison with soil organic matter. Organic Geochemistry 11/12, 1023-1028.

Knicker, H., Almendros, G., Gonzalez-Vila, F.J., Martin, F., Ludemann, H-D., 1996. ^{13}C and ^{15}N-NMR spectroscopic examination of the transformation of organic nitrogen in plant biomass during thermal treatment. Soil Biology and Biochemistry 28, 1053-1060.

Knicker, H., Hatcher, P.G., 1997. Survival of protein in an organic-rich sediment – possible protection by encapsulation in organic matter. Naturwissenschaften 84, 231-234.

Knicker, H., Scaroni, A.W., Hatcher, P.G., 1997. ^{13}C and ^{15}N-NMR spectroscopic investigation on the formation of fossil algal residues. Organic Geochemistry 24, 661-669.

Knicker, H., Skjemstad, J.O., 2000. Nature of organic carbon and nitrogen in physically protected organic matter of some Australian soils as revealed by solid-state ^{13}C and ^{15}N-NMR spectroscopy. Australian Journal of Soil Research 38, 113-127.

Leenheer, J.A., Rostad, C.E., Barber, L.B., Schroeder, R.A., Anders, R., Davisson, M.L., 2001. Nature and chlorine reactivity of organic constituents from reclaimed water in groundwater, Los Angeles County, California. Environmental Science and Technology 35, 3869-3876.

Najm, I., Trussel, R.R., 2001. NDMA formation in water and wastewater. Journal of American Water Works Association 93, 92-99.

Patience, R.L., Baxby, M., Bartle, K.D., Perry, D.L., Rees, A.G.W., Rowland, S.J., 1992. The functionality of organic nitrogen in some recent sediments from the Peru upwelling region. Organic Geochemistry 18, 161-169.

Poinar, H.N., Stankiewicz, B.A., 1999. Protein preservation and DNA retrieval from ancient tissues. Proceedings of the National Academy of Sciences of the United States of America 96, 8426-8431.

Rostad, C.E., Leenheer, J.A., Daniel, S.R., 1997. Organic carbon and nitrogen content associated with colloids and suspended particulates from the Mississippi River and some of its tributaries. Environmental Science and Technology 31, 3218-3225.

Schenk, H.J., Horsfield, B., 1993. Kinetics of petroleum generation by programmed-temperature closed- versus open-system pyrolysis. Geochimica et Cosmochimica Acta 57, 623-630.

Schulten, H-R., Sorge-Lewin, C., Schnitzer, M., 1997. Structure of 'unknown' soil nitrogen investigated by analytical pyrolysis. Biology and Fertility of Soils 24, 249-254.

Schulten, H.-R., Schnitzer, M., 1998. The chemistry of soil organic nitrogen: A review. Biology and Fertility of Soils 26, 1-15.

Schulten, H.-R., Gleixner, G., 1999. Analytical pyrolysis of dissolved organic matter in aquatic systems: Structure and origin. Water Research 33, 2489-2498.

Shang, C., Gong, W.L., Blatchley, E.R., 2000. Breakpoint chemistry and volatile by-product formation resulting from chlorination of model organic-N-compounds. Environmental Science and Technology 34, 1721-1728.

Sharma, R.K., Chan, W.G., Hajaligol, M.R., 2005. Product compositions from pyrolysis of some aliphatic α-amino acids. Journal of Analytical and Applied Pyrolysis 75, 69-81.

Sigleo, A.C., Hoering, T.C., Helz, G.R., 1982. Composition of estuarine colloidal material: Organic components. Geochimica et Cosmochimica Acta 46, 1619-1626.

Simoneit, B.R.T., Rushdi, A.I., Bin Abas, M.R., Didyk, B.M., 2003. Alkyl amides and nitriles as novel tracers for biomass burning. Environmental Science and Technology, 37, 16-21.

Sinninghe-Damsté, J.S., Eglinton, T.I., de Leeuw, J.W., 1992. Alkylpyrroles in a kerogen pyrolysate: Evidence for abundant tetra-pyrrole pigments. Geochimica et Cosmochimica Acta 56, 1743-1751.

Stankiewicz, B.A., van Bergen, P.F., Duncan, I.J., Carter, J.F., Briggs, D.E.G., Evershed, R.P., 1996. Recognition of chitin and proteins in invertebrate cuticles using analytical pyrolysis/gas chromatography and pyrolysis/gas chromatography/mass spectrometry. Rapid Communications in Mass Spectrometry 10, 1747-1757.

Tehrani, K.A., Kersiene, M., Adams, A., Nenskutonis, R., De Kimpe, N., 2002. Thermal degradation studies of glucose/glycine melanoidins. Journal of Agricultural and Food Chemistry 50, 4062-4068.

Templier, J., Derenne, S., Croué, J.-P., Largeau, C., 2005a. Comparative study of two fractions of riverine dissolved organic matter using various analytical pyrolytic methods and a ^{13}C CP/MAS NMR approach. Organic Geochemistry 36, 1418-1442.

Templier, J., Derenne, S., Mercier, F., Barre, N., Miserque, F., Largeau, C., 2005b. Nitrogen functionality in various fractions of riverine dissolved organic matter. Abstract. International Meeting of Organic Geochemistry, Seville, September 2005.

Voorhees, K.J., Zhang, W., Hendricker, A.D., Murugaverl, B., 1994. An investigation of the pyrolysis of oligopeptides by Curie-point pyrolysis-tandem mass spectrometry. Journal of Analytical and Applied Pyrolysis 30, 1-16.

Westerhoff, P., Mash, M., 2002. Dissolved organic nitrogen in drinking water supplies: A review. Journal of Water Supply: Research and Technology-AQUA 51, 415-448.

Yamamoto, S., Ishiwatari, R., 1992. A study of the formation mechanism of sedimentary humic substances.III. Evidence for the protein-based melanoidin model. The Science of the Total Environment 117/118, 279-292.

Zang, X., van Heemst, J.D.H., Dria, K.J., Hatcher, P.G., 2000. Encapsulation of protein in humic acid from a histosol as an explanation for the occurrence of organic nitrogen in soil and sediment. Organic Geochemistry 31, 679-695.

Zeng, L., Jingyu, S., Yongli, Z., Xiong, F., Tangsheng, P., Ye, Z., Yanhui, M., Yingzhou, C., Xiaohua, X., Yaohua, Z., Guiyangsheng, W., 1999. Search for new compounds and biologically active substances from Chinese marine organisms. Pure and Applied Chemistry 71, 1147-1151.

List of Figures

List of Tables

Table 2
N organic products tentatively identified from MSSV pyrolysis of a peptide, an amino sugar, a porphyrin and a cultured bacterium.

Table 3
Alkyl pyrrole products tentatively identified from MSSV pyrolysis of pentaglycine and porphyrin at three different temperatures.

Fig 1

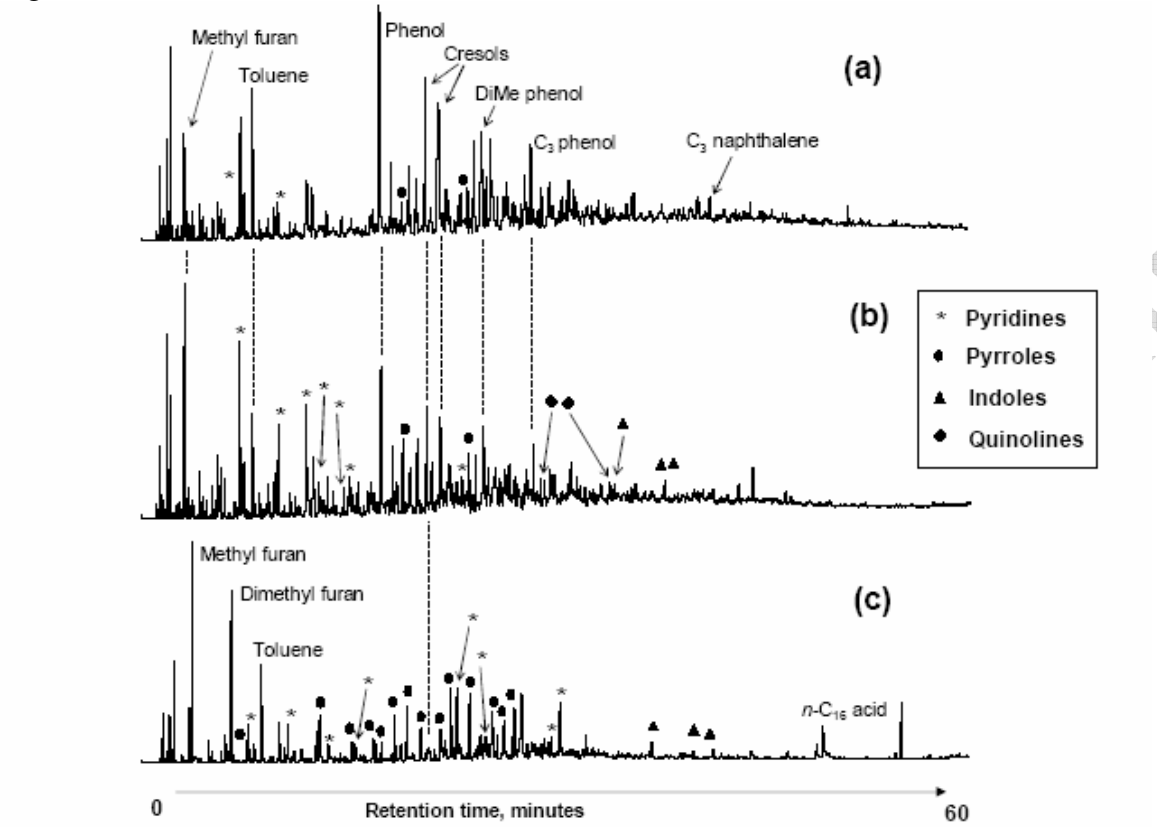
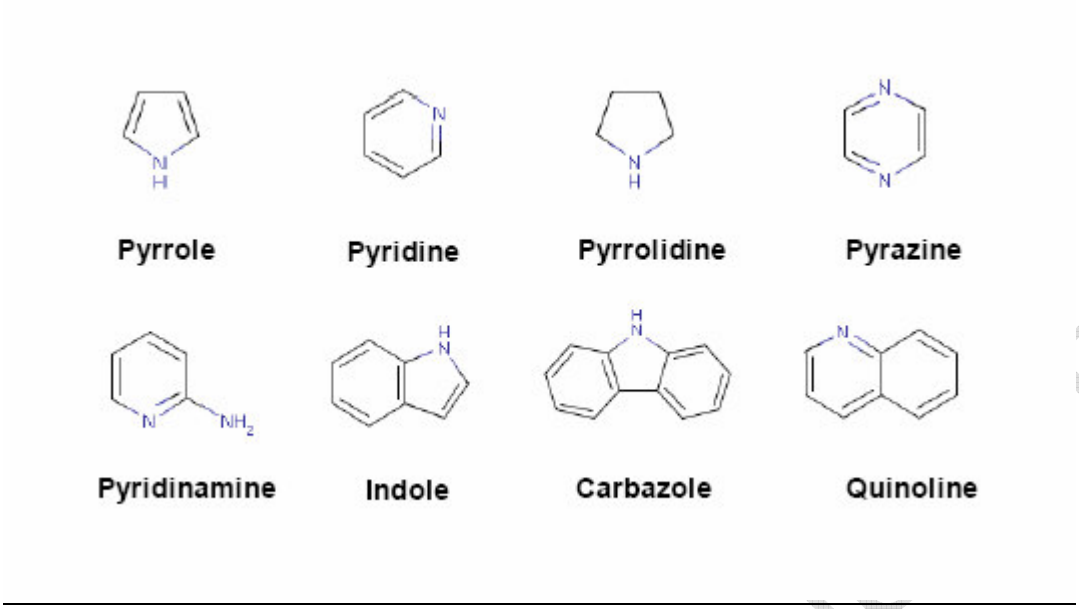


Fig 2



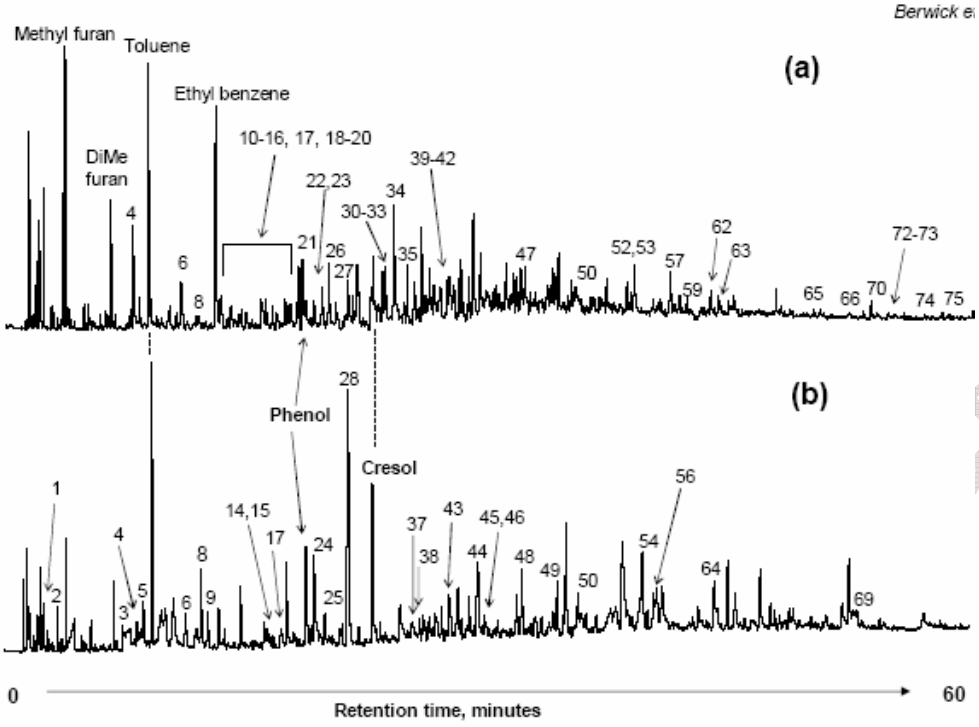


Fig 3

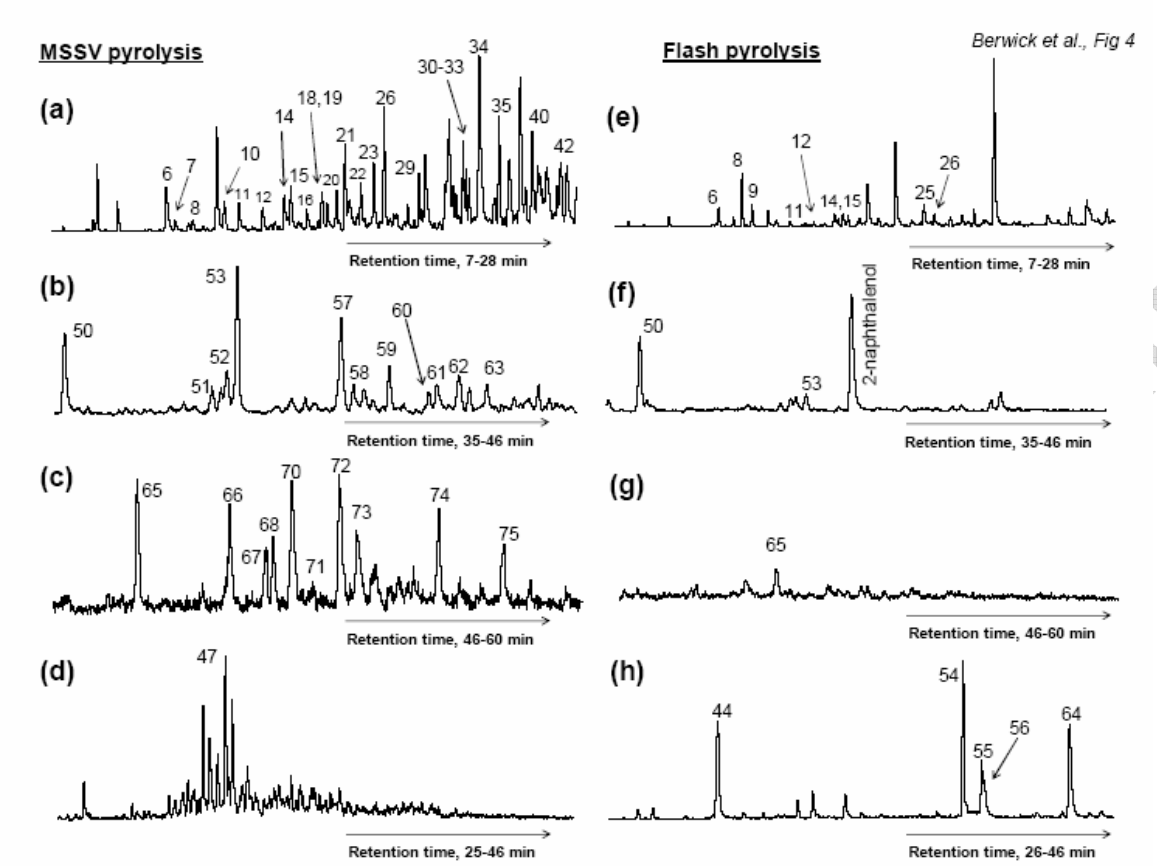


Fig 4

Fig 5

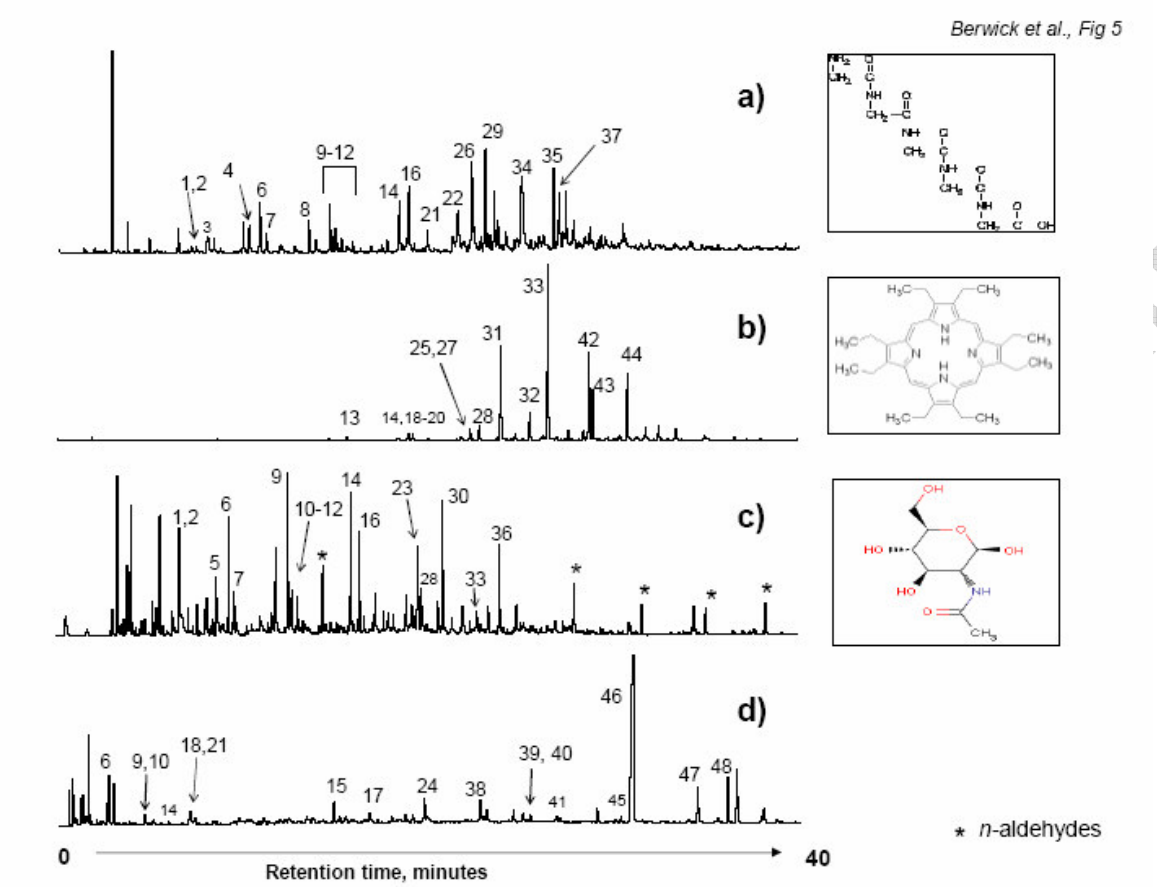


Fig 6

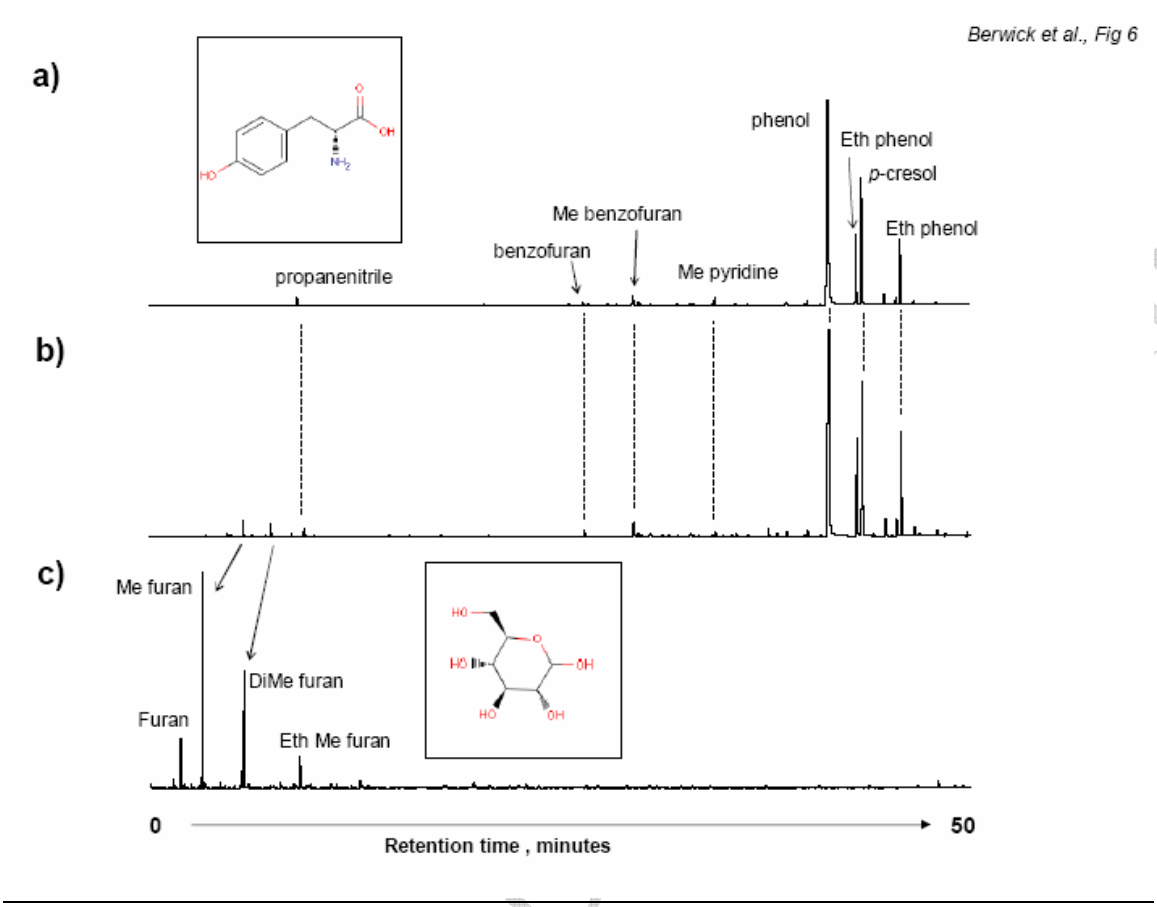
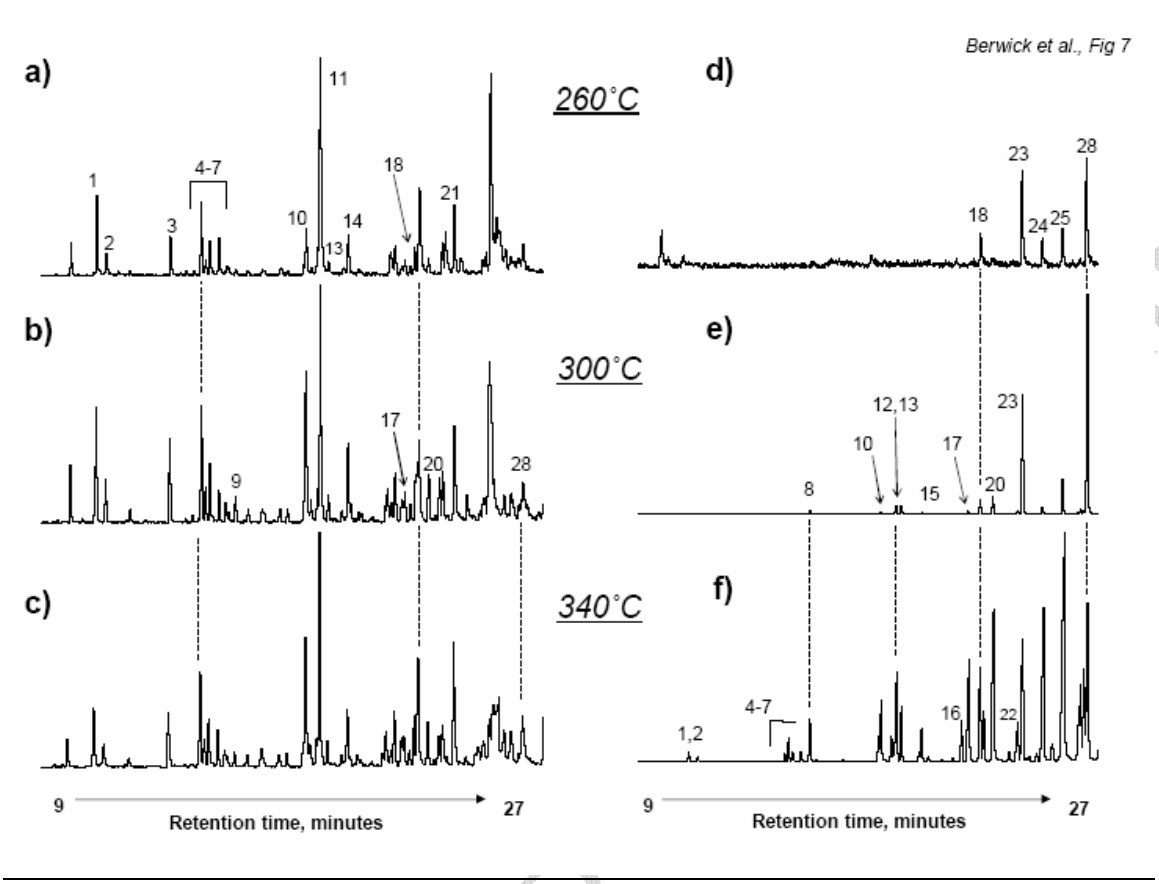


Fig 7



Berwick et al., 2006, Table 1.

Peak #	Tentative identification	MSSV pyrolysis 300˚C/72hr	Flash pyrolysis 550˚C/10 sec
1	2-propenenitrile	–	*
2	propanenitrile	–	*
3	acetamide	–	**
4	pyridine	***	*
5	pyrrole	–	*
6	methyl pyridine	**	*
7	methyl pyrazine	*	–
8	methyl pyrrole	*	**
9	methyl pyrrole	–	*
10	dimethyl pyrrole	*	–
11	dimethyl pyridine	*	*
12	ethyl pyridine	*	*
13	ethyl pyrazine	*	–
14	dimethyl pyrrole	*	*
15	dimethyl pyridine	*	*
16	dimethyl pyridine	*	
17	N-acetyl pyrrole	–	*
18	trimethyl pyrrole + ethyl pyridine	*	–
19	ethyl methyl pyridine	*	–
20	trimethyl pyrrole	*	–
21	aniline	**	–
22	trimethyl pyrrole	*	–
23	trimethyl pyridine + ethyl methyl pyrrole	*	–
24	methyl-2,4-imidazolidinedione	–	**
25	1-pyrrole-2-carboxaldehyde	–	*
26	trimethyl pyrrole	**	*
27	methyl pyrrolidinone	*	–
28	unknown (*m/z* 128, 113, 87)	–	***
29	ethyl dimethyl pyrrole	*	–
30	ethyl dimethyl pyrrole	*	–
31	tetramethyl pyrrole	*	–
32	dimethyl ethyl pyrrole + n-alkane	*	–
33	ethyl dimethyl pyrrole	*	–
34	3-pyridinamine	***	–
35	tetramethyl pyrrole	**	–
36	C5 pyrrole	*	–
37	2,5-pyrrolidinedione	–	*
38	benzeneacetonitrile	–	*
39	ethyl trimethyl pyrrole	*	–
40	methyl pyridinamine	*	–
41	ethyl trimethyl pyrrole	*	–
42	C5 pyrrole	*	–
43	benzene diacetonitrile	–	*
44	acetamido furan	–	**
45	acetyl formyl pyrrole	–	*
46	benzene propanenitrile	–	*
47	1-piperidino-1-cyclopentene?	*	–

48	indole	–	*
49	unknown acetamide derivative	–	*
50	methyl indole	*	*
51	ethyl indole	*	–
52	dimethyl indole	*	–
53	dimethyl indole	**	*
54	dihydroxy phenyl acetamide	–	**
55	dihydroxy phenyl acetamide	–	*
56	amino phenyl acetamide	–	*
57	acetyl indole	**	–
58	trimethyl indole	*	–
59	trimethyl indole	*	–
60	trimethyl indole	*	–
61	methyl acetyl indole	*	–
62	indole derivative + n-alkane	*	–
63	unknown indole derivative	*	–
64	acetamido acetyl furan	–	*
65	carbazole	*	–
66	methyl carbazole	*	–
67	methyl carbazole	*	–
68	methyl carbazole	*	–
69	2,5-diketopiperazine	–	*
70	1-methyl-9H-pyrido-[3,4-β]-indole (harman)	*	–
71	9H-pyrido-[3,4-β]-indole (norharman)	*	–
72	methyl carbazole	*	–
73	1-ethyl-9H-pyrido-[3,4-β]-indole?	*	–
74	C2 carbazole	*	–
75	C2 carbazole	*	–

*, **, *** provide an indication of relative abundance

Berwick et al., 2006, Table 2

Peak #	Tentative Identification	Pentaglycine	N-acetyl-D-glucosamine	Porphyrin	F.*aurantia*
1	pyridine	*	*	–	*
2	pyrrole	*	*	–	*
3	acetamide	*	–	–	–
4	N-methyl acetamide	*	–	–	–
5	methyl pyridine	–	*	–	–
6	methyl pyrrole	*	*	*	*
7	methyl pyrrole	*	*	–	–
8	dimethyl pyrazine	*	–	–	–
9	dimethyl pyrrole	*	*	*	*
10	ethyl pyrrole	*	*	*	*
11	dimethyl pyrrole	*	*	*	–
12	dimethyl pyrrole	*	*	*	–
13	ethyl pyrrole	–	–	*	–
14	ethyl methyl pyrrole	*	*	*	*
15	benzenepropanenitrile	–	–	–	*
16	trimethyl pyrrole	*	*	–	–
17	indole	–	–	–	*
18	ethyl methyl pyrrole	–	–	*	*
19	ethyl methyl pyrrole	–	–	*	–
20	ethyl methyl pyrrole	–	–	*	–
21	trimethyl pyrrole	*	–	–	*
22	dimethyl imidazole + ethyl dimethyl pyrrole	*	–	–	–
23	C_4 pyrrole + *n*-C_9 aldehyde	–	*	–	–
24	methyl indole	–	–	–	*
25	ethyl dimethyl pyrrole	–	–	*	–
26	1-methyl-2,5-pyrrolidinedione	*	–	–	–
27	ethyl dimethyl pyrrole	–	–	*	–
28	ethyl dimethyl pyrrole	*	*	*	–
29	methyl nitroimidazole	*	–	–	–
30	dimethyl aniline + tetramethyl pyrrole	–	*	–	–
31	ethyl dimethyl pyrrole	–	–	*	–
32	diethyl methyl pyrrole	–	–	*	–
33	ethyl trimethyl pyrrole	–	*	*	–
34	trimethyl pyrazole	*	–	–	–
35	1-piperidinecarboxaldehyde	*	–	–	–
36	C_4 pyridine + *n*-C_{10} aldehyde	–	*	–	–
37	ethyl dimethyl pyrrolidinedione	*	–	–	–
38	ethyl indole	–	–	–	*
39	acetyl indole	–	–	–	*
40	tryptophan methyl ester	–	–	–	*
41	indolyl acetone	–	–	–	*
42	Unknown	–	–	*	–
43	Unknown	–	–	*	–
44	Unknown	–	–	*	–
45	N-acetyl-tryptophan	–	–	–	*
46	tetradecanenitrile	–	–	–	*

47	hexadecanenitrile	–	–	–	*
48	octadecanenitrile	–	–	–	*

* detected
– not detected

Berwick et al., 2006, Table 3

Peak #	Tentative identification	Pentaglycine 260˚C	Pentaglycine 300˚C	Pentaglycine 340˚C	Porphyrin 260˚C	Porphyrin 300˚C	Porphyrin 340˚C
1	methyl pyrrole	*	*	*	–	–	*
2	methyl pyrrole	*	*	*	–	–	*
3	dimethyl pyrazine	*	*	*	–	–	–
4	dimethyl pyrrole	*	*	*	–	*	*
5	ethyl pyrrole	*	*	*	–	*	*
6	dimethyl pyrrole	*	*	*	–	*	*
7	dimethyl pyrrole	*	*	*	–	*	*
8	ethyl pyrrole	–	–	–	–	*	*
9	dimethyl pyrrole	–	*	*	–	–	*
10	ethyl methyl pyrrole	*	*	*	–	*	*
11	trimethyl pyrrole	*	*	*	–	–	–
12	ethyl methyl pyrrole	–	–	–	–	*	*
13	ethyl methyl pyrrole	*	*	*	–	*	*
14	trimethyl pyrrole	*	*	*	–	–	–
15	ethyl methyl pyrrole	–	–	–	–	*	*
16	dimethyl ethyl pyrrole	–	–	–	–	–	*
17	dimethyl ethyl pyrrole	–	*	*	–	*	*
18	ethyl dimethyl pyrrole	*	*	*	*	*	*
19	ethyl dimethyl pyrrole	–	–	–	–	–	*
20	ethyl dimethyl pyrrole	–	*	*	–	*	*
21	tetramethyl pyrrole	*	*	*	–	–	–
22	dimethyl ethyl pyrrole	–	–	–	–	–	*
23	ethyl dimethyl pyrrole	–	–	–	*	*	*
24	C5 pyrrole	–	–	–	*	*	*
25	C5 pyrrole	–	–	–	*	*	*
26	C5 pyrrole	–	–	–	–	–	*
27	C5 pyrrole	–	–	–	–	–	*
28	C5 pyrrole	–	*	*	*	*	*