

A GC×GC-ToFMS Investigation of the Unresolved Complex Mixture and Associated Biomarkers in Biodegraded Petroleum

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Abstract: Heavy biodegraded crude oils have larger numbers of coeluting compounds than non-biodegraded oils, and they are typically not resolved with conventional gas chromatography (GC). This unresolved complex mixture (UCM) has been investigated using comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC-ToFMS) within a set of biodegraded petroleum derived from distinct sedimentary basins, including northwestern Sichuan (Neoproterozoic, marine), Tarim (Early Paleozoic, marine), Bohai Bay (Eocene, saline/brackish) and Pearl River Mouth (Eocene, freshwater). In general, the hydrocarbons that constitute the UCM in petroleum saturate fractions can be classified into three catalogues based on the distributions of resolved compounds on two dimensional chromatograms. Group 1 is composed mainly of normal and branched alkanes, isoprenoid alkanes and monocyclic alkanes; Group 2 comprises primarily terpanes ranging from two to five rings, and Group 3 is dominated by monoaromatic hydrocarbons such as tetralins and monoaromatic steranes. In addition, the UCM is source dependent and varies between oil populations. i.e., the UCM of petroleum derived from Precambrian and Early Paleozoic marine, Eocene saline/brackish and freshwater source rocks is specifically rich in higher homologues of A-norsteranes, series of 1,1,3-trimethyl-2-alkylcyclohexanes (carotenoid-derived alkanes), and tetralin and indane compounds, respectively.

Key words: unresolved complex mixture (UCM), biomarker, source, biodegradation, comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC-ToFMS)

1 Introduction

The characterization of petroleum components is key in oil exploration, exploitation, refining and spill identification. The unresolved complex mixture (UCM) or “hump”, consisting of complex organic compounds not resolved by the column, appearing as an elevated baseline in gas chromatograms, is common in biodegraded petroleum (Gough and Rowland, 1990). It contains numerous unresolved compounds and a variety of tools have been applied to identify some of the individual compounds. For example, oxidation of UCM hydrocarbons resulted in the formation of many products

that could be resolved using gas chromatography (GC; e.g. carboxylic acids; Gough and Rowland, 1990; Killops and Al-Juboori, 1990). Multi-step and multi-dimensional chromatography can also lead to the identification of individual compounds (Warton, 1999; Sutton et al., 2005). Comprehensive two dimensional gas chromatography (GC×GC), allowing the separation of complex mixtures using two GC columns of different selectivity coupled with a modulator, when connected to a time-of-flight mass spectrometry instrument (GC×GC-ToFMS) is powerful for characterizing petroleum biomarkers, source rock extracts and UCM composition (Frysiner and Gaines, 2001; Tran et al., 2006, 2010; Ventura et al., 2008, 2012; Eiserbeck et al., 2011, 2012; Wang et al., 2013a, 2013b; Li et al., 2015). The method provides some clues that the

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UCM consists in part of simple aliphatic and aromatic hydrocarbons such as highly branched alkanes, cycloalkyl systems comprised mainly of single ring units, substituted benzenoid structures, and alkylated tetralins or decalins (Gough and Rowland, 1990; Killops and Al-Juboori, 1990; Tran et al., 2010; Thomas, 1995; Booth, 2004). The UCM of heavy oils also comprises monocyclic, bicyclic and tricyclic ring compounds (Wang et al., 2010, 2013c, 2014). In addition, it has been proposed that differences in UCM composition probably reflect differences in the kerogen from which the oils were derived (Gough and Rowland, 1990; Killops and Al-Juboori, 1990).

In this study, we have investigated the UCM composition by way of GC×GC-ToFMS of a suite of biodegraded petroleum samples derived from various depositional environments and ages. We provide further evidence for the occurrence and origin of the UCM.

2 Geological Setting and Methods

2.1 Samples and geological setting

In total 14 petroleum samples were collected from a variety of petroleum basins in China (Fig. 1; Table 1). The 5 crude oils produced from the Panyu uplift, Pearl River Mouth Basin (PRMB), are derived from the Eocene Wenchang Formation shales deposited in fresh water and have similar thermal maturities (Wang et al., 2016; Hao et al., 2017). The 5 heavy oils collected from the Dongying Depression, Bohai Bay Basin, were sourced from Eocene saline/brackish sedimentary rocks of the Shahejie Formation (Zhang et al., 1999, 2006; Wang et al., 2006, 2011, 2013d; Zhao et al., 2016; Zhang et al., 2017). One extra-heavy petroleum (soft tar) and 3 other crude oils were obtained from the Tahe oilfield, Tarim Basin, the largest marine oil reservoir in China, with one or two sets

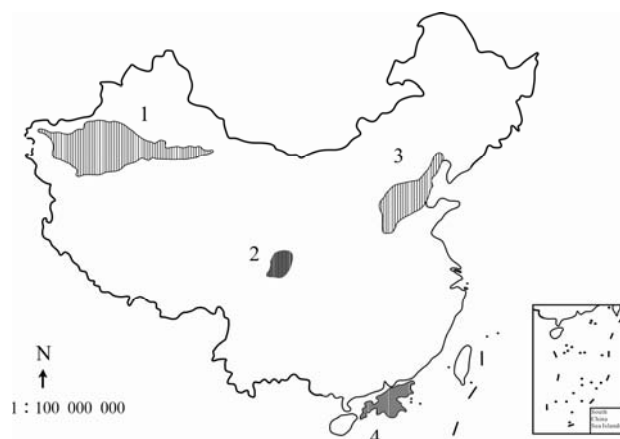


Fig. 1. Map showing the petroleum basins studied (China basemap after China Bureau of Surveying and Mapping Geographical information).

1, Tarim Basin; 2, Sichuan Basin; 3, Bohai Bay Basin; 4, Pearl River Mouth Basin (PRMB)

Table 1 Basic information and biodegradation level of the petroleum samples

No.	Well name	Depth(m)	Property	Basin	Source	Biodegradation level (Peters et al., 2005)	Composition	Column sets
1	B16H	1540 (top)	Crude oil	PRMB	Eocene, fresh lake	Moderate, 5	Minor <i>n</i> -alkanes and acyclic isoprenoids, no 25-norhopane	P/NP, NP/P
2	B27H	1994 (top)	Crude oil	PRMB	Eocene, fresh lake	Moderate, 4	<i>n</i> -alkanes substantially removed, acyclic isoprenoids altered	P/NP, NP/P
3	A16H	2252 (top)	Crude oil	PRMB	Eocene, fresh lake	Light, 2	<i>n</i> -alkanes partially removed, acyclic isoprenoids unaltered	P/NP, NP/P
4	A20H	2415 (top)	Crude oil	PRMB	Eocene, fresh lake	Light, 1	Lower homologs of <i>n</i> -alkanes removed	P/NP, NP/P
5	CG126	449-534	Heavy oil	Bohai Bay Basin	Eocene, saline	Heavy, 7	Steranes substantially degraded, 25-norhopanes appeared	P/NP, NP/P
6	ZX41	1490	Heavy oil	Bohai Bay Basin	Eocene, saline	Heavy, 6	Steranes partly degraded, 25-norhopanes appeared	P/NP, NP/P
7	C27-2		Heavy oil	Bohai Bay Basin	Eocene, saline	Heavy, 7	Steranes substantially degraded, 25-norhopanes appeared	P/NP, NP/P
8	D83-03	1172	Heavy oil	Bohai Bay Basin	Eocene, saline	Heavy, 7	Steranes substantially degraded, 25-norhopanes appeared	P/NP, NP/P
9	Z362	1196.3-1210	Heavy oil	Bohai Bay Basin	Eocene, saline	Heavy, 7	Steranes substantially degraded, 25-norhopanes appeared	P/NP, NP/P
10	B5	outcrop	Solid bitumen	Sichuan Basin	Precambrian, marine	Moderate, 6	<i>n</i> -alkanes and acyclic isoprenoids absent, 25-norhopanes present	P/NP, NP/P
11	S99		extra-heavy petroleum (Tar)	Tarim Basin	Lower Paleozoic, marine	Heavy, 6	<i>n</i> -alkanes and acyclic isoprenoids, 25-norhopanes present	P/NP, NP/P
12	TK410		Crude oil	Tarim Basin	Lower Paleozoic, marine	Mix (0 + 6)*	<i>n</i> -alkanes and acyclic isoprenoids complete, terpanes and steranes intact, 25-norhopanes present	P/NP, NP/P
13	T801		Crude oil	Tarim Basin	Lower Paleozoic, marine	Mix (0 + 6)*	<i>n</i> -alkanes and acyclic isoprenoids complete, terpanes and steranes intact, 25-norhopanes present	P/NP, NP/P
14	TK817		Crude oil	Tarim Basin	Lower Paleozoic, marine	Mix (0 + 6)*	<i>n</i> -alkanes and acyclic isoprenoids complete, terpanes and steranes intact, 25-norhopanes present	P/NP, NP/P

The fresh non-biodegraded oils (Level 0) mixed with earlier oils that had been heavily biodegraded (Level 6).

of source rocks hosted in the Lower Paleozoic as potential contributor (Zhang et al., 2002; Wang et al., 2008a; Hu et al., 2016; Zhan et al., 2017). One outcrop oil-bearing sand sample was taken from the northwestern Sichuan Basin, and is attested to originate from Precambrian marine carbonate source rocks (Wang et al., 2015). All the samples had been biodegraded to various degrees (Table 1).

2.2 Methods

About 20 mg crude oils or extracts of the solid bitumen were weighed and then deasphalted by addition of ca. 150 mL *n*-hexane and fractionated using column chromatography (silica gel:alumina, 3:2) into saturate, aromatic and polar fractions (Wang et al., 2008b). The sequential elution solvents were *n*-hexane, CH₂Cl₂:*n*-hexane (2:1) and CH₂Cl₂:CH₃OH (98:2).

GC×GC-ToFMS analyses of the saturate fractions were performed with an Agilent 7890A gas chromatograph coupled to a Pegasus 4D time-of-flight mass spectrometer. Two column sets were used: one (P/NP) comprised of a polar (SGE BPX50; 50% phenyl polysilphenylene-siloxane) phase column (30 m×0.25 mm i.d.×0.25 μm df) for the one-dimensional phase, coupled to a non-polar (SGE BPX5; 5% phenyl polysilphenylene-siloxane) phase column (1.0 m×0.10 mm i.d.×0.10 μm df) for the second-dimensional column. The other (NP/P) was a HP-5 column (30 m×0.25 mm i.d.×0.25 μm df) as 1D phase, connected to a Rxi-17 column (1.7 m×0.10 mm i.d.×0.10 μm df) as 2D phase. The carrier gas was He at constant flow 1.5 mL/min. Each saturate sample was injected into a heated (300°C) injector, with a split ratio of 200:1. The GC oven was programmed from 35°C (hold 5 min) to 280°C (held 20 min) at 2°C/min. The second oven was heated from 45°C (held 5 min) to 290°C at 2°C/min (hold 20 min). The modulator temperature was 30°C higher relative to the GC oven and the modulation period was 4 s. The temperatures of the transfer line and ion source were 280°C and 250°C, respectively. Ionization was performed with electron ionization 70 eV and the MS detector voltage was 1350 V. The mass spectral data acquisition rate was 100 Hz and data were collected over a range of 40–500 da. Data were processed using automated data processing software (ChromaTOF 4.43 with NIST11), with a signal-to-noise (S/N) threshold of 40.

GC-MS analysis was conducted with an Agilent 6890N gas chromatograph coupled to an Agilent 5973i mass spectrometer. An HP-5MS column (60 m×0.25 mm, 0.25 μm film thickness) was used, with the oven temperature programmed from 50°C (5 min hold) to 310°C (30 min hold) at 3°C/min. He was the carrier gas at a constant 1 mL/min. The temperatures of the injector and transfer line

were 300°C and 280°C, respectively. The mass spectrometer was operated in full scan mode from 40–550 da, with an ionization energy of 70 eV.

A great number of compounds occurred in the saturate UCM obtained from GC×GC-ToFMS and GC-MS. Assignment of the biomarkers and some other compounds was based on library and literature data. In addition, the tentative distinction of various tetralins and indanes was according to their unique peak pattern and mass spectra as well as comparison with the reported retention indices.

3 Results

Analysis of the UCM will provide critical information towards understanding complicate processes constraining the occurrence of petroleum in the subsurface environment. It appears that the UCM derives from diagenetic alteration of biogenic material via a coupled process of isomerization and hydrocarbon cracking caused by thermal stress (Ventura et al., 2008, 2012; Rushdi and Simoneit, 2011; Simoneit and Lonsdale, 1982; Simoneit et al., 2004). On the other hand, the sources have been defined for all the petroleum samples studied within the respective basins from detailed oil-oil and oil-source correlations utilizing a set of more resistant biomarkers (e.g. Wang et al., 2008a, 2008b, 2013d, 2015, 2016).

3.1 Petroleum biodegradation level

The extent of biodegradation has been evaluated using different scales based on the alteration/removal of saturated and aromatic compounds. For example, Volkman et al. (1983) developed a biodegradation scale based on the alteration of compounds in the saturated hydrocarbon fractions. Later, Volkman et al. (1984) expanded this scale to include aromatic hydrocarbons. The PM biodegradation scale (Peters and Moldowan, 1991) is based on saturated biomarkers and select aromatic compounds (mainly aromatic steroids). Another scale that considers both saturated and aromatic hydrocarbons was developed by Wenger and Isakson (2002). Therefore, different biomarker biodegradation scales have been developed to assess the extent of oil biodegradation based on the comparisons of the relative abundance of a selection of hydrocarbon compound classes in saturated (e.g. *n*-alkanes, pristane, phytane, terpanes, steranes) and aromatic (e.g. C₁-, C₂-, C₃- and C₄-naphthalenes, C₂-phenanthrenes, and aromatic steroids) hydrocarbon fractions with different susceptibility to biodegradation (Volkman et al., 1983, 1984; Peters and Moldowan, 1991; Wenger and Isakson 2002). Larter et al. (2012) developed a new biodegradation scale called Manco (Modular Analysis and Numerical Classification of Oils) scale,

based on integrating the extent of biodegradation of various aromatic compounds such as alkyl aromatic compounds (e.g. alkyltoluenes, naphthalenes, methylnaphthalenes, phenanthrene, methylphenanthrenes, methyl dibenzothiophenes) and steranes. The authors noted that this scale can be applicable to heavily and severely biodegraded oils from western Canada, with biodegradation PM levels 4–8. In fact, nearly all the scales for ranking the levels of microbial degradation were developed based on the relative abundances of saturated and/or aromatic hydrocarbons with different susceptibilities to biodegradation (Peters et al., 1991; Volkman, 1984; Wenger and Isakson, 2002; Larter et al., 2012). However, the extent of biodegradation can be ranked on a scale of 1–7 based on the scale proposed by Peters and Moldowan (1991) in this study (Table 1, Fig. 2a–d). In addition, the UCMs display different elution shapes and distributions in m/z 95 chromatograms (a common key ion for UCM, Fig. 2). The UCM was closer to a Gaussian distribution for the extra-heavy petroleum (tar) sample S99 and other oils from Tarim Basin, and bimodal for outcrop bitumen sample B5, but not as apparent for crude oil sample B16H and heavy oil sample C27-2, which could be suppressed by the higher biomarker (mainly $17\alpha,21\beta$ -hopane and homohopanes) concentration. However, this is only an approximation of the actual conditions in the reservoirs. Some of the more

resistant compounds (e.g. isoprenoids) are depleted before complete destruction of less resistant compounds (e.g. n -alkanes), or steranes and hopanes are degraded to the same stage (Table 1). In fact, many dynamic processes may be involved in the biodegradation of hydrocarbon classes, indicating a more complicated process than has been reported (Bennett and Larter, 2008; Head et al., 2003; Wang et al., 2013d). Moreover, the UCM consisting of compounds that resist biodegradation is predominant for highly degraded oils, accounting for nearly all to about half of the total mass (Peters et al., 2005).

The PRMB crude oils displayed an increase in biodegradation level from slight (A20) to moderate (B16H), where normal and acyclic alkanes have been more or less altered (Table 1; Wang et al., 2016). Oils are additionally vulnerable to water washing, which favors removal of compounds with high polarity or aqueous solubility, such as gasoline range and aromatic hydrocarbons (Palmer, 1993; Wang et al., 2016). All 5 heavy oils from the Bohai Bay Basin have experienced substantial biodegradation in the reservoirs that impacted both steranes and hopanes (Table 1, Fig. 2b; Wang et al., 2013d). Solid bitumen sample B5 (Fig. 2d) from the northwestern Sichuan Basin and the tar sample S99 (Fig. 2c) from Tarim Basin had a high abundance of 25-norhopanes but the steranes seemed to be unaffected (Wang et al., 2008a, 2015). The other three crude oils

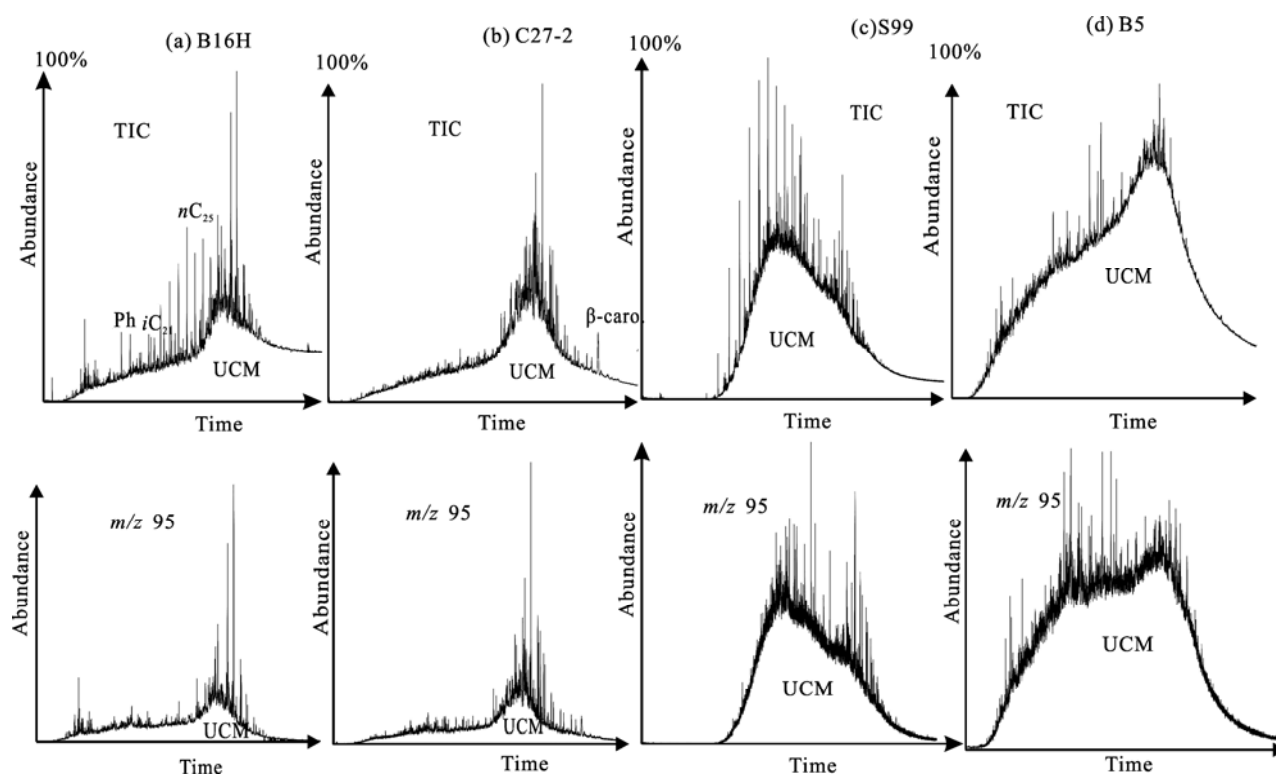


Fig. 2. Examples of GC-MS total ion chromatograms (TICs) and a key ion chromatogram, m/z 95, showing the UCM in petroleum saturates.

(TK410, T801 and TK817) from Tarim Basin may be mixtures of non-biodegraded and heavily biodegraded petroleum, based on the appearance of the UCM and 25-norhopanes along with some *n*-alkanes (Table 1). This may indicate that a second oil charge was mixed with the initial oil which had undergone severe biodegradation within the reservoir (Peters et al., 2005; Wang et al., 2008a).

3.2 Composition of the UCM

Hydrocarbons that constitute the UCM of a petroleum saturate fraction could be divided into three zones based on the elution of compounds in the first and second dimensions of GC×GC-ToFMS chromatograms from the P/NP column set (Fig. 3). The compounds in Zone 1 were mainly normal and branched alkanes, isoprenoid alkanes and monocyclic alkanes; compounds in Zone 2 consisted primarily of cyclic terpanes with two to five rings; and compounds in Zone 3 were dominantly monoaromatic hydrocarbons such as tetralins, indanes, or monoaromatic steranes.

3.2.1 Compounds in Zone 1

The hydrocarbon classes had higher 2nd dimensional retention times on the P/NP column set, showing weak or no polarity for the compounds. Apart from the normal, branched and isoprenoid alkanes, the other significant

components were mono- and bicyclic hydrocarbons (Fig. 4).

Monocyclic alkanes typically included 1,1,3-trimethyl-2-alkylcyclohexanes (i.e. carotenoid-derived alkanes), *n*-alkylcyclohexanes and methyl-*n*-alkylcyclohexanes. A series of abundant carotenoid-derived alkanes ranging from C₁₈ to C₃₁ was assigned from the key fragment ion at *m/z* 125 (Appendix I; Jiang and Fowler, 1986) in the heavy oil sample C27-2, a heavily biodegraded oil, together with β-carotane and lexane (Fig. 4a). While reduction of the double bonds of β-carotene is regarded as the most important diagenetic process (Koopmans et al., 1996), the higher β-carotane (Appendix II) and the elevated C₃₃ carotenoid, lexane, could be from further enrichment, perhaps due to a greater resistance to microbial alteration by the terminal cyclohexyl ring (Jiang and Fowler, 1986; Wang et al., 2013d). However, limited homologues of 1,1,3-trimethyl-2-alkylcyclohexanes were present in the crude sample B16H, as well as the tar sample S99 and the solid bitumen sample B5 (Appendix I). This is in accord with the conclusion that 1,1,3-trimethyl-2-alkylcyclohexanes are preferably formed in anoxic lacustrine depositional environments (Rullkötter and Philp, 1981; Jiang and Fowler, 1986; Hall and Douglas, 1983; Chen et al., 1996; Hanson et al., 2001). The few 1,1,3-trimethyl-2-alkylcyclohexanes in the petroleum derived from marine carbonate source rocks of

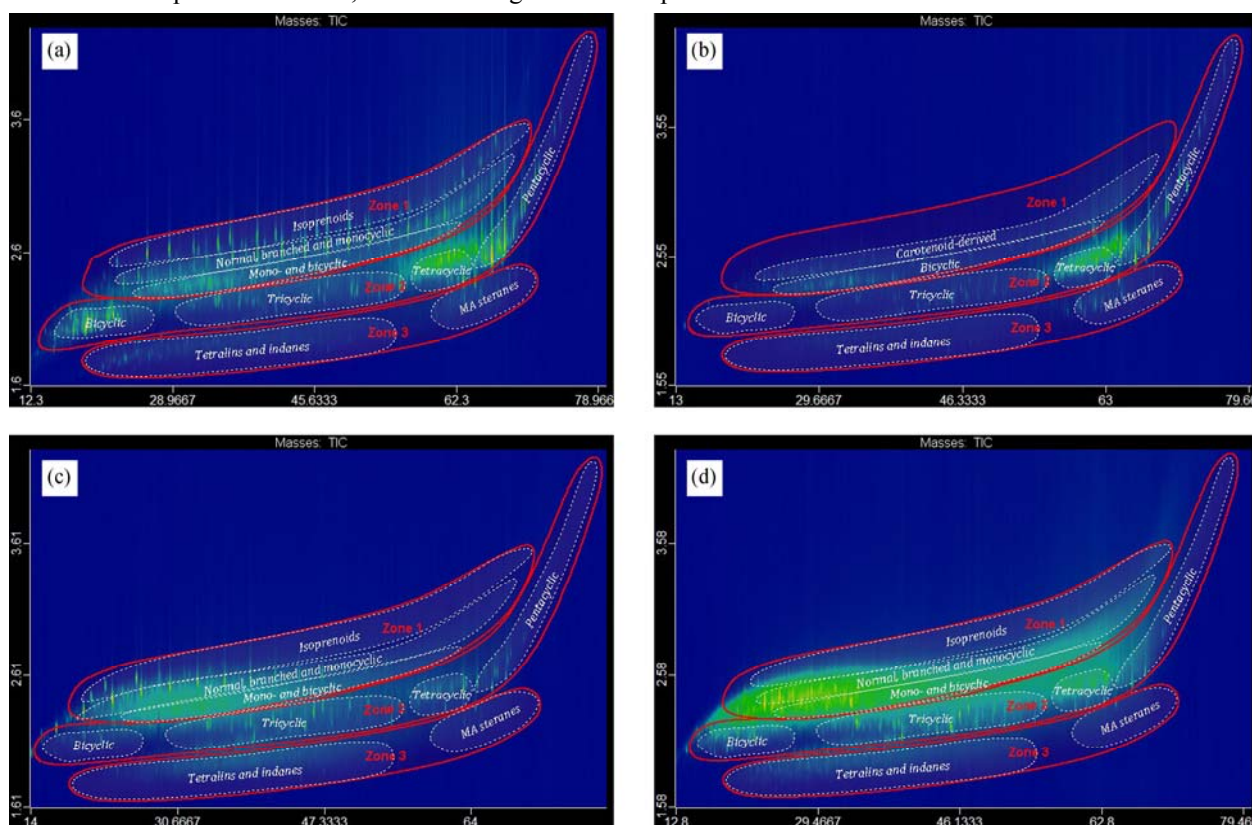


Fig. 3. Examples of GC×GC-ToFMS TIC traces of petroleum saturates.

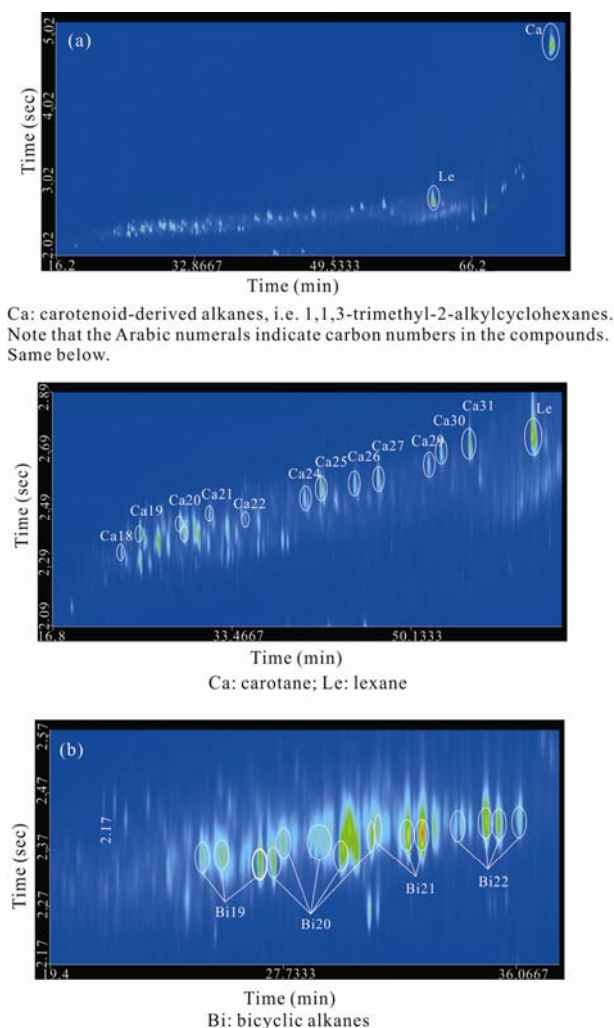


Fig. 4. Some biomarkers included in Zone 1.

(a) Carotenoid-derived alkanes (m/z 125) in C27-2, and (b) bicyclic alkanes (m/z 110 + 124) in the tar sample S99.

the NW Sichuan and Tarim basins could result from alternative reactions of carotenoids, such as aromatization (Rullkötter and Philp, 1981) or sulfurization into kerogen (Sinninghe Damsté and Koopmans, 1997) during diagenesis.

The alkylcyclohexanes and methyl alkylcyclohexanes, which could be derived from the cyclization of fatty acids with or without decarboxylation, respectively, were abundant in B16H, but in low abundance in other petroleum samples derived from marine and saline source rocks. A clay matrix may provide the medium or circumstances for the origin of these two types of monocyclic alkanes. In contrast, a set of bicyclic alkanes was recognized from key ions m/z 110 or m/z 124, ranging from C_{19} to C_{22} in the heavy oil sample C27-2 and the tar sample S99, and comprising C_{21} and C_{22} in the solid bitumen sample B5, while they were absent from the crude sample B16H (Appendix I, Fig. 4b). Various precursors have been proposed for bicyclic alkanes, such as vitamin D; or

lupane, oleanane and onocerane triterpanes; and extended di- or tricyclic terpanes (Anders and Robinson, 1971; Bendoraitis, 1974; Kagramanova et al., 1976; Philp et al., 1981; Rullkötter and Wendisch, 1982; Alexander et al., 1983). Alternatively, they could be derived from sterol precursors via opening of ring B (Jiang et al., 1990).

3.2.2 Compounds in Zone 2

Zone 2 compounds consisted principally of bicyclic to pentacyclic terpanes (Appendix I), such as sesquiterpanes (Fig. 5a), tricyclic terpanes (Fig. 5b), A-norsteranes (Fig. 5c), steranes (Fig. 5d), hopanes (Fig. 5e), and 25-norhopanes (Fig. 5f). All are linked across Earth history as biomarkers, or molecular fossils (Peters et al., 2005). The characteristics and distributions of these biomarkers vary among the crude oils and have been described elsewhere (Zhang et al., 2003; Li et al., 2008; Wang et al., 2013d, 2016). For instance, diasteranes are abundant in crude oil derived from siliciclastic source rocks (Peters et al., 2005; Jiang et al., 2016) as sample B16H, together with higher oleananes derived from angiosperms (Moldowan et al., 1994) and elevated 4-methylsteranes contributed by dinoflagellates (Volkman et al., 1990). Elevated gammacerane is found in petroleum from source rocks that result from stratified water column environments commonly due to elevated salinity (Fig. 5e; Sinninghe Damsté et al., 1995). The C_{19} and C_{20} A-norsteranes (Appendix II) were detected in all the petroleum samples, but more C_{23} homologues were encountered in the two marine oils S99 and B5 (Fig. 5c). A-norsteranes have been reported in the Neoproterozoic-Cambrian South Oman Salt Basin (Grosjean et al., 2009). On the other hand, as biodegradation reduces hydrocarbon classes to non-detectable levels in the petroleum samples, other new compounds are enhanced. For example, 25-norhopanes from C_{28} to C_{34} were present in the heavy oil C27-2 and the tar sample S99 (Fig. 5f), whereas 18 α -22,25,29,30-tetranorhopane (DTs, Appendix II), 17 α -22,25,29,30-tetranorhopane (DTm) and 25,30-bisnorhopane (DH28 and DH28Ts; Appendix II) were enriched in the solid bitumen sample B5.

3.2.3 Compounds in Zone 3

The Zone 3 compounds generally contained one aromatic ring, increasing the polarity. It has been estimated that they can constitute up to 30% of the UCM components in petroleum (Killops and Al-Juboori, 1990). Tetralins and indanes, aryl isoprenoids, and ring-C monoaromatic steranes were major compounds in Zone 3.

Tetralins and indanes were most abundant in the oils from the PRMB, and at least four types were identified on the basis of the M^+ chromatograms of m/z 188 (Fig. 6).

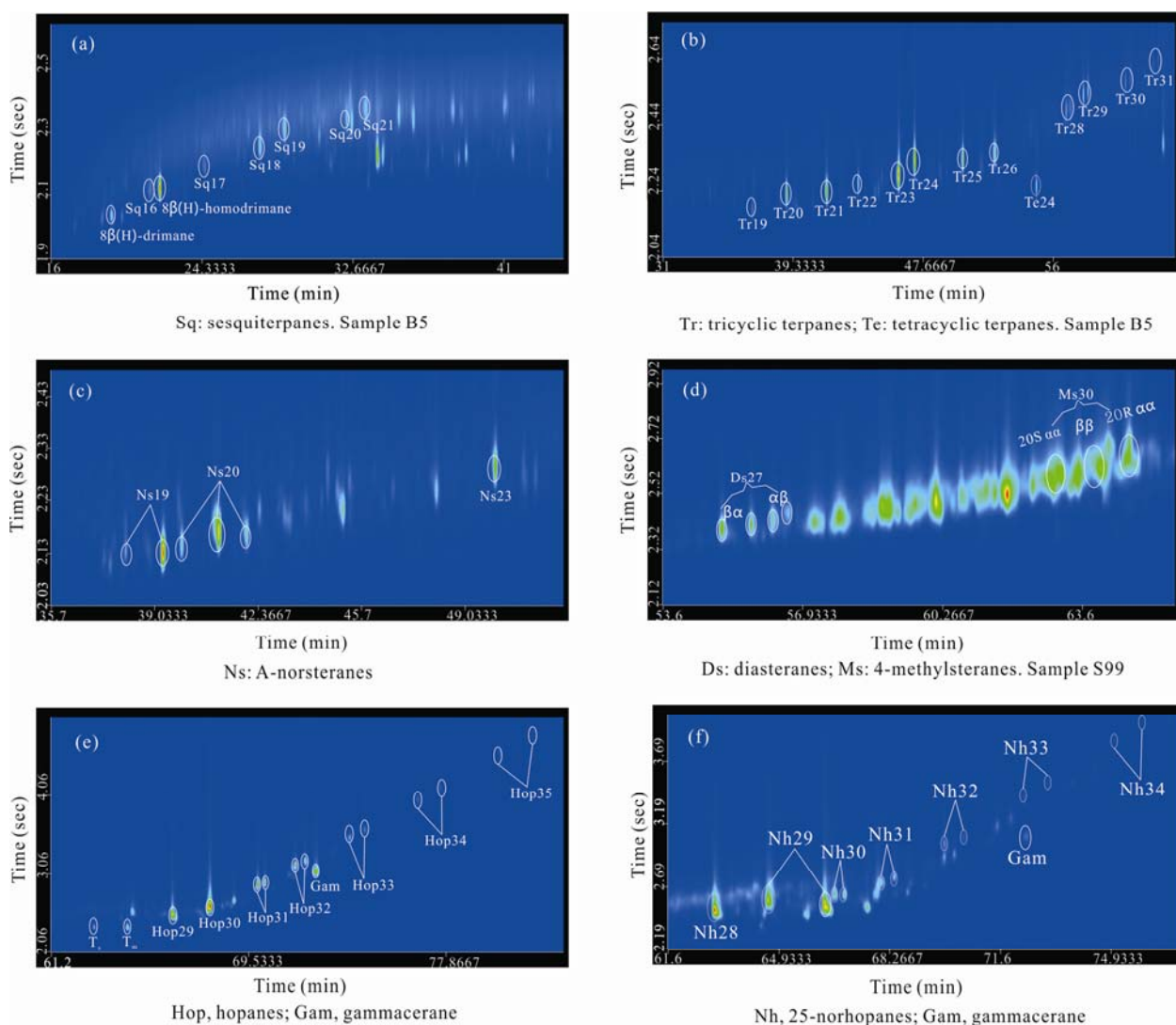


Fig. 5. Some biomarkers included in Zone 2.

(a) Bicyclic sesquiterpanes (m/z 123) in solid bitumen sample B5, (b) tricyclic terpanes (m/z 191) in solid bitumen sample B5, (c) A-norsterpanes (m/z 203) in tar sample S99, (d) steranes (m/z 217) in tar sample S99, (e) hopanes and gammacerane (G) (m/z 191) in tar sample S99, and (f) 25-norhopanes (m/z 177) in tar sample S99.

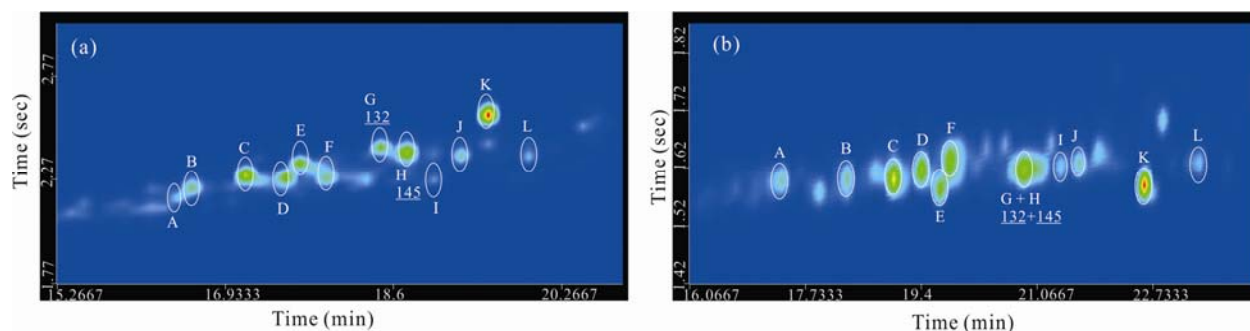


Fig. 6. Chromatograms of m/z 188 for tetralin and indane compounds.

(a) NP/P column set and (b) P/NP column set of GC×GC-TOFMS of the PRMB B16H saturate fraction, which have been indicated by letter A to L in turn.

The spectra for all the $C_{14}H_{20}$ isomers are shown in Appendix III. The first type included only compound B, characterized by the base peak at m/z 118. The second

included D and G, characterized by a base peak at m/z 132. For example, 2,2,7,8-tetramethyl-1,2,3,4-tetrahydronaphthalene (tetralin) can fragment by a retro

Diels-Alder reaction to yield the ion at m/z 132 (Forster et al., 1989). The data from analysis of synthetic standards showed that m/z 118 is the base peak for some disubstituted tetralins such as 2,4-, 2,6-, and 2,7-dimethyltetralins, and m/z 104 is the base peak of tetralin. Thus, the fragments at m/z 104, 118 and 132 could be used to diagnose tetralin or its substitution patterns. However, peaks D and G have similar mass spectra and were specifically assigned as 2,2,5,7-tetramethyltetralin (synthetic standard) and 2,2,7,8-tetramethyltetralin, respectively. The latter can be regarded as a biological marker specific for angiosperms, which provides a means of dating sedimentary organic materials as Cretaceous or more recent (Forster et al., 1989). Generally, under highly reducing depositional environments oleanane skeletons are preferentially preserved, whereas degradation and aromatization processes occur extensively in terrestrial sediments or under acidic conditions (Murray et al., 1997). In the oxidative scheme proposed for the evolution of β -amyrin, 2,2,7,8-tetramethyltetralin is a key end product (Forster et al., 1989; Püttmann and Villar, 1987). A high abundance of 1,2,5-trimethylnaphthalene (TrMN), 1,2,7-trimethylnaphthalene (TrMN) and 1,2,5,6-tetramethylnaphthalene (TeMN) in the aromatic fractions, together with elevated oleanane and 2,2,7,8-tetramethyltetralin in the saturate fractions, supported the proposal that the PRMB petroleum was derived from shale source rock horizons deposited in Eocene freshwater environments (e.g. Bao et al., 2016; Wang et al., 2016).

The third type included compounds F, H, J and L, which have a base peak at m/z 145; the fourth included only C, with dominant ions at m/z 159, 144, and 128, and the fifth included A, E, I and K, which have a base peak at m/z 173 (Appendix III). This implies that these hydrocarbons consisted mainly of substituted tetralins or indanes, which can be analogues and expressed as m/z 145 + 14*n* (*n* = 0 to 2).

However, the abundance of tetralins and indanes varied with petroleum basins, being for example very low in the heavy oil sample C27-2, medium in the tar sample S99 and higher in the solid bitumen sample B5. This reveals that certain phytoplankton or microorganisms could also contribute to these compounds considering their occurrence in these early Paleozoic and Precambrian-derived crude oils, although an angiosperm input can produce significant tetralins within diagenesis (Alexander et al., 1983).

Two sets of benzenoid hydrocarbons (Appendix II) were distinguished in all of the four petroleum catalogues, with unique fragments at m/z 119/120 and m/z 133/134 (Fig. 7a). These hydrocarbons were tentatively identified as alkyl substituted xylenes and aryl isoprenoids,

respectively (Appendix III; Summons et al., 1987; Williams et al., 1988). The latter compounds could be derived from carotenoid precursors from the Chlorobiaceae family of sulfur bacteria (Summons et al., 1987). This is in agreement with geological and geochemical studies which all the heavy oil sample C27-2, the tar sample S99 and the solid bitumen sample B5 petroleum source rocks were deposited under a saline and sulfide-rich water column, which resulted in elevated gammacerane, C_{35} hopanes and dibenzothiophenes (Peters et al., 2005). However, B5 contained numerous isomers of aryl isoprenoids ranging from C_{14} to C_{20} , which is quite different from the others and might reveal a special depositional environment or organic matter (OM) input in the Precambrian era.

Ring-C monoaromatic steranes (MA) and their methyl analogues (MMA) are recognized by the key ion fragmentograms at m/z 253 and 267 that varied among the samples (Fig. 7b,7c). They were extremely abundant in C27-2, ranging from C_{20} to C_{29} and from C_{21} to C_{29} for MA and MMA, respectively; much higher MA than MMA occurred in crude oil sample B16H; and high short chain MA appear in tar sample S99 and oil sand sample B5. These distributions matched their respective sterane counterparts, and fit with the presumed geological fate of steroids, where ring-C monoaromatic steranes are intermediates from sterenes to triaromatic steroid hydrocarbons (Mackenzie et al. 1982; Moldowan and Fago, 1986).

4 Discussion

While the identifiable compounds that constitute the petroleum UCM can be divided into three zones on GC×GC-ToFMS chromatograms, those hydrocarbons bearing two or more saturated rings in Zone 2 and one aromatic ring in Zone 3 are normally major components for heavily biodegraded oils. In addition, these compounds are generally “masked” in petroleum but become dominant as the major resolved components decrease or disappear during microbial attack, and thus appear as a big “hump” or “UCM” on GC and GC-MS as suggested Killops and Al-Juboori (1990), using other analytical techniques. The occurrence and distribution of the UCMs vary between petroleum groups that derived from distinct depositional environments. For example, tetralins are important in the crude sample B16H and can be partially attributed to an angiosperm source (Forster et al., 1989). For coal and shale samples, these compounds are key end products intermediate in the proposed degradation and aromatization scheme of triterpenoids, e.g. β -amyrin (Forster et al. 1989; Püttmann and Villar, 1987), or the

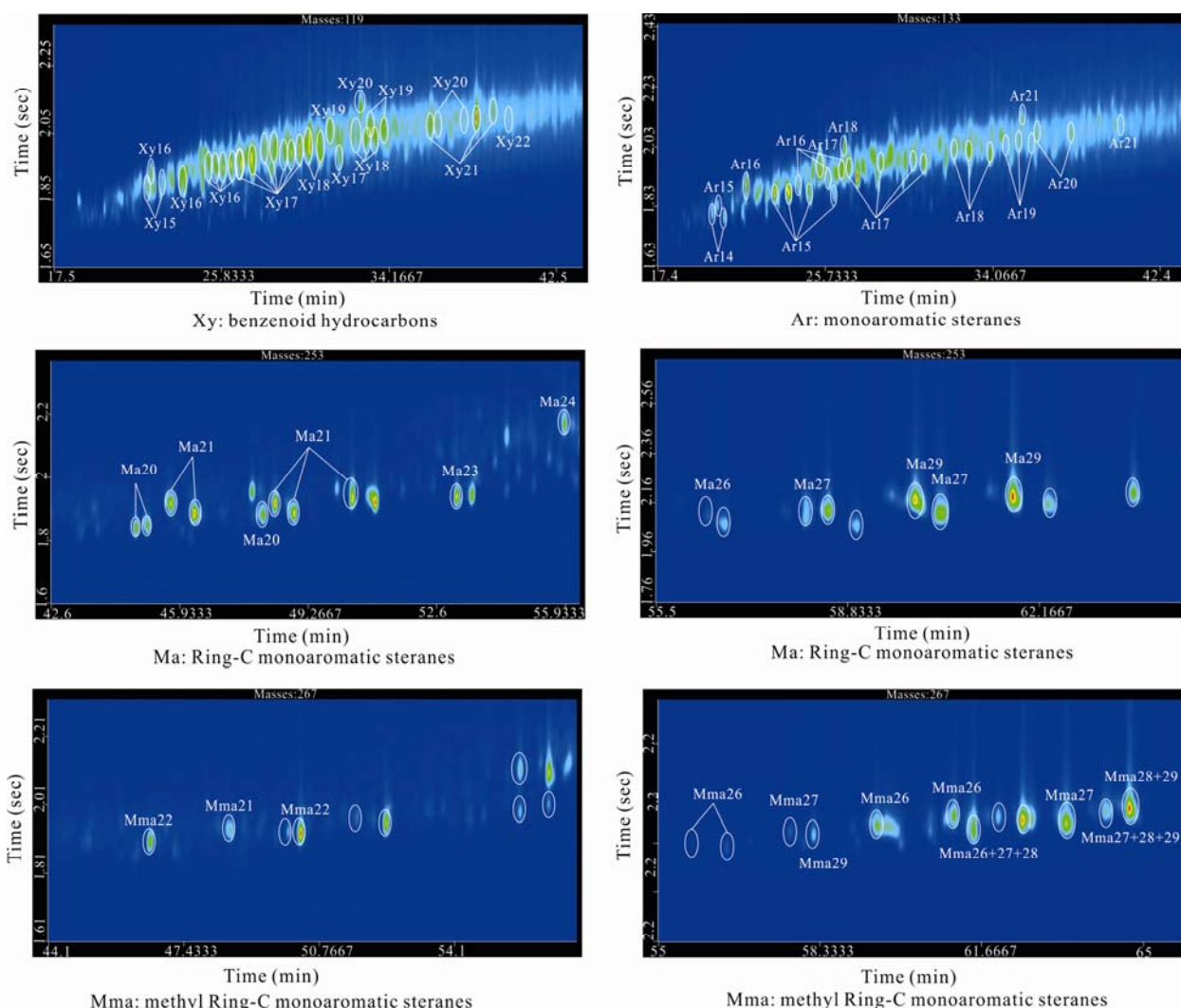


Fig. 7. Some biomarkers included in Zone 3.

(a) Benzenoid hydrocarbons and monoaromatic steranes (m/z 119 and 133) in solid bitumen sample B5, and (b) monoaromatic steranes (m/z 253) and methyl Ring-C monoaromatic steranes (m/z 267) in heavy oil sample C27-2. The carbon numbers range from 20–29 for the monoaromatic steranes in the m/z 253 chromatogram, and from 21–29 for the methyl monoaromatic steranes in the m/z 267 chromatogram. Note that both are shown as two segments based on their retention times.

second ring may form by ring closure of an alkylated single ring compound (Williams et al., 1988). The tetralins also occur in both the Lower Paleozoic-derived oil (tar sample S99) and the Precambrian-derived oil (solid bitumen sample B5), indicating that they have extensive alternate precursors besides angiosperms.

Eukaryote-derived steroids, including steranes and monoaromatic steranes, are widely distributed in the oils and show distinct characteristics among the samples. This reflects the different alteration pathways of the precursors during maturation. Abundant steranes and monoaromatic steranes, as well as their 4-methyl counterparts, occur in the heavy oil C27-2 from a hypersaline deposit. 4-Methylsteranes and 4-methyl monoaromatic steranes are especially significant in the crude sample B16H, from freshwater depositional conditions. Short chain steroids,

such as the 5α , 14β , 17β H stereoisomer of pregnane called diginane and ring-C aromatic diginane (Wingert and Pomerantz, 1986), emerge in the carbonate source rock-derived petroleum samples B5 and S99. They could be geological products derived from steroid precursors bound to kerogen by a sulfurized side chain (Wang et al., 2015). Furthermore, aryl isoprenoids are enriched in the solid bitumen sample B5, reflecting restricted, stratified marine or lagoon depositional conditions (Wang et al., 2015).

It can be enclosed that UCM components are primarily inherited from source or kerogen that depend on depositional environment and OM input. They generally become enriched following the reduction or disappearance of the other dominant hydrocarbon classes during microbial alteration or water washing of petroleum. Thus, the UCM comprises countless compounds that derived

from minor components in petroleum, remnants of altered hydrocarbons, and several biomarker classes being enriched during biodegradation. Comparison of the oxidation products with those obtained by laboratory simulations from kerogen (Killops and Al-Juboori, 1990) suggests that the UCM forms part of the hydrocarbon material expelled from the kerogen matrix during the petroleum generation processes. However, it is generally not apparent in non-biodegraded oils at the sample concentrations employed when determining *n*-alkane distributions from GC analysis (Killops and Al-Juboori, 1990).

5 Conclusions

Based on the investigation of hydrocarbon compositions in a range of subsurface biodegraded petroleum from various basins, it is revealed that:

(1) The identifiable compounds in the unresolved complex mixture (UCM) consist mainly of groups of tetralins and indanes, substituted xylenes and aryl isoprenoids, bicyclic to pentacyclic terpanes, and steranes and monoaromatic steranes.

(2) The composition and distribution of UCM are source dependent and varies between oil populations.

(3) The UCM is enriched in alkyl and methyl alkylcyclohexanes, and tetralins and indanes in the petroleum derived from freshwater source rocks (Eocene Pearl River Mouth basin); shows higher C_{18} to C_{31} 1,1,3-trimethyl-2-alkylcyclohexanes (carotenoid-derived alkanes) and C_{20} - C_{29} Ring-C monoaromatic steranes (MA) and their C_{21} - C_{29} methyl analogues (MMA) in the crude oils generated from saline/brackish source horizons (Eocene Bohai Bay basin); comprises enriched C_{28} - C_{34} 25-norhopanes and C_{23} homologues of A-norsteranes in the tar mat from Early Paleozoic marine source beds (Tarim basin); and contains elevated C_{23} homologues of A-norsteranes, 18 α -22,25,29,30-tetranorhopane (DT_s), 17 α -22,25,29,30-tetranorhopane (DT_m), and 25,30-bisnorhopane (DH28 and DH28T_s) for the hydrocarbons derived from Precambrian marine organic matter sources (northwestern Sichuan basin).

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About the First author

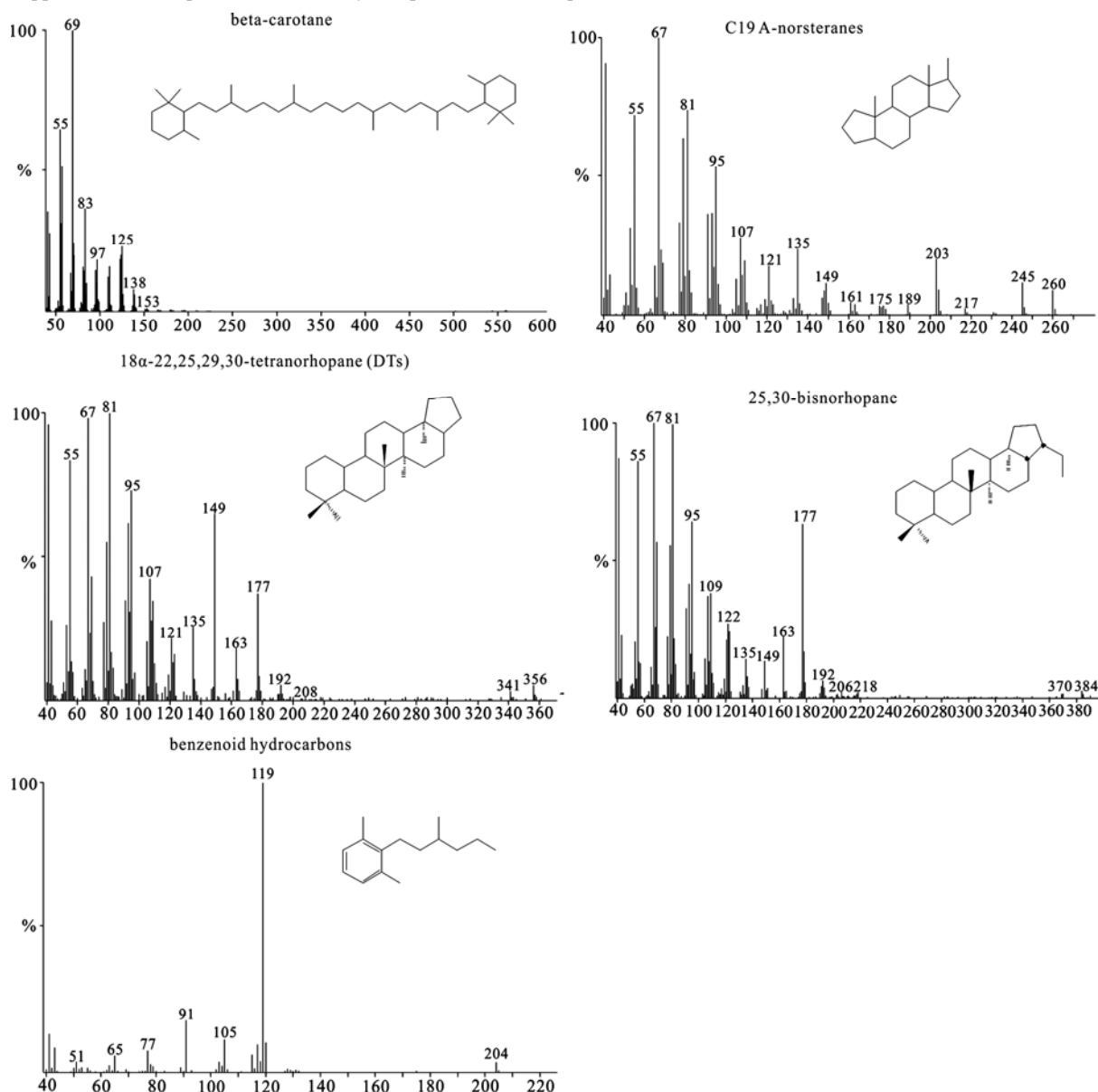
WANG Guangli, obtained his PhD degree in 2006, and currently as Associate Professor in China University of Petroleum, Beijing. His major interests include petroleum geochemistry and biomarkers. E-mail: sydxwgl@cup.edu.cn.

Appendix I Molecular composition of the crude oil samples

Sample	Caro	Hex	BC	SQ	TT	AS	Ste	Hop	Gam	NH	TI	AI	MA
B16H	C ₁₄ -C ₂₁	C ₂₁ -C ₃₀	+	C ₁₄ -C ₁₆	C ₁₉ -C ₂₆	+++	+++	C ₂₇ -C ₃₄	+	+	+++	C ₁₅ -C ₂₉	++
C27-2	C ₁₈ -C ₃₁	+	C ₁₉ -C ₂₂	+	C ₁₉ -C ₃₁	+++	++	C ₂₇ -C ₃₅	++	C ₂₈ -C ₃₄	+	C ₁₈ -C ₂₀	+++
B5	C ₁₈ -C ₂₀	+	C ₂₁ -C ₂₂	C ₁₆ -C ₂₁	C ₁₉ -C ₃₁	++	+++	C ₂₇ -C ₃₃	+	C ₂₆ -C ₃₁	++	C ₁₄ -C ₂₀	+
S99	C ₁₈ -C ₂₀	+	C ₁₉ -C ₂₂	+	C ₁₉ -C ₃₀	++	++	C ₂₇ -C ₃₅	++	C ₂₇ -C ₃₅	+	C ₁₅ -C ₂₂	++

Caro= carotenoid-derived alkanes (m/z 125); Hex= alkylcyclohexanes (m/z 83); BC= bicyclic alkanes (m/z 110); SQ= bicyclic sesquiterpane (m/z 123); TT= tricyclic terpanes (m/z 191); AS= A-norsterane (m/z 203); Ste= steranes (m/z 217); Hop= hopanes (m/z 191); Gam= gammacerane (m/z 191); NH= 25-norhopane (m/z 177); TI= tetralins and indanes (m/z 188); AI= aryl isoprenoids (m/z 119, 133); MA= monoaromatic steranes (m/z 253). +++, high; ++, medium; +, low or absent.

Appendix II Mass spectra of several key compounds in the samples.



Appendix III Mass spectra of all the $C_{14}H_{20}$ isomers with molecular weight 188 in the samples.