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Research Paper

An examination by GC×GC-TOFMS of organic molecules present in highly degraded oils emerging from Caribbean terrestrial seeps of Cretaceous age

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ABSTRACT

For our ancestors, oil seeps were both a fascination and a resource but as the planet's reserves of high quality low density oil becomes increasingly depleted, so there is now a renewed interest in heavier, biodegraded oils such as those encountered in terrestrial seeps. One such seep is Pitch Lake in the Caribbean island of Trinidad, which is the largest natural deposit of asphalt in the world. At the northern end of the Caribbean, oil emerges along a tectonic contact on the island of Cuba. The sources of the oils from these seeps are relatively recent and both are subject to intense weathering due to the tropical conditions. When analysed by gas chromatography (GC) both oils appear as unresolved complex mixtures (UCM) and show a very high degree of biodegradation thus presenting an analytical challenge. In this case study, these two Caribbean seep oils were analysed by comprehensive two dimensional GC with time of flight mass spectrometry (GC×GC-TOFMS) to expose many thousands of the individual compounds that comprise the UCM. The high chromatographic resolution of the GC×GC-TOFMS produced good quality mass spectra allowing many compounds including molecular fossil 'biomarkers' to be identified. Compound classes included diamondoid hydrocarbons, demethylated hopanes and *seco*-hopanes, mono- and tri-aromatic steroids. D-ring aromatised structures of the 8,14-*seco*-hopanes, including demethylated forms were present in both oils but further demethylation, probably at position C-25 during biodegradation, was only observed in the Pitch Lake oil. Many polycyclic aromatic hydrocarbons (PAHs) were absent although the fungal-derived pentacyclic PAH perylene was present in both oils. The presence of the angiosperm biomarker lupane in the Pitch Lake oil constrained the age to the Late Cretaceous. The higher degree of biodegradation observed in the Cuban oil was likely due to relatively slow anaerobic processes whereas oil within Pitch Lake was probably subject to additional more rapid aerobic metabolism within the lake.

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1. Introduction

Since humankind first walked the Earth, oil oozing from the ground has fascinated us. For many thousands of years individuals and civilisations have made use of the oil from seeps, e.g. caulking the hulls of boats, without understanding what it was. Our understanding of the nature of oil has only really developed in the

last few decades, since gas chromatography (GC) and, later, mass spectrometry (MS) has allowed us to interpret the chemical structures of the tens of thousands of compounds present in oil (Sutton et al., 2005). However, oils from seeps can be particularly analytically challenging as they have likely experienced considerable biodegradation, either from extensive periods at temperatures below 80 °C and contact with the water interface at the margin of the basin or at the Earth's surface (Head et al., 2003). When the seeps are emerging into the hot tropical sun, evaporative processes further reduce the number of peaks resolved by GC-MS and severely limit our ability to identify structures.

The vast oil seep known as Pitch Lake, situated on the island of Trinidad, at the southern end of the Lesser Antilles, has become a

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tourist destination and, like our early ancestors, we come to gaze at the bitumen emerging from the earth (Fig. 1). Pitch Lake is the largest natural deposit of asphalt in the world covering 40 ha at La Brea in southwest Trinidad (Chaitan and Graterol, 1991). Scientific interest in Pitch Lake has a long history. It was first reported by Europeans when Sir Walter Raleigh was shown the lake by local natives and apparently used the tar to caulk his ships in March 1595 (Chaitan and Graterol, 1991). It was once considered that Pitch Lake lies within a depression of a former volcanic crater but a more recent explanation is that it lies above the intersection of two major faults (Chaitan and Graterol, 1991). The oil rises to the surface whereupon it loses most of its volatiles from exposure to the tropical heat and gradually turns into the asphalt we observe today. With exposure to the surface, this asphalt is also subject to microbial degradation, thus adding to its structural complexity. But it is not just recent biodegradation that the Pitch Lake oil has been subjected to: Meckenstock et al. (2014) recently reported that the oil at Pitch Lake was biodegraded by a diverse community of microbes within minuscule water droplets (1–3 μm). Archaea, mainly methanogens within the Methanosarcinales and Methanomicrobiales, were present in the majority of the analysed droplets indicating that acetotrophic and hydrogenotrophic methanogenesis was involved in the biodegradation of the Pitch Lake oil (Meckenstock et al., 2014). Halotolerant or halophilic Halobacteriales were also present in all water droplets that contained archaea, suggesting high salinity of the formation waters (Meckenstock et al., 2014). Indeed, Pitch Lake has been used as an analogue for what conditions might be like on Saturn's largest moon Titan, with the potential for extra-terrestrial life (Schulze-Makuch et al., 2011).

At the opposite end of the chain of Caribbean islands in the Greater Antilles lies Cuba (Fig. 1). Oil seeps were discovered in the mountains of Cuba hundreds of years ago (Lewis, 1932). Rather than emerging from sedimentary limestones and sandstones typically associated with oil, here they appeared to be dripping from igneous

rocks (see insert photograph, Fig. 1). As far back as the 1930s, the potential for large reserves of oil locked in rock along the north-central Cuban coast was reported with an estimated >200 million barrels of "asphalt grade" oil (Lewis, 1932). A much more recent estimate by the U.S. Geological Survey (2005) suggested there was 4.6 billion barrels of undiscovered oil plus nearly 10 trillion cubic feet of undiscovered natural gas in the North Cuba basin. Deposition of Cuban oils occurred in the Late Jurassic and Early Cretaceous and can vary from light to highly biodegraded (Magnier et al., 2004).

Although the formation process of oil is measured in millions of years, its degradation is much more rapid with many compounds metabolised within years or months under anaerobic conditions (e.g. Aitken et al., 2018 and references therein) and on the timescale of days and hours under aerobic conditions (e.g. Aeppli et al., 2014). Depending on the extent of biodegradation and evaporative processes the seep oils experience, they can be difficult to analyse using traditional GC-MS methods (reviewed by Eiserbeck et al., 2012). The underlying unresolved complex mixtures, or 'humps', of hydrocarbons and heterocyclic compounds observed in the GC-MS chromatograms (Fig. 2) result from the co-elution of numerous relatively small peaks (Gough and Rowland, 1990). Obtaining sufficiently resolved peaks to obtain good mass spectra is therefore challenging. Two dimensional gas chromatography with time-of-flight mass spectrometry (GC \times GC-TOFMS) has proven to be a powerful tool for the analysis of extreme UCM-dominated samples (e.g. Frysinger et al., 2003; Arey et al., 2007; Booth et al., 2007; Ventura et al., 2008; Rowland et al., 2011a; Eiserbeck et al., 2012; Nelson et al., 2016). This improved separation permits the generation of cleaner mass spectra facilitating identification of chemical structures including 'biomarkers'. Biomarkers can be considered chemical fossils as their signatures yield information about the presence and relative abundance of specific classes of organisms that can help us gain insights into past environments (reviewed by Brocks and Grice, 2011; Grice and Brocks, 2011). Such



Figure 1. Locations of Pitch Lake, Trinidad, and oil seeps in western Cuba with photographs of sample collection sites (inserts).

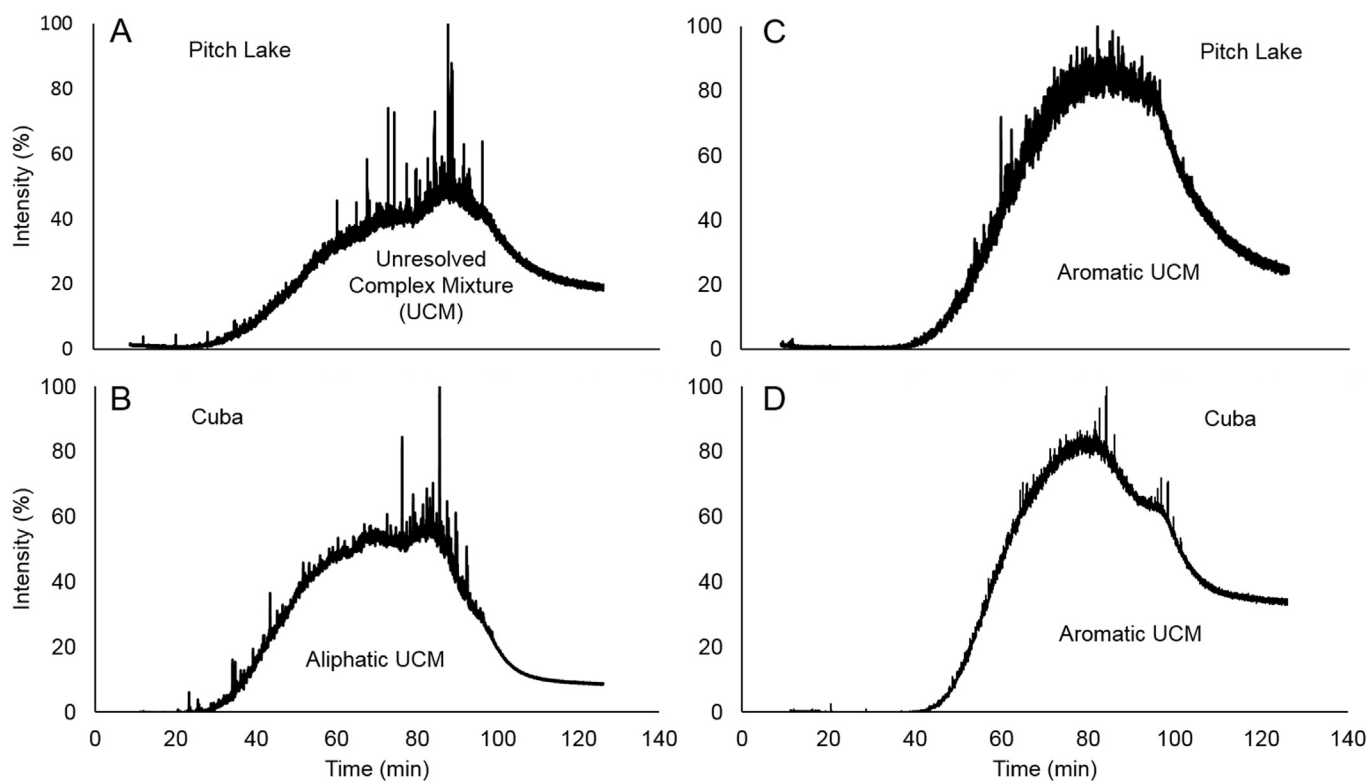


Figure 2. GC-MS total ion chromatograms of Pitch Lake and Cuba saturates (A and B respectively), and aromatics (C and D respectively) fractions.

biomarkers are routinely used for characterisation of oils, gas, and source rocks in petroleum systems and can aid comparisons and correlations between different oils or oils and their source rocks.

As the planet's reserves of low density oil diminish, so there is an increasing interest in heavier, biodegraded oils such as those encountered in terrestrial seeps. In many respects, the analytical challenges posed by the samples in the current study are not only representative of other terrestrial oil seeps worldwide but also of environmental oil spills where recent aerobic biodegradation and weathering can rapidly alter the nature of the spilled oil (Aeppli et al., 2014). The different nature of the two seeps investigated herein i.e. oil exuded recently from Cuban rock fractures and the highly exposed Pitch Lake of Trinidad, provide examples of different weathering conditions within a similar climatic region. The aim of this case study was to explore within the UCM to compare a wide range of compound classes especially those associated with weathering processes. The strategy of using GC-MS extracted ions to explore full scan chromatograms combined with multiple analyses in selected ion monitoring (SIM) mode has proved to be highly successful in the past (e.g. Peters et al., 2007), but this has its drawbacks e.g. the restricted fragment ions visible in mass spectra obtained by SIM. By applying GC×GC-TOFMS analysis following minimal sample preparation, we were able to obtain good quality mass spectra for a range of biomarkers and other informative chemical structures, including diamondoids, demethylated hopanes and aromatic steroids present in terrestrial seep oils from Cuba and Pitch Lake.

2. Geological setting

2.1. Caribbean island chain

The Caribbean islands are a continuous volcanic arc chain of calc-alkaline composition (Nelson et al., 2011). Although the motion causing the creation of the Caribbean Plate is thought to

have begun in the Cenozoic, its formation history is still under debate (Bachmann, 2001). The Greater Antilles group of islands saw initiation of volcanic activity in the Early to Late Cretaceous, whereas the Lesser Antilles represent a separate volcanic event commencing in the Oligocene (Bouysse et al., 1990).

2.2. Trinidad

One of the earliest reports by someone with geological knowledge, following a visit to Pitch Lake in 1807, describes how 'We now perceived a strong sulphureous and pitchy smell, like that of burning coal, and soon after had a view of the lake, which at first sight appeared to be an expanse of still water, frequently interrupted by clumps of dwarf trees or islets of rushes and shrubs: but on a nearer approach we found it to be in reality an extensive plain of mineral pitch' (Nugent, 1812). Nearly 150 years ago, in a publication in 'Transactions of the Edinburgh Geological Society', it was stated: "Where the bitumen came from was almost impossible to answer, but there was a mud volcano near the lake. Petroleum appeared to have more to do with the activity of the mud volcano than the mud volcano had in making it. The tar in the lake did not contain any paraffin." (Taylor, 1873). In addition, Crosby (1879) provided one of the earliest reports that contains sound scientific logic and is critical of the amount of miss-information published about Pitch Lake.

Although a natural curiosity, little scientific work has been published on Pitch Lake, that is until very recent times when the interest in micro-organisms alluded to above has become of great interest. A geophysical survey identified a NNW–SSE gravity low beneath the lake, and that it was irregular in shape (Chaitan and Graterol, 1991). Importantly, two large faults were found to intersect below the lake, considered to be splays off the major E–W Los Bajos Fault that cuts through the island south of the lake (Chaitan and Graterol, 1991). The source of the pitch has been related to underlying Miocene strata, based on palynomorphs extracted from

the bitumen (Kumar, 1981). A geotechnical survey revealed that the Brighton Anticline also passes beneath Pitch Lake and that there are abundant high-angle faults in the area, forming part of the Naparina Thrust System (Maharaj, 2000). It is considered that extensive tectonic transport to the SE from the Late Miocene to Pliocene led to the Upper Miocene to Pliocene Morne L'Enfer Formation, consisting of clay, carbonaceous shales and interbedded sandstones, being thrust over more coherent Oligocene sediments. The source of the pitch is thus considered to be the Morne L'Enfer Formation, with bitumen concentrated due to fluid migration along imbricate thrusts, coupled with local high fluid pressure that led to expulsion to the surface along thrusts and a network of oblique faults (Maharaj, 2000).

2.3. Cuba

The origin of the Caribbean plate is still very controversial (e.g. Meschede and Frisch, 1998; Pindell et al., 2006). The most accepted model emphasizes that the plate formed in the Pacific (Farallon) and that a Cretaceous island arc was originally built due to subduction of the Proto-Caribbean (Atlantic) beneath the Caribbean plate (Pindell et al., 2006; Boschman et al., 2014). Consumption of the Proto-Caribbean crust allowed the eastward drift of the Caribbean plate (relative to the Americas) until the volcanic arc finally collided with the passive margin of North America in the Tertiary. This drifting caused the collision of the Caribbean volcanic arc with the continental margins of North and South America in the Late Cretaceous–Cenozoic. GPS measurements and paleomagnetic studies show the Caribbean plate is currently moving east-north-eastwards in relation to North and South America (Mann et al., 2002) and references therein, thus favouring its origin in the Pacific.

The geology of Cuba consists of oceanic and passive continental margin units forming a NE–SW trending fold and thrust belt, overlain by Cenozoic cover rocks (Iturralde-Vinent et al., 1996). The continental margin units include Jurassic to Cretaceous sedimentary rocks, including units from the borderlands of the Maya block and the southern Bahamas platform, and latest Cretaceous–Eocene basin sediments (e.g. Iturralde-Vinent et al., 1996; Garcia-Casco et al., 2008; Cruz-Orosa et al., 2012) (Fig. S1). Since the Palaeocene–Early Eocene, Cuba has been divided into several mega-blocks by a system of strike-slip faults related to the opening of the Cayman ridge (Sommer et al., 2011 and references therein) (Fig. S1).

3. Material and methods

3.1. Sample collection

The Pitch Lake at La Brea in Trinidad was visited in February 2014 (Fig. 1). A prominent feature is the rainwater that forms ponds and small lakes on the surface (see insert photograph, Fig. 1). The bitumen is firm under foot, but can be penetrated easily by pushing small tree branches through the outer crust. Where there was even slight variation in topography, wrinkling of the surface was evident (Fig. S2), analogous to pahoehoe lava and indicating that the substrate was capable of viscous flow. The sample was collected from the eastern side of Pitch Lake (Lat: 10°13'34"N; Long: 61°37'24"W), after first removing the outer crust.

The sample site in Cuba was an old quarry close to the Pinar del Rio-Soroa Road (Lat: 22°46'45.5"N; Long: 83°0'13.43"W). Here, bitumen can be seen oozing from several locations in the quarry, but principally at the contact between serpentinite and limestone (Figs. 1 and S2). The lithology consists of strongly foliated serpentinite enclosing limestone blocks of the Artemisa Formation (Jurassic–Oxfordian). The sample comes from a tectonic contact along the Pinar Fault, one of the main faults in Cuba (Fig. S1).

Asphalt is abundant in fractures along the tectonic contact (Figs. 1 and S2). The sample was taken from recently-extruded material in January 2016.

3.2. Authentic standards

Hydrocarbon authentic standards included NIST SRM2266 (steranes and hopanes) and angiosperm biomarkers, available from a previous study (Eiserbeck et al., 2011). Alkyldiamantane mixture (>98% purity, also containing adamantanes, triamantanes and tetramantanes) was supplied by PolyDiamond Technologies (Pleasanton, CA). Adamantane-1-carboxylic acid methyl ester was available from a previous study (Rowland et al., 2011a).

3.3. Sample preparation

Asphaltenes were removed by precipitation in chilled pentane. The resulting maltene fractions were analysed as whole fractions (GC×GC-TOFMS only, Fig. 3) and following pre-activated silica gel fractionation as 'Saturates', eluting in hexane, and 'Aromatics', eluting in 7:3 hexane:dichloromethane, fractions by GC-MS (Fig. 2). A further 'Resin' fraction eluting in 1:1 dichloromethane:methanol was also collected and the acids present derivatised by refluxing with boron trifluoride-methanol (Sigma-Aldrich, Pty Ltd, Castle Hill, NSW, Australia) and extracting into hexane.

3.4. GC-MS analysis

GC-MS analyses were performed using a HP-6890A gas chromatograph (Agilent, Santa Clara, CA, USA) interfaced to a HP-5973 mass selective detector (MSD) (Agilent). The column was a DB-5-MS 60 m × 0.25 mm × 0.25 μm (Agilent, Santa Clara, CA, USA). The GC oven was programmed from 40 °C (1 min) to 325 °C at 3 °C min⁻¹ (30 min isothermal). Ultrahigh purity helium was used as carrier gas with a constant flow of 1 mL min⁻¹. Sample injection was 1 μL pulsed splitless at 290 °C. The MSD was operated at 70 eV with a source temperature of 230 °C. Mass spectra were acquired in both full scan and SIM modes. Selected ions were: *m/z* 123, 177, 191, 205, 217, 218, 355, 357, 370, 371, 372, 383, 386, 397, 398, 400, 412, 414, 426, 428.

3.5. GC×GC-TOFMS analysis

Whole maltene plus saturate and aromatic fractions were analysed using a Leco Pegasus IV system equipped with dual stage cryogenic modulator (Leco, Saint Joseph, MI, USA). The primary column was a 60 m × 0.25 mm × 0.25 μm Rxi 5Sil MS (Restek, Bellefonte, PA, USA) coupled to a secondary column 1.4 m × 0.25 mm × 0.25 μm DB-17 ms (Agilent). The carrier gas was ultrahigh purity helium with constant flow of 2 mL min⁻¹. The inlet temperature was 310 °C with 1 μL injection. Maltenes and fractions were initially analysed with a relatively fast temperature program: 50 °C (1 min isothermal), then 3.6 °C min⁻¹ to 320 °C, (isothermal 20 min) and a modulation period of 4 s, with 2° oven and modulator offset 15 °C. Additional analyses of the saturate and aromatic fractions were conducted with a slower heating ramp of 2.5 °C min⁻¹ and a 3 s modulation which improved peak resolution but resulted in some 'wrapping', i.e. failure to trap all compounds in a single modulation period, of high molecular weight aromatic compounds. The mass spectrometer was operated in positive ion electron ionisation mode at 70 eV with ion source at 250 °C and transfer line 320 °C. The scan speed was 100 Hz with a range of 45–580 Da. ChromaTOF (LECO) software package was used for instrument control and data analysis. Mass spectra were compared with National Institute of Standards &

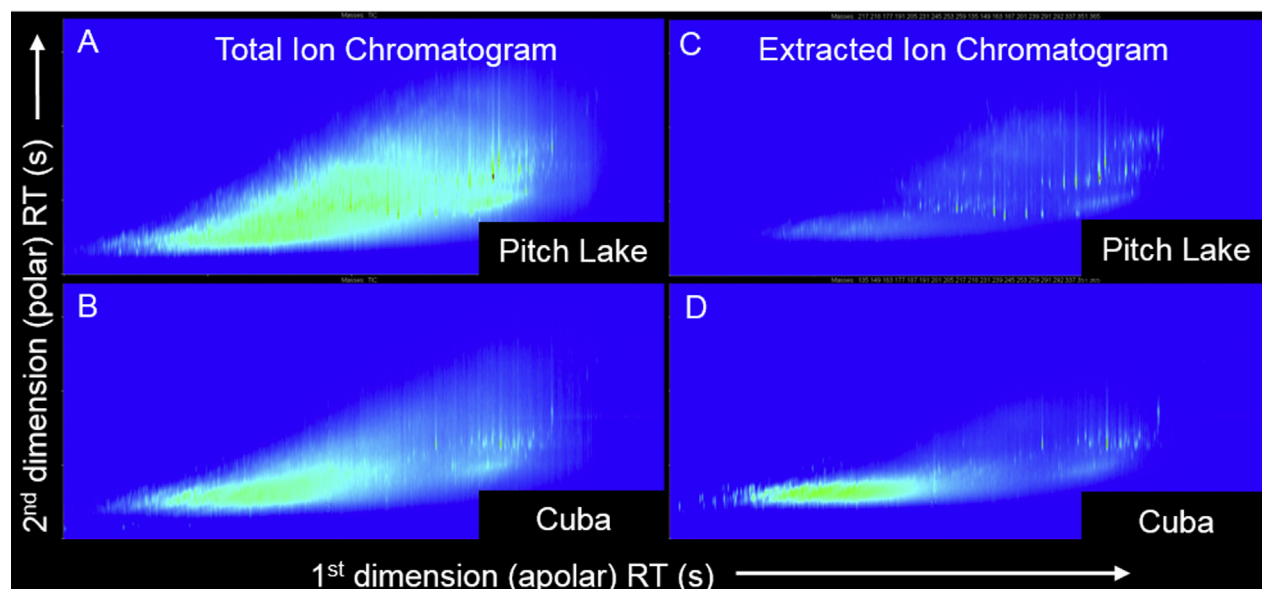


Figure 3. GC×GC-TOFMS total ion chromatograms of Pitch Lake and Cuban oil seep maltene fractions (A and B respectively), and corresponding extracted ion chromatograms (C and D respectively) for compound classes discussed in text. Key ions m/z 135, 149, 163, 177, 187, 191, 201, 205, 217, 218, 231, 239, 245, 253, 259, 291, 292, 337, 351 and 365.

Technology (NIST, Gaithersburg, MD, USA) libraries plus in-house TOFMS libraries.

4. Results and discussion

4.1. GC-MS analyses

Analyses by full scan GC-MS of the saturate and aromatic fractions of the seep oils produced chromatograms dominated by large UCMs (Fig. 2). Targeted analysis using SIM still produced unresolved humps but resolution of hopanes (m/z 191 + confirming ions) was much improved (Fig. S3). Multiple GC-MS SIM analyses would be required for different compound classes in order to explore the composition of these oils. Furthermore SIM has the disadvantage of producing incomplete mass spectra which can be a drawback when peaks of unknown identity are present. A full comparison of what can be achieved by GC-MS and GC×GC-TOFMS analyses is outside the scope of this study and the focus will largely be on the latter.

4.2. GC×GC-TOFMS analyses

The 2D total ion chromatograms (TIC) produced by GC×GC-TOFMS analyses of the maltene extracts of the Pitch Lake and Cuban oils were still highly complex (Fig. 3A and B) but the resolution was dramatically improved with good quality mass spectra obtained for thousands of individual peaks. The advantage of analysing extracts with so little preparation is that losses are minimised. For example, structures containing a single benzene ring but with large aliphatic components, such as arylisoprenoids and monoaromatic steroids, can easily split between fractions during solid phase liquid chromatography sample preparation so having all compounds in a single fraction is highly desirable and hence a major advantage of GC×GC-TOFMS analysis. Even with the reduced complexity of the saturate fractions, there were typically at least five peaks eluting at exactly the same time in the apolar (1st) dimension. As expected, both oils showed a high level of biodegradation. There was a complete absence of alkanes and even the isoprenoid biomarkers, pristane and phytane (largely derived from chlorophyll *a*), that are resistant to microbial attack were confirmed to be absent in both

oils using GC×GC-TOFMS. The use of extracted ion chromatograms (EIC) of the GC×GC-TOFMS analyses enabled targeted compound classes to be revealed within the 'hump' (Fig. 3C and D). For this discussion, these compound classes have been grouped into three sections: alicyclic hydrocarbons with a particular focus on biodegraded compounds (section 4.3); alicyclic hydrocarbons with cage-like diamondoid structures (section 4.4); and, aromatic hydrocarbons (section 4.5) with a focus on aromatic steroids.

4.3. Alicyclic hydrocarbons

Steranes in the Cuban oil were in very low abundance and were co-eluting with more abundant peaks, even when analysed by GC×GC-TOFMS, but were in higher abundance and more distinct in the Pitch Lake oil. Cholestane and its methyl and ethyl substituted structures were absent in both oils but some C_{27-29} diasteranes were present supporting a source rock containing clay (e.g. marlstone). Short chain, thermally more stable (Requejo et al., 1997), C_{21} and C_{22} diapregnanes were relatively much more abundant than pregnanes in the Pitch Lake oil. Biodegradation of the short chain steranes is limited to enzymatic attack of the core structure giving them high resistance (Peters et al., 2007). Regular hopanes were relatively low or absent especially in the Cuban oil. The tris-nor-hopanes, $18\alpha(H)-22,29,30$ -Trinorneohopane (Ts) and $17\alpha(H)-22,29,30$ -Trinorhopane (Tm) were prominent in the Pitch Lake sample as were tricyclic terpanes (Fig. 4A). The demethylated tris-nor-hopanes (25 -nor-Ts and 25 -nor-Tm) were much more prominent in the Cuban oil but absent in Pitch Lake sample (Fig. 4B). The stability of Ts is greater than that of Tm during catagenesis (Seifert and Moldowan, 1978), hence their relationship provides a measure of maturity although source also influences this (Peters et al., 2007). The ratio $Ts/(Ts + Tm)$ was 0.31 (± 0.009 standard deviation (sd), $n = 3$) for Pitch Lake and 0.46 (± 0.035 sd) for Cuban oil suggesting greater maturity for the latter but source may be a factor. A recent study (Kayukova et al., 2016) reported an almost identical maturity based on Ts/Tm for a sample from Pitch Lake despite the oil displaying far less indications of biodegradation e.g. presence of *n*-alkanes. The C_{30} triterpane gammacerane has been used as an indicator of water stratification and is often associated with hypersalinity (Damste et al., 1995; Grice et al., 1998) was very prominent in both oils

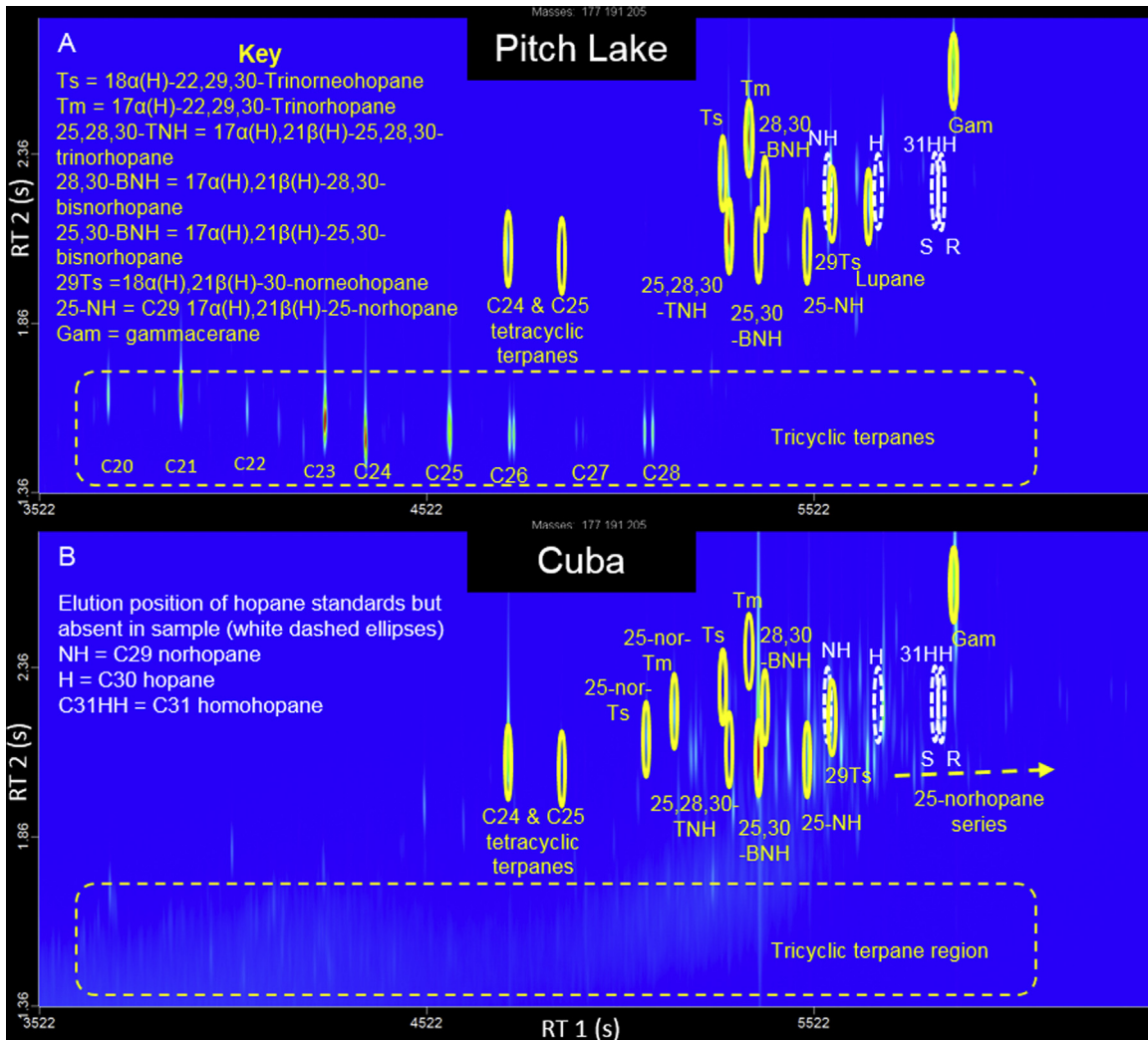


Figure 4. GC×GC-TOFMS extracted ion chromatograms (m/z 177, 191, 205) showing elution positions of major tri, tetra and pentacyclic terpanes present in Pitch Lake (A) and Cuban (B). Yellow ellipses placed around peaks identified by mass spectra and elution position and/or comparison with authentic standards. White dashed ellipses show positions of NIST SRM2266 hopane standards absent or below detection limits.

(Fig. 4). Gammacerane is highly resistant to biodegradation but this appears to be dependant on environmental conditions (Huang, 2017). The gammacerane (gam) index ($10 \times \text{gam}/(\text{gam} + \text{hopane})$) was >8 for both oils. Hypersaline stratification is consistent with the study by Meckenstock et al. (2014) reporting the presence of Halobacteriales in water droplets suggesting high salinity of the formation waters. A distinct band of demethylated hopanes, the 25-norhopanes, eluted just below the region where the regular hopanes elute in the polar dimension (Fig. 4). The 25-norhopanes are thought to result from severe biodegradation of the hopanes (Peters et al., 1996). Ring-opened pentacyclic structures, 8,14-*seco*-hopanes, are also known to be highly resistant to biodegradation, and possibly created as a result of bacterial attack (Wenger and Isaksen, 2002). These compounds were abundant in both of the oils with C₂₇ and C₂₉ structures predominating (Fig. S4). In the Cuban oil the peaks for these were of similar chromatographic areas ($C_{27}/C_{29} = 0.9$) but the larger compound was considerably more abundant in the Pitch Lake oil ($C_{27}/C_{29} = 0.25$). The C₂₉ 8,14-*seco*-hopane chromatographic peaks were about 3x larger than gammacerane peaks in both oils. Angiosperm biomarkers were also observed. Lupane and β-oleanane co-elute on typical GC columns but are resolved by GC×GC (Eiserbeck et al., 2011). Comparison of retention times and mass spectra of authentic standards revealed the presence of lupane in

the Pitch Lake oil (Fig. 4). A prominent peak eluting above the C₂₆ tricyclic terpanes with mass spectra very similar to the angiosperm des-A-triterpenoids with M^+ 330 (C₂₄ tetracyclic), reported by Eiserbeck et al. (2012), and a smaller peak with M^+ 344 (C₂₅ tetracyclic) were present in both oils; such compounds have previously been attributed to a terrigenous source (Grice et al., 2001). The presence of angiosperm biomarkers constrains the source of the Pitch Lake oil, and possibly the Cuban oil, to the Late Cretaceous.

4.4. Diamondoids

One class of compound that is particularly resistant to biodegradation are the diamondoids (Dahl et al., 1999; Grice et al., 2000; Schulz et al., 2001). These thermally stable hydrocarbons possessing cage-like structures have been used to predict the extent of oil cracking, compare biodegraded oils and to distinguish oil mixtures (Dahl et al., 1999; Grice et al., 2000; Schulz et al., 2001). Numerous diamondoid structures were found to be present in both Pitch Lake and Cuban oils but the relative abundance was greater in the latter for most diamondoids. The smallest diamondoid structures are the tricyclic adamantanes with a cage structure containing C₁₀H₁₆. Methyl- and ethyl-substituted structures were present in Pitch Lake but absent in Cuban oil (Fig. 5), likely due to evaporative loss

(Li et al., 2014) although biodegradation was also a possibility as diamondoid acids, thought to be metabolic products of microbial attack, have been detected in extracts derived from highly biodegraded oils and commercial mixtures of naphthenic acids (Rowland et al., 2011a,b,c). To test for this, the acids present in the polar fractions of the oils were converted to their methyl esters. Adamantane acids were not detected in the Cuban oil but a small peak with a mass spectrum very similar to adamantane-1-carboxylic acid methyl ester, and eluting at exactly the same 2D elution times as an authentic standard, was found in the Pitch Lake sample (Fig. S5) supporting a metabolic product source from the bacteria degrading the oil. No other diamondoid acids were found in either oil. Dimethyl adamantanes were more prominent in the Cuban oil than Pitch Lake (Fig. 5). Until recently alkyl chains > C2 on adamantane had not been observed, consistent with the hypothesis that the creation of alkylated adamantanes proceeded via catalytic isomerization of tricyclic hydrocarbons on activated clays or aluminosilicates (Gordadze, 2008). However, propyladamantanes have now been tentatively identified (Silva et al., 2013; Wang et al., 2013) although their mass spectra were not shown. Both 1- and 2-*n*-propyladamantanes were tentatively assigned by 2D elution order and mass spectral interpretation (base peak m/z 135 and M^+ 178) (Figs. 5 and S6).

Pentacyclic diamondane possess two cage structures ($C_{14}H_{20}$) which provides greater resistance to evaporative losses. Parent and methyl-substituted structures were present in oils from both locations but ethyl- and 1,4,9-trimethyl- were absent from the Pitch Lake sample (Fig. 6). Many tri- and tetra-substituted structures were present in the Cuban oil only (Table S1). Triamantane ($C_{18}H_{24}$) three cages, plus its methyl-substituted structures were in high abundance in the Cuban oil with dimethyl structures also present (Fig. S7). Only the parent structure and 9-methyltriamantane were present in the Pitch Lake oil (Fig. S7). A series of tetramantanes ($C_{22}H_{28}$) four cages, skew-, anti- and iso-tetramantane isomers plus methyl-substituted structures were present in Cuban oil only (Fig. S7C). Diamondoids incorporating sulfur in the cage structure e.g. thiaadamantane are thought to be indicator products of thermochemical sulfate reduction between hydrocarbons and sulfates under high temperature in deep-buried strata (Wei et al., 2012). These compounds were therefore searched for using extracted ions and GC×GC elution regions as reported by Wei et al. (2012) and Zhu et al. (2016) but thiaadamantoids were not found in either oils. A comparison of the diamondoid structures present and absent in the two oils is presented in Table S1.

The most stable diamondoid structures possess a methyl group in the bridgehead position. Hence, the order of

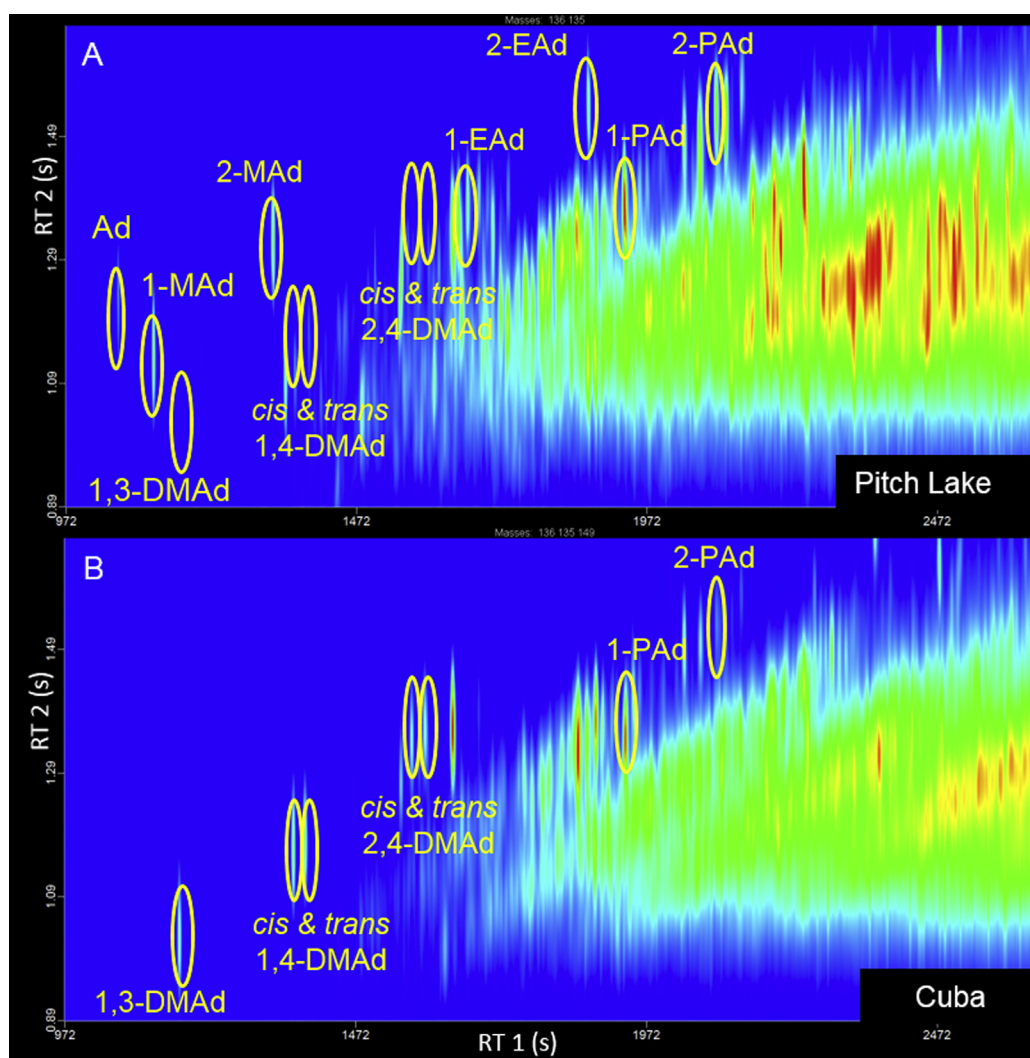


Figure 5. GC×GC-TOFMS extracted ion chromatograms (m/z 136, 135) of Pitch Lake (A) and Cuban (B) oils showing adamantane (Ad), methyl- (M), dimethyl (DM), ethyl- (E) and (tentatively assigned) propyl-substituted structures. Mass spectrum provided in Fig. S6.

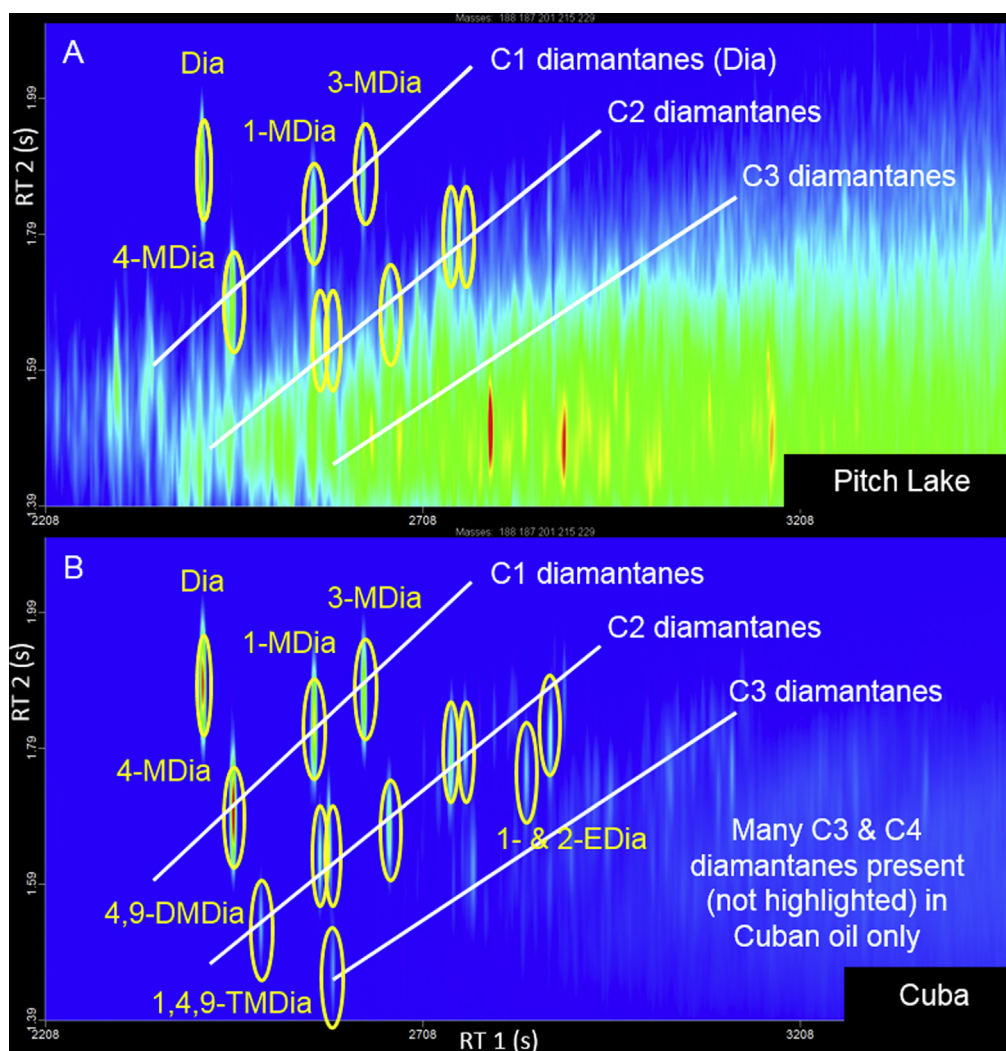


Figure 6. GC×GC-TOFMS extracted ion chromatograms (m/z 188, 187, 201, 215, 229) of Pitch Lake (A) and Cuban (B) oils showing diamantane (Dia), methyl- (M), dimethyl (DM), ethyl- (E) and trimethyl (TM) substituted structures. 1 & 2E-, 4,9-DM, 1,4,9-TM plus many C3 and C4 substituted structures absent in Pitch Lake oil.

stability is 1-methyladamantane (1-MAd) > 2-MAd and 4-methyldiamantane (4-MDia) > 3-MDia and 1-MDia. This difference in stability lead Chen et al. (1996) to introduce two indices: the methyl adamantane index ((MAI = 1-MAd)/(1-MAd + 2-MAd)) and the methyl diamantane index ((MDI = 4-MDia)/(4-MDia + 1-MDia + 3-MDia)) which were found to increase with increasing vitrinite reflectance in source rocks from Chinese basins. Pitch Lake oil was found to have a mean MAI of 0.46 (± 0.030 sd, $n = 3$) but this should be treated with caution as the sample was likely subject to evaporation and biodegradation. The absence of low molecular weight diamondoids in the Cuban oil excluded the use of the MAI for comparison but the MDI could be used. The Pitch Lake oil had a mean MDI of 0.40 (± 0.013 sd, $n = 3$) similar to that of the Cuban oil 0.43 (± 0.004 sd, $n = 3$). The slightly higher ratio implies greater maturity, consistent with the Ts/Tm data. However, the interpretation of diamondoid ratios and abundances is not straight forward as some conflicting results have been reported, e.g. see Schulz et al. (2001) and Fang et al. (2013). Using pyrolysis, the latter reported that diamondoids are formed during the generation of oil, that they are released at relatively low levels of maturity, and further generated during oil cracking. The possibility that they are also subject to biodegradation further complicated interpretation.

4.5. Aromatic hydrocarbons

The aromatic fractions of the oils were even more complex than the saturates fractions with very prominent UCMs evident in the GC-MS traces (Fig. 2C and D). Alkylbenzenes, indans and tetralins could be detected by GC×GC-TOFMS with elution positions and mass spectra consistent with highly branched structures (Booth et al., 2007). Numerous small peaks consistent with arylisoprenoids with either a monocyclic structures (basepeak m/z 133 or alkylated indan structures (basepeak m/z 173) were present in both oils suggesting not only a stratified water column, as suggested by the large gammacerane peaks, but possibly photic zone euxinic conditions, however, diaromatic structures indicative of anaerobic photosynthetic sulfur bacteria, such as isorenieratane (Brocks and Schaeffer, 2008; Melendez et al., 2013; Whiteside and Grice, 2016), were not detected in the aromatic fractions. Similarly, only highly alkylated naphthalenes and biphenyls were observed. Phenanthrene and its methylsubstituted isomers are commonly used for various biomarker indices including maturity e.g. the methylphenanthrene ratio (2-methylphenanthrene/1-methylphenanthrene) but these were not present in either of the samples analysed. Naphthalenes and phenanthrenes have been shown to degrade quite rapidly, < two years, under sulphate-reducing conditions giving rise to their

carboxylic acid metabolites (Aitken et al., 2018). Such aromatic acids and their succinic acid intermediates (as methyl esters) were searched for in the polar fractions of the oils but were not found. Additional indicators of maturity are the aromatic steroids (Mackenzie et al., 1981; Yang et al., 2013) and these were however present. C-ring monoaromatic steroids (MAS) are comprised of two cyclohexane rings (A + B) and one cyclopentane ring (D ring) fused with an aromatic ring (C-ring) in their molecular structure (Fig. S8). During catagenesis, MAS undergo degradation of the aliphatic side chains and are slowly transformed into triaromatic hydrocarbons. Except under extreme conditions, the C_{26–28} homologues of triaromatic steroids (TAS) are very resistant to weathering even over geological timescales including in-reservoir biodegradation (Volkman et al., 1984). Hence, the abundance of MAS relative to TAS provides information related to various geochemical correlations, particularly oil maturity (Mackenzie et al., 1981). The C_{27–29} MAS were found to be more abundant in the Pitch Lake oil but C₂₁ structures were more dominant in Cuban oil, consistent with greater biodegradation in the latter (Fig. S8). Only negligible TAS were observed in the Cuban oil and lower than MAS in Pitch Lake suggesting low maturity but very severe biodegradation is likely a factor as noted by Volkman et al. (1984). Methyl-substituted MAS and TAS (*m/z* 267 and 245 respectively) were also present in both oils (Fig. S9). Oliveira et al. (2012) previously demonstrated the benefits of the improved separation of these types of compounds using GC×GC-TOFMS. A series of peaks consistent with C₂ and C₃ substituted TAS (base peaks *m/z* 259 and 273 respectively) were also present in the Pitch Lake oil only (Fig. S9). Interestingly, a study into the degradation of oil components following the Deepwater Horizon spill found that TAS were relatively rapidly degraded, i.e. within a few years, leading the authors to hypothesize a combination of biodegradation and photo-oxidation to be responsible (Aeppli et al., 2014). The exposed nature of Pitch Lake may well permit photo-oxidation to occur at the surface. As the sample investigated in the current study was taken from beneath the outer crust, it would likely be protected from this rapid degradation. As the Cuban oil was collected soon after emergence, photo-oxidation was unlikely and therefore extreme biodegradation is more probable.

D-ring aromatised structures of the 8,14-*seco*-hopanes, both normal (EIC *m/z* 365) and demethylated (EIC *m/z* 351, probably due to loss at C-28 position) were also present in both oils. The demethylated structures were first reported by Killops (1991) but have also been reported in non-degraded oils (Nytoft et al., 2000). As is the case with their saturated counterparts, the demethylated D-ring aromatised 8,14-*seco*-hopanes can lose an additional methyl group (probably C-25) during biodegradation, observed using EIC *m/z* 337 (Nytoft et al., 2000). The latter compounds were only observed in the Pitch Lake sample. The mass spectrum of tentatively assigned C₂₈ aromatic 25,28-bisnor-8,14-*seco*hopane is provided in supplementary information (Fig. S10).

The common polycyclic aromatic hydrocarbons (PAHs) fluoanthrene, pyrene and their alkyl-substituted homologues were present in Pitch Lake but absent in the Cuban oil. Higher molecular weight PAHs with 5 aromatic rings such as benzopyrenes and benzofluoranthrenes were present in low abundances in both oils. Although atmospheric deposition was a possible source of the pyrogenic PAHs, especially for Pitch Lake which is very exposed, this seems less likely for the Cuban oil emerging from seeps. Perylene was also present at similar abundance in both oils. Perylene is now assigned an origin from perylene quinone pigments of fungi degrading wood (e.g. Grice et al., 2009; Bertrand et al., 2013). In the Pitch Lake oil, the benzo(*b,j,k*)fluoranthrenes were in similar abundance as perylene, but benzo(*e*)pyrene (B(*e*)P) was about 3.5× larger. In contrast, the benzo(*b,j,k*)fluoranthrenes were relatively more abundant (4× perylene) in the Pitch Lake oil with negligible

B(*e*)P. Benzo(*a*)pyrene which is commonly the largest of the 5-ring PAHs in samples with high inputs of atmospheric pyrogenic contamination was very low in both oils (×0.15 perylene).

5. Conclusions

The use of GC×GC-TOFMS to analyse such highly complex samples, represented by the oils from a Cuban seep and Pitch Lake, Trinidad, that were virtually unresolved by GC-MS, produced thousands of individual resolved peaks with good quality mass spectra although many were unidentifiable. A major advantage of using GC×GC-TOFMS analyses of the whole maltene fraction was that chromatograms could be viewed with minimal losses occurring during sample preparation. However, the reduced complexity when analysing these samples following silica-gel fractionation certainly allowed improved both 1D and 2D chromatographic resolution.

Both oils would be considered to display severe biodegradation i.e. >6 on the scale by Peters and Moldovan (1993) with the Cuban oil showing characteristics of >8. The latter possessing an extensive series of demethylated hopanes. Although there were many similarities between the oils e.g. the presence of D-ring aromatised structures of the 8,14-*seco*-hopanes, there were distinct differences such as their further demethylation within the Pitch Lake oil only. The distribution of diamondoids suggested the Cuban oil was subject to greater evaporative losses, evidenced by the lack of low weight structures and a concentration of higher weight triamantanes and tetramantanes. The surface crust on Pitch Lake probably protected the oil below from evaporative losses and photo-oxidation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.gsf.2018.03.011>.

Supplementary data

A Table of diamondoids identified in Cuban and Pitch Lake oil samples is provided. Also, figures showing the regional geology of Cuba; photographs of the surface of Pitch Lake and asphalt oozing from fractures in the rock in Cuba; SIM chromatograms of both oils; GC×GC-TOFMS EIC (*m/z* 123) showing elution positions of 8,14-*seco*-hopanes; GC×GC-TOFMS EIC (*m/z* 135) and mass spectra of tentatively assigned adamantane-1-carboxylic acid methyl ester in Pitch Lake chromatogram and that of standard and; GC×GC-TOFMS mass spectra of tentatively assigned 2-*n*-propyladamantane and NIST library standard; GC×GC-TOFMS EIC (*m/z* 240, 239, 253) showing elution positions of triamantanes and tetramantanes; GC×GC-TOFMS EIC (*m/z* 231, 253) showing relative elution regions of triaromatic steroids and monoaromatic steroids; GC×GC-TOFMS EIC showing series of C1 and C2 substituted triaromatic and monoaromatic steranes, with examples of mass spectra, in maltene fractions of Pitch Lake oil, and; GC×GC-TOFMS EIC of Pitch Lake oil showing series of demethylated monoaromatic 8,14-*seco*-hopanes.

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