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Identification of C_{24} and C_{25} lanostanes in Tertiary sulfur rich crude oils from the Jinxian Sag, Bohai Bay Basin, Northern China

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ABSTRACT

Unusual short chain lanostanes (C_{24} and C_{25}) and C_{30} lanostane were identified in sulfur rich crude oils from the Jinxian Sag, Bohai Bay Basin, northern China. Besides the regular steranes (C_{27-30}), a series of 4-methyl steranes (C_{22-23}, C_{27-30}), 4,4-dimethyl steranes (C_{22-24}, C_{28-30}), short chain steranes (C_{23-26}), abundant pregnanes (C_{21-22}) and androstanes (C_{19-20}), together with sulfur containing steroids (20-thienylpregnanes and thienylandrostanes) were detected in the aliphatic and branched-cyclic hydrocarbon fraction of these crude oils. A literature survey of some long chain sterane analogues (e.g., A-nor-steranes, norcholestanes, C_{30} steranes, lanostanes) and pregnanes seems to point to a sponge and/or dinoflagellate source. 4-Methyl, 4,4-dimethyl steroids and lanosterols (4,4,14-trimethyl steroids as the basic skeleton of lanostanes) can be derived from methanotrophic bacteria. Thus, a biological origin from a prokaryotic methylotroph can be used to explain the common source of abundant short chain steranes (C_{23-26}), 4-methyl (C_{22-23}) and 4,4-dimethyl steranes (C_{22-24}), as well as lanostanes (C_{24-25} and C_{30} analogues) in our oil samples. Generally, the steroids appear to have been extensively sulfurized with sulfur substitution at the C-22 position in the side chain during the early stage of diagenesis, which was readily subject to attack by bacterial degradation (enzymatic cleavage) and/or abiotic oxidation. As a consequence, short chain sterane analogues (e.g., abundant pregnanes and androstanes in this study) and short chain lanostanes (C_{24}–C_{25}) might later be released through cleavage of weak C–S bonds at the C-22 carbon in the sulfurized steroids and lanostane sulfides. Finally, the formation of the short chain C_{24}–C_{25} lanostanes and distinctive occurrence of short chain steranes in this study can be well explained by microbial biodegradation of sulfurized lanostanoids and steroids in the reservoir.

1. Introduction

C_{30}–C_{32} lanostanes were first identified in Tertiary saline sediments and biodegraded tar sands of China (Chen et al., 1989; Chen and Summons, 2001). Murae et al. (1990) isolated several 19, 28-bisnorlanostane ketones from a plant fossil and suggested that lanosterols could be biodegraded in the early stages of diagenesis. Peng et al. (1998) reported the occurrences of C_{30} and C_{31} lanostane sulfides in a low maturity crude oil sample from China. Lanostane (C_{30}) was detected in a middle Miocene Monterey Formation (California, USA) rock sample (Finck et al., 2004) and nor-lanostane (C_{29}) and methyl-lanostane (C_{31}) were reported in a Miocene methane seep limestone sample (Pietralunga, Italy) (Peckmann et al., 2004; Birgel and Peckmann, 2008). All of these reports were associated with C_{30}–C_{32} lanostanes. Typically, it was suggested that C_{30} squalene is biologically transformed to lanosterols (C_{30}) and then to C_{27}–C_{29} sterols (Chen et al., 1989). The carbon number range of identified lanostanes (C_{30}–C_{32}) in the geological record corresponds to the carbon number of regular steranes (C_{27}–C_{30}), which are the diagenetic product of sterols, ubiquitous components of Phanerozoic sediments and crude oils.

Compared with steranes, lanostanes are rarely found in geochemical samples. Furthermore, the carbon number relationship between lanosterol (C_{30}), sterols (C_{27}–C_{29}) and steranes (C_{27}–C_{29}) cannot be used to explain the widely occurring short chain C_{21}–C_{22} steranes (e.g., androstane, Tokes and Amos, 1972; diginane and homodiginane, Wingert and Pomerantz, 1986; pregnane and homopregnane, Wingert and Pomerantz, 1986; diapregnane and diahomopregnane, Requejo et al., 1997) because we could find no report of short chain lanostanes in the geologic record. Here we report for the first time the occurrence and identification of short chain lanostanes (C_{24} and C_{25}) in crude oils from the Jinxian Sag, Bohai Bay Basin of northern China (Fig. 1).

2. Geologic setting

Two crude oils were collected from the Zhao-7 (2256.2–2280 m, E*k*) and Zhao-9 (2360.6–2390 m, E*k*) wells, in the northern part of the Jinxian Sag. Also, 17 drill core samples (2366, 2454, 2494, 2459,

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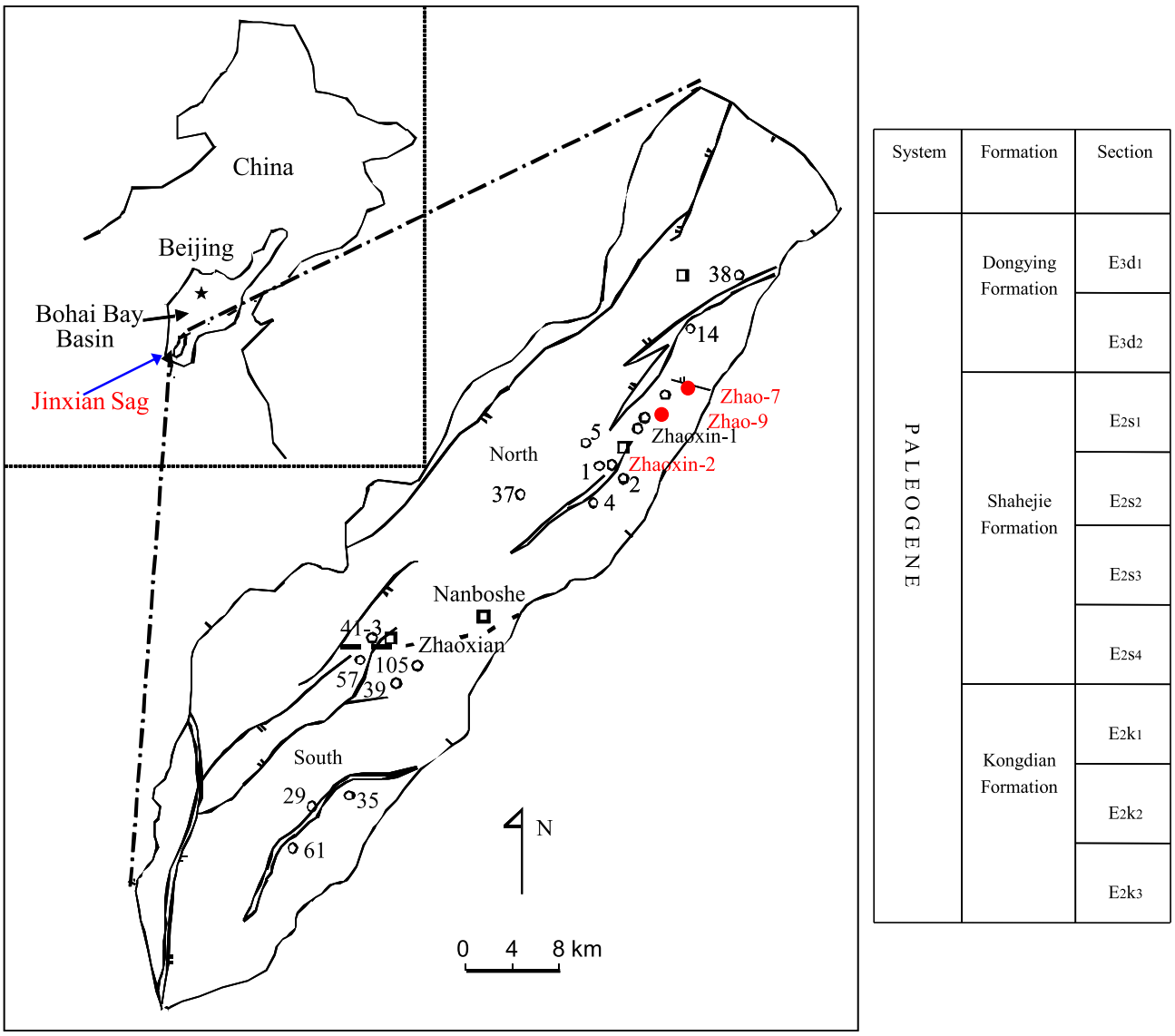

Fig. 1. Map showing the geological setting, stratigraphic section and location of wells Zhao-7 and Zhao-9 in the Jinxian Sag of the Bohai Bay Basin, northern China. A total of 17 drill core samples were collected from the nearby Zhaoxin-2 well.

2543, 2625, 2708, 2792, 2859, 2899, 2937, 2968, 2940, 3009, 3087, 3211 and 3232 m) were collected from the same formation in the Zhaoxin-2 well near the Zhao-7 and Zhao-9 wells (Fig. 1), for the purpose of comparison and source study. The Jinxian Sag is one of several faulted Eocene depressions in the Bohai Bay Basin in northern China. It is characterized by carbonates and evaporites. The sulfur content of some of the crude oils in this region can be as high as 14.7% and H_2S frequently occurs in the associated gas produced with crude oils in this region (as high as 92% of the gas in the Zhao-2 well). Paleogene sediments in the Jinxian Sag are subdivided into the Eocene Kongdian Formation (E_2k), the Oligocene Shahejie Formation (E_2s_4–E_2s_1) and the Dongying Formation (E_3d) (Fig. 1). The carbonate and evaporitic deposits in the first member of the Kongdian Formation (E_2k_1) and the fourth member of the Shahejie Formation (E_2s_4) are of late Paleocene and early Eocene age, respectively. The sediments in the E_2k_1–E_2s_4 Formations were deposited in a hypersaline lacustrine environment (Bao and Li, 2001; Cai et al., 2005; Zhang et al., 2005; Li et al., 2008; Lu et al., 2009). The structural geology, stratigraphy and petroleum geology of the Jinxian Sag were summarized in detail by Bao and Li (2001), Cai et al. (2005), Zhang et al. (2005) and Lu et al. (2009).

3. Analytical methods

The surface of the core samples was first washed thoroughly with dichloromethane (DCM) before extraction. The samples were then ground and Soxhlet extracted with DCM (72 h). Asphaltenes from crude oil and sediment extracts were removed by centrifugal precipitation with *n*-heptane (at least 40 times as much chilled *n*-heptane as the volume of extract). Aliquots of the maltenes (ca. 50 mg) were then separated by silica gel chromatography (120 °C overnight). After pre-elution with *n*-hexane on a silica gel column, gradient elution of maltenes was obtained using solvents of increasing polarity with two column volumes of solvent in all cases. Fractionation of the extracts yielded three fractions: saturated hydrocarbons (*n*-hexane), aromatic hydrocarbons (20% dichloromethane in *n*-hexane) and a polar fraction (50% dichloromethane in methanol). The solvent from each fraction was carefully removed on a sand bath (80 °C). The saturated fraction was further separated into straight chain and branched/cyclic hydrocarbon fractions using a 5 Å molecular sieve (Merck, Germany).

The saturated and aromatic fractions were initially analysed using a Micromass Platform II mass spectrometer in full scan mode

at the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China. The analysis was subsequently repeated at the Centre of Applied Organic Geochemistry, Curtin University of Technology, Perth, Australia, using an HP 5973N GC–MS system in full scan mode. Furthermore, the branched/cyclic fraction obtained from the 5 Å molecular sieve method was analysed by the HP 5973N GC–MS system in full scan mode. All ratios discussed in the paper and listed in Table 1 were computed from integrated areas of peaks in the GC–MS full scan analysis.

The Guangzhou GC–MS full scan analysis was conducted on a Micromass Platform II mass spectrometer coupled to an HP 6890 gas chromatograph. Chromatographic separations were performed using a 30 m × 0.25 mm i.d. fused silica capillary column coated with a 0.25 μm film of HP-5. The oven temperature was set at 65 °C for 1 min and then 65–290 °C at 3 °C/min and held for 30 min. Helium was used as the carrier gas with a flow rate of 1 ml/min. The transfer line temperature was 250 °C and the ion source temperature was 200 °C. The ion source was operated in the electron impact mode at 70 eV.

The Curtin GC–MS analyses of the saturated/aromatic and branched/cyclic fractions were carried out on an HP 5973 Mass Selective Detector (MSD) attached to an HP 6890 gas chromatograph. The column used was a fused silica capillary column (60 m × 0.25 mm i.d.) with a 0.25 μm 5% phenyl-methyl-silicon stationary phase (DB-5). The gas chromatograph oven was programmed from 40 °C to 315 °C at 3 °C/min, with an initial and final hold time of 1 and 30 min, respectively. Samples were dissolved in hexane and injected on-column by an HP 6890 auto-sampler. Helium was used as carrier gas.

4. Results and discussion

4.1. Identification and structural elucidation of C_{24} and C_{25} lanostanes

During routine GC–MS analysis it was noted that the saturated fractions of oil from the Zhao-7 (2256.2–2280 m, E*k*) and Zhao-9 (2360.6–2390 m, E*k*) wells contain three compounds with a strong fragment at *m/z* 259 (mass chromatograms are shown in Fig. 2e). Careful inspection of the mass spectral data for these three compounds showed the *m/z* 259 fragment as well as an unusual and abundant ion at *m/z* 190 (Fig. 3). Based on comparison with the previously published mass spectra of authentic lanostanes (Philp, 1985; Chen et al., 1989), the compound with a molecular ion at *m/z* 414 (Fig. 3a) is identified as C_{30} lanostane. The other two peaks with molecular ions at *m/z* 330 and 344, respectively (Fig. 3b and c), are tentatively identified as C_{24} and C_{25} lanostanes, based on elution time and by analogy with the mass spectrum of the C_{30} component. The $C_{24–25}$ lanostanes eluted between homopregnane and the C_{26} steranes.

Table 1
Detected biomarkers and organic geochemical parameters in the crude oils from the Zhao-7 and Zhao-9 wells in the Jinxian Sag, Bohai Bay Basin, northern China.

Detected compounds	Parameter/well	Zhao-7	Zhao-9
Isoprenoids	Pristane/phytane (Pr/Ph)	0.34	0.21
	Pr/*n*-C_{17}	0.29	0.28
	Ph/*n*-C_{18}	0.89	0.92
Hopanes	C_{29}Ts/C_{29}-hopane	0.11	0.22
	C_{29}-hopane/C_{30}-hopane	0.51	0.5
	C_{31}-hopane 22*S*/(22*S* + 22*R*)	0.57	0.64
	C_{32}-hopane 22*S*/(22*S* + 22*R*)	0.47	0.53
	C_{31}-hopane/$C_{31+32+33+34+35}$	0.41	0.46
	C_{32}-hopane/$C_{31+32+33+34+35}$	0.26	0.19
	C_{33}-hopane/$C_{31+32+33+34+35}$	0.11	0.11
	C_{34}-hopane/$C_{31+32+33+34+35}$	0.10	0.08
	C_{35}-hopane/$C_{31+32+33+34+35}$	0.13	0.17
Gammacerane	Gammacerane/C_{30}-hopane	0.99	0.54
Regular Steranes (C_{27}–C_{30})	C_{27}/$C_{27+28+29}$ sterane	0.51	0.43
	C_{28}/$C_{27+28+29}$ sterane	0.23	0.22
	C_{29}/$C_{27+28+29}$ sterane	0.26	0.34
	C_{29}-sterane αββ/(ααα + αββ)	0.68	0.76
	C_{29}-sterane 20S/(20S + 20R)	0.53	0.58
Norcholestane (C_{26})	C_{26}/C_{27} sterane	0.61	0.39
	C_{26}/C_{28} sterane	1.32	0.74
	C_{26}/C_{29} sterane	1.2	0.48
Pregnanes (C_{20}–C_{22})	C_{21+22}/$C_{27+28+29}$ sterane	1.55	1.17
Short chain steranes (C_{23}–C_{25})			
A-nor-steranes (C_{19}–C_{20})	(androstanes)		
A-nor-steranes (C_{26}–C_{28})			
4-Methylsteranes (C_{22}–C_{23})			
4-Methylsteranes (C_{27}–C_{29})			
4-Methylsteranes (C_{30})			
4,4-Dimethylsteranes (C_{22}–C_{24})			
4,4-Dimethylsteranes (C_{28}–C_{30})			
Lanostanes (C_{24}–C_{25})			
Lanostanes (C_{30})			
Aromatic steroids	Monoaromatic steroids		
	Methyl-triaromatic steroids		
Sulfur containing steroids	C_{30} (20-thienylpregnane)		
	C_{30} (thienylandrostane)		
	C_{29} (sterane thiophene)		

Note: The ratios of terpenoids and steroids listed in the table are computed from integrated areas of peaks in the chromatograms of *m/z* 191 and *m/z* 218 in the GC–MS full scan analysis. The ααα and αββ isomers were used to compute the abundance of C_{26} norcholestanes relative to C_{27}, C_{28} and C_{29} steranes.

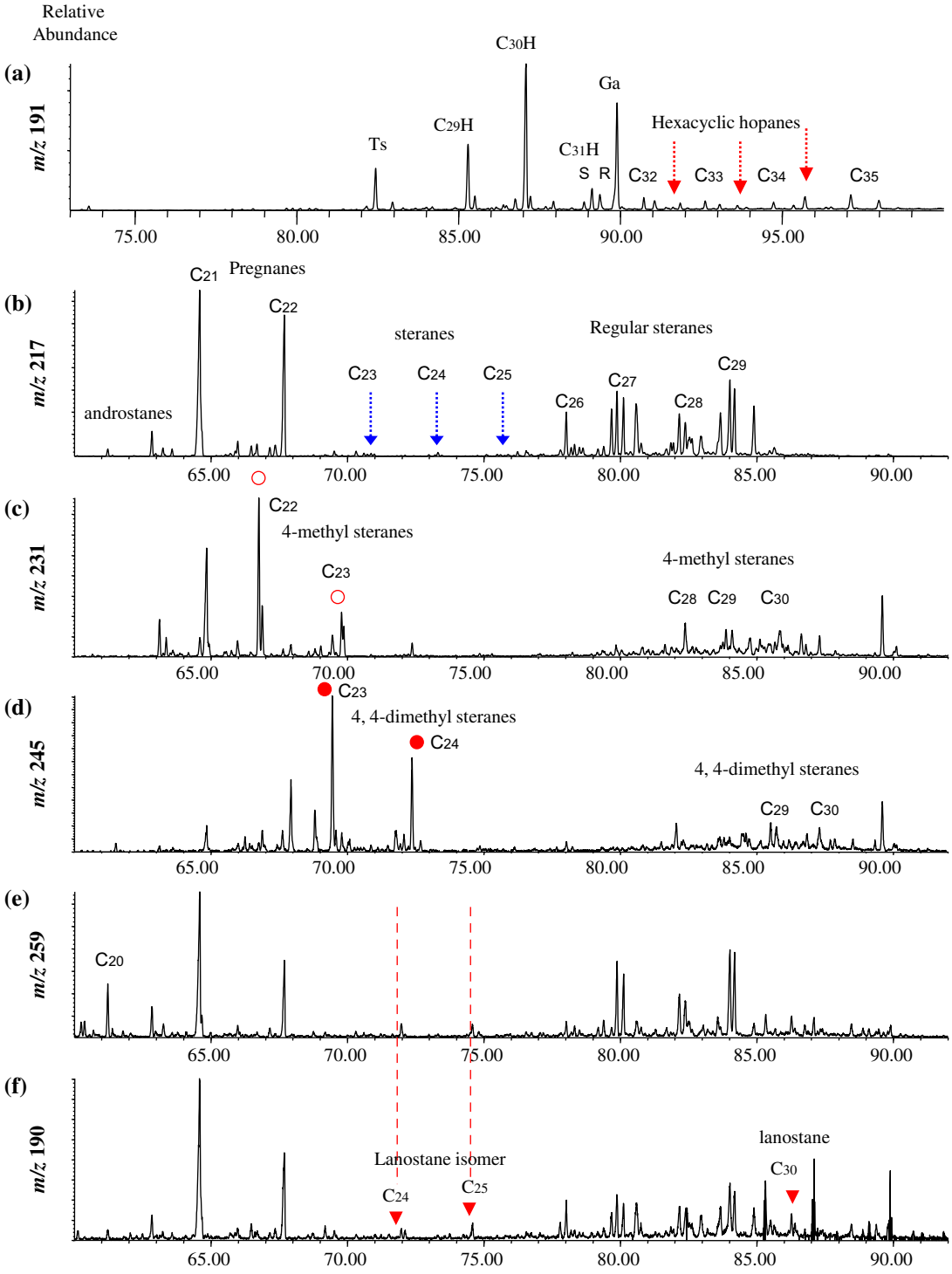

Fig. 2. *M/z* 191, 217, 231, 245, 259 and 190 chromatograms from the full scan analysis (Agilent 5973N) of the saturated fraction of crude oil from the Zhao-9 well in the Jinxian Sag, Bohai Bay Basin, northern China.

In structural elucidation efforts, Muccino and Djerassi (1974) used electron impact induced fragmentation of ring D in lanostanes and in 14α-methylcholestane with extensive deuterium labelling of the steroid nucleus to show that major differences result from the presence of the C-14 methyl group. Unlike cholestane, cleavage of lanostane ring D involves methyl loss from a partial ring D fragmentation and a minor cleavage process occurs with a single hydrogen transfer (contributed predominantly from the C-32 position). In contrast to steroids lacking a 14α-methyl group, partial ring D cleavage plays a dominant role in directing all subsequent fragmentation. The base peak *m/z* 259 in the mass spectra of lanostanes (C_{30}) corresponds to the substituted ring without the side-chain, thus it is not as strong as fragments such as *m/z* 55. Another notable feature of these spectra is the diminished intensity of the parent and M–CH_3 ion, indicative of the increased substitution of ring D, whose preferential rupture could trigger new and important fragmentation sequences (Muccino and Djerassi, 1974).

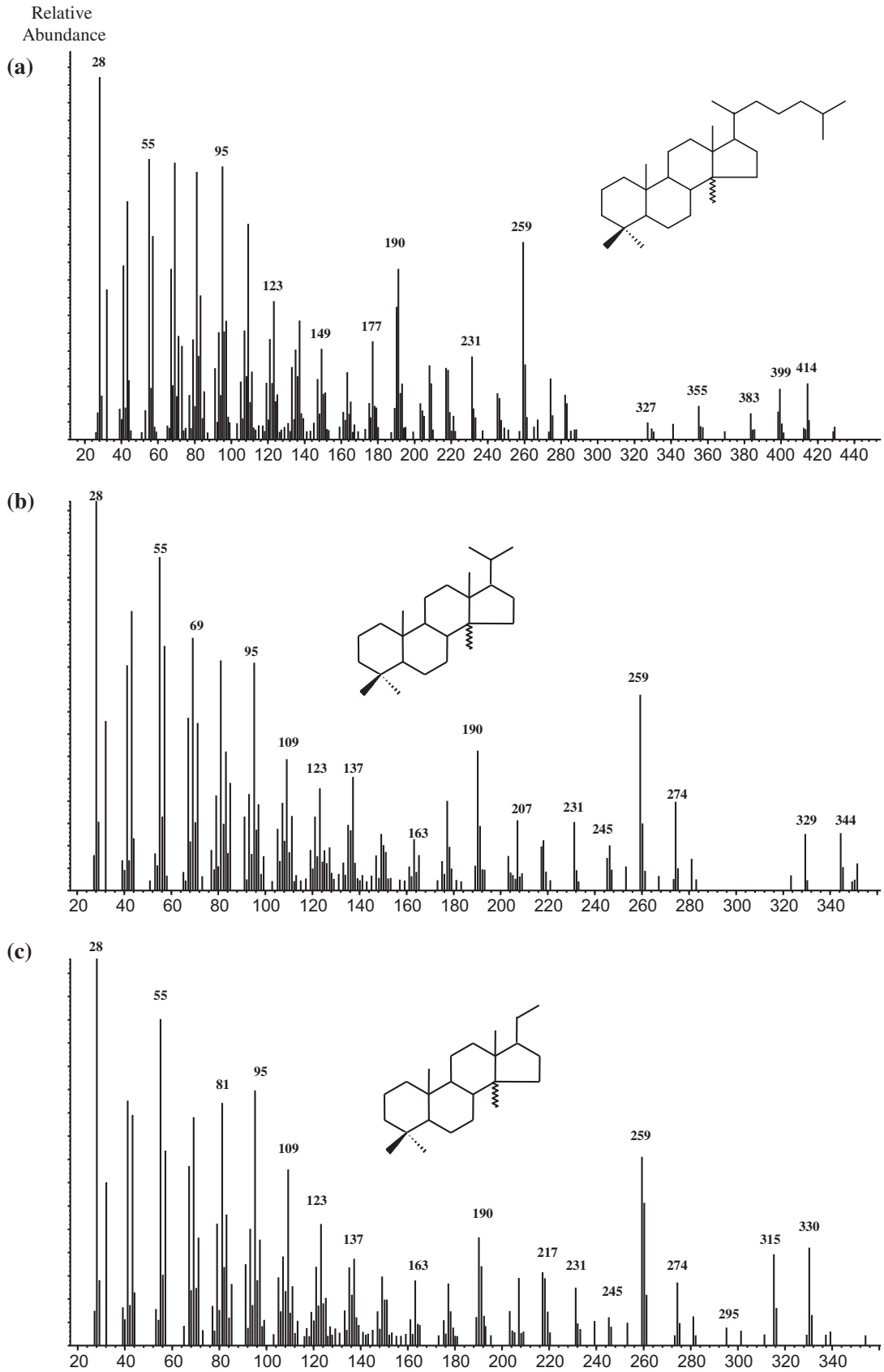

Fig. 3. Mass spectra (Agilent 5973N) for the C_{30}-, C_{25}- and C_{24}-lanostanes detected in the saturated fraction of crude oil from the Zhao-7 well in the Jinxian Sag, Bohai Bay Basin, northern China.

A pronounced *m/z* 274 ion is also observed in the lanostane mass spectra, representing 45–50% of the base peak, corresponding to partial ring D cleavage (loss of the side chain together with a C_2H_3 moiety). Two intense peaks at *m/z* 231 and *m/z* 190 in the spectra are due to the presence of the 14α-methyl substituent. The loss of ring C and hydrogen from C-7 and C-9 resulted in the occurrence of the ionized butadiene having *m/z* 190. The C_{24} and C_{25} lanostanes have mass spectra roughly consistent with published spectra of C_{30} lanostane (Philp, 1985; Chen et al., 1989) and their occurrence in these samples with lanostane (C_{30}) is strong support for these assignments.

4.2. General geochemical characteristics

The GC–MS distributions of the aliphatic hydrocarbons in the crude oils from the Zhao-7 and Zhao-9 wells are given in Fig. 4. The crude oil from the Zhao-7 well shows *n*-alkanes ranging from C_{11} to C_{26} and maximizing at *n*-C_{17}. There is no obvious odd/even carbon number preference. There is, however, a predominance of short chain *n*-alkanes, suggesting input from algae/bacteria. In contrast, the aliphatic hydrocarbon fraction of the crude oil from the Zhao-9 well displays *n*-alkanes ranging from C_{11} to C_{26}, maximizing at *n*-C_{18}, with an obvious even/odd carbon number preference. We interpret this to indicate a saline carbonate environment for the source rock (Bao and Li, 2001; Cai et al., 2005; Zhang et al., 2005; Li et al., 2008; Lu et al., 2009).

The regular isoprenoids range from C_{13} to C_{20} and are dominated by phytane in both oils. The pristane/phytane ratios of the two crude oil samples are 0.34 and 0.21, respectively. The Pr/*n*-C_{17} ratios of the two crude oil samples are 0.28 and 0.29 respectively. The Ph/*n*-C_{18} ratios of the two crude oil samples are 0.89 and 0.92, respectively (Table 1). Chlorophyll *a* is the major natural precursor for these isoprenoids, but other sources can be included, such as tocopherol for pristane (Goossens et al., 1984)

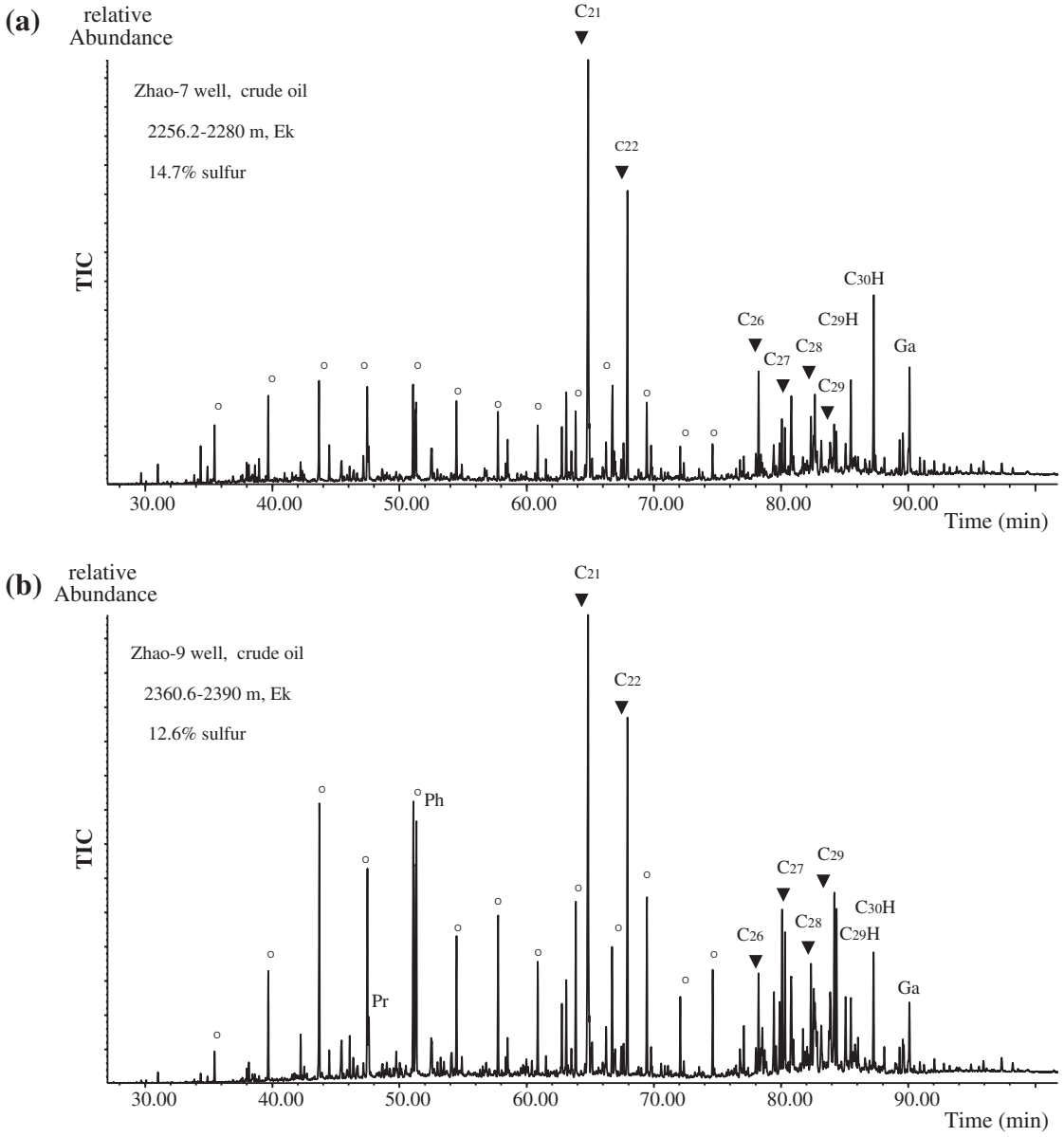

Fig. 4. TIC chromatograms (Agilent 5973N) of the saturated hydrocarbon fractions of crude oils from the Zhao-7 (a) and Zhao-9 (b) wells in the Jinxian Sag, Bohai Bay Basin, northern China. Components shown are *n*-alkanes (labelled as ○); C_{21}–C_{29} steranes (labelled as ▼); C_{29}H, hopane; C_{30}H, C_{30} hopane; Ga, gammacerane; Ph, phytane; Pr, pristane.

and halophilic archaea for phytane as reported in studies of Dead Sea organic matter (Nissenbaum et al., 1972).

The *m/z* 191 chromatogram of the aliphatic hydrocarbon fraction of crude oil from Zhao-9 well (Fig. 2a) showed that the following compounds are identified: 17α(H)-22,29,30-trisnorhopane (Ts), 17α(H),21β(H)-norhopane (C_{29}H), 17α(H),21β(H)-hopane (C_{30}H), homohopane (C_{31}H), bishomohopane (C_{32}H), trishomohopane (C_{33}H), tetrakishomohopane (C_{34}H) and pentakishomohopane (C_{35}H). In addition, C_{33}–C_{34} hexacyclic hopanes, alkyl benzothiophenes and alkyl dibenzothiophenes were also detected in both oil samples, and we attribute this to an anoxic carbonate-anhydrite paleoenvironment (Connan and Dessort, 1987; Hughes et al., 1995). Abundant gammacerane (Ga) in the samples (Fig. 2a, Table 1) pointed to a stratified water column environment (Sinninghe Damsté et al., 1995). Gammacerane is thought to have originated by the dehydration and hydrogenation of tetrahymanol (ten Haven et al., 1989). An alternative route involves natural sulfurization of the functionalized precursor and/or its corresponding ketone, followed by the early thermal release of gammacerane (Sinninghe Damsté et al., 1995). Tricyclic terpanes (C_{19}–C_{26}) that ordinarily occur widely, were surprisingly absent in these oil samples. Similarly, there was no report in the relevant work of the occurrence of lanostanes in prokaryotic methylotrophs (Peckmann et al., 2004; Lamb et al., 2007; Birgel and Peckmann, 2008).

A wide variety of abundant steranes (with different base peaks and carbon number ranges), aromatic steroids and S-containing steroids, were present in these two oils. They are listed in Table 1 and roughly described as follows.

(1) Regular steranes (*m/z* 217/218, C_{20}–C_{30})

The regular C_{27}–C_{30} steranes in the two crude oils are dominated by the C_{27} series (Fig. 2b). Furthermore, 5α(H),14β(H),17β(H)–20*S*, 20*R* isomers are present in much greater amounts than their 5α(H),14α(H),17α(H)–20*S*, 20*R* counterparts. The ratios of αββ/(ααα + αββ) (0.68 and 0.76), together with ααα–C_{29} steranes 20*S*/(20*S* + 20*R*) (0.5 and 0.6) for the C_{29} steranes in the two crude oils indicate equilibrium. In addition, the C_{27} sterenes (MW = 370) likely having a double bond within the ring system (Lu et al., 2009) were detected in both oils.

C_{26} norcholestanes with αββ configuration are present in both oils (Fig. 2b). Based on the *m/z* 217 and 218 mass chromatograms and tentatively identified C_{26}–C_{28} 21-norcholestanes in Eocene evaporitic sediments in the same Jinxian Sag (Bao and Li, 2001), we tentatively identify the C_{26} component as 20*R*–21-norcholestane.

Unusual C_{25} (MW = 344), C_{24} (MW = 330) and C_{23} (MW = 316) steranes with αββ configuration were also detected in these two oils (Fig. 2b). They were previously reported in saturated hydrocarbon fractions after fermentation of immature organic matter (Ding et al., 2001). The most abundant steranes, pregnanes (C_{20-22}) with αββ configuration were present in the aliphatic hydrocarbon fraction of the two oils (Figs. 2b and 4). The short chain steranes (C_{21}–C_{22}) are regarded as an indicator of a hypersaline environment (ten Haven et al., 1985; Requejo et al., 1997), or derivation from the side chain cleavage of regular steranes during thermal evolution (Wingert and Pomerantz, 1986; Huang et al., 1994; Requejo et al., 1997). Their precursors, the sterols with short side chains (namely C–0 to C–6 side chains) have been reported as trace constituents in marine organisms, such as gorgonians and sponges (Carlson et al., 1978; Delseth et al., 1978).

(2) 4-Methyl steranes (*m/z* 231/232, C_{22-23}, C_{27-30})

C_{27}–C_{30} and C_{22}–C_{23} 4-methyl steranes were also present in these two oils (Fig. 2c) and short chain species apparently dominated their distribution.

(3) 4,4-Dimethylsteranes (*m/z* 245/246, C_{28-30}, C_{22-24})

A series of 4,4-dimethylsteranes (C_{28-30}, C_{22-24}) were observed with a base peak at *m/z* 245 and 246, and ions at *m/z* 177 (Fig. 2d). The enhanced intensity of the *m/z* 177 ion indicates a 4,4-dimethyl not a 4,14-dimethyl moiety (Kimble et al., 1974). The 330 Da compound was tentatively identified as a 4,4-dimethyl-5α(H),14β(H),17β(H)-homopregnane by ten Haven et al. (1985).

(4) Androstane (*m/z* 203, C_{19-20})

Androstanes (C_{19-20}) were present in both oils (Fig. 2b). C_{26}–C_{28} A-nor-steranes with base peaks at *m/z* 203 and molecular ions at *m/z* 358, 372 and 386 were previously identified in some Cretaceous black shales by van Graas et al. (1982). They were regarded as biomarkers for certain types of sponges, because sterols with a 5α(H)-A-nor-nucleus and 3β-hydroxymethyl group have been found as major sterols in several sponges (van Graas et al., 1982). The C_{19} and C_{20} androstanes detected in this study should be short chain species of those C_{26}–C_{28} A-nor-steranes.

(5) Sulfur containing steroids

Abundant sulfur containing steroids were also detected in the aliphatic and branched/cyclic hydrocarbon fractions of these two oils, possibly due to over elution by hexane because of the low percentage of the saturated hydrocarbon fraction (14.5%). The sulfur containing steroids were composed mostly of 20-thienylpregnanes (base peak = 167, MW = 426), thienylandrostanes (B.P. = 383, MW = 426) and sterane thiophene (B.P. = 341, MW = 412), based on mass spectra comparison (Schmid, 1986).

5. Discussion

Owing to their low concentrations, it was not possible to obtain stable carbon isotope values for the C_{24}-, C_{25}- and C_{30}-lanostanes. Lanosterol and other 4-methylated steroids are the most likely precursors of lanostane (Birgel and Peckmann, 2008). Lanosterol is biosynthesized as an intermediate or accumulated natural product in a very large number of extant taxa, including plants, animals, fungi, dinoflagellates, sponges and bacterial methylotrophs (Lamb et al., 2007). First, the C_{24} and C_{25} lanostanes in our oils are probably not derived from higher plants, because no high carbon numbered *n*-alkanes ($\geqslant n$-C_{27}) were detected (Fig. 4). Second, among the microalgae, there are no reports of lanosterol in diatoms. Geiner et al. (1991) reported that five species of dinoflagellates produced lanosterol. C_{30} 4-methyl steranes and C_{26} norcholestanes are present in our oils and this supports a dinoflagellates origin for the lanostanes. However, there appear to be more reports of the occurrence of lanosterol in marine and freshwater sponges or other non-photosynthetic organisms (Chaffee et al., 1986). Thus, we consider that the C_{24} and C_{25} lanostanes in our samples were derived mainly from sponges and/or dinoflagellates, as indicated by the following: (1) In our literature survey (Van Graas et al., 1982; Wolff et al., 1986; Summons et al., 1987; Chen et al., 1989; Moldowan et al., 1991; Volkman et al., 1993; Peters et al., 2005), all reports of long chain steranes (A-nor-steranes, norcholestanes, 4-methyl steranes and lanostanes) and pregnanes refer to a sponge and/or a dinoflagellate source; (2) lanosterol and cycloartenol in some sponges are both transformed to 4,4,14-trimethyl sterols (Kerr et al., 1989), the basic skeleton of lanostanes; (3) although some sponges are incapable of *de novo* biosynthesis, they can also selectively modify the molecular nucleus to give rise to unique A-nor or 19-nor skeletons (Kerr et al., 1989), hydrocarbon equivalents of which are described as androstane, which is present in our two oil samples; (4) short chain sterols have been more frequently identified in gorgonians and sponges than in dinoflagellates (Carlson et al., 1978). For

example, short side chain sterols of the androstane and pregnane type, and of the 3-hydroxy-pregnan-20-type, were found in sponges (*H. flavescens*) from the Black Sea (Elenkov et al., 1999); (5) an unusually large number of C_{21} and C_{22} short chain steranes, counterparts of short chain sterols, and much more abundant in sponges (Carlson et al., 1978), are distinctly dominant in our samples.

4-Methyl and 4,4-dimethyl steroids have been identified in cultures of methanotrophic bacteria (Bouvier et al., 1976; Schouten et al., 2000). One aerobic methanotroph (*Methylococcus capsulatus*) is known to have the unusual ability to synthesize 4-methylated steroids (Bird et al., 1971; Bouvier et al., 1976; Summons et al., 1994). Other bacteria, e.g., *Methylobacterium organophilum*, have been reported to contain 4,4-dimethylsterols, including lanosta-8, 22, 24-trien-3β-ol (Patt and Hanson, 1978). Another methylotroph, *Methylosphaera hansonii*, containing the same 4-methyl and 4,4-dimethylsterols, as well as lanosterol and lanost-8(9)-en-3β-ol, was reported in sediments from an Antarctic lake (Schouten et al., 2000). In that case, these compounds were isolated and proven not to have been derived from eukaryotes, but rather from a prokaryotic methylotroph on the basis of the $\delta^{13}C$ values (Schouten et al., 2001). The sterols of methanotrophic bacteria (e.g., *Methylococcus capsulatus*) encompass primitive C–4 monomethylated and C–4 dimethylated structures derived from the lanostane carbon skeleton (Bouvier et al., 1976). Lanosterols were also found in a prokaryotic methylotroph. Lanosterol biosynthesis occurs in the prokaryote *Methylococcus capsulatus* (Lamb et al., 2007). Lanostanes with a depleted ^{13}C signature, reflecting a major incorporation of methane carbon, were also found in a fossilized microbial mat preserved in a Miocene methane seep limestone (Pietralunga, Italy), believed to originate from methylotrophic bacteria (Peckmann et al., 2004). The 4,4,14-trimethyl steroids share the basic skeleton of lanostanes. Thus, from a viewpoint of methyl moieties, an origin from a prokaryotic methylotroph can be used to explain the detected 4-methyl (C_{22-23}, C_{27-30}), 4,4-dimethyl steranes (C_{22-24}, C_{28-30}) and lanostanes (C_{24-25}, C_{30}, with 4,4,14-trimethyl moieties) in this study.

The C_{26}–norcholestanes in our samples most likely originated by bacterially mediated alteration of C_{27} sterols, as suggested by Moldowan et al. (1991). Low molecular weight C_{21}–C_{25} steranes and C_{22}–C_{26} methyl steranes were reported in saturated hydrocarbon fractions derived from fermentation of immature organic matter (Ding et al., 2001), which also indicates a bacterial source for the unusual short chain steranes (C_{20}–C_{26}) in this study. Short chain lanostanes (C_{24} and C_{25}) were not detected in the core samples from the Zhaoxin-2 well in the vicinity of the Zhao-7 and Zhao-9 wells (Fig. 1) but were present only in the crude oil samples, indicating an origin by biodegradation in the reservoir (Peckmann et al., 2004; Birgel and Peckmann, 2008). Furthermore, a high concentration of H_2S associated gas in this region (92% volume/volume in the Zhao-2 well), especially in the oil reservoir of this study, was once attributed to microbial sulfate reduction (Cai et al., 2005; Worden and Cai, 2006), although thermochemical sulfate reduction (TSR) as a mechanism was debated for this region (Zhang et al., 2005, 2006). Sulfate reducing bacteria generally live in metabolic association with the methanotrophic bacteria, which may help to explain the methanotrophic source of some lanostanes in this study.

The dominance of short chain steranes is a distinctive characteristic of our oil samples, e.g., extremely high $C_{21}+C_{22}$ pregnane/C_{27}–C_{29} values as shown in Table 1. The concentration of pregnanes was higher than that of regular steranes, phytane and *n*-alkanes, as shown in Figs. 2 and 4. Moreover, concentrations of short chain 4-methyl and 4,4-dimethyl steranes (C_{22}–C_{24}) were also apparently higher than their higher carbon number (C_{27}–C_{30}) analogues. Thus, further explanation for the carbon number range was needed for the distribution of short chain steroids and short chain lanostanes in this study.

It has been suggested that thermal degradation of regular steranes (C_{27}–C_{29}) is responsible for the formation of C_{21} and C_{22} short chain steranes (Huang et al., 1994), but this explanation cannot apply to the low maturity crude oils having C_{27} sterenes in this study. The incorporation of sulfur into precursor molecules at an early stage of diagenesis leads to formation of sulfur rich macromolecules (Adam et al., 2000; Kok et al., 2000). Previous work on samples from Ace Lake in Antarctica (Kok et al., 2000) illustrated that the sulfurization process seems to be highly substrate specific and of the many compound types available for sulfurization, only the steroids seem to have been sulfurized extensively. Thus, diagenetic sulfur substitution has potentially occurred in biological functionalized steroids at the C-22 site. Abiotic oxidation or enzymatic cleavage of precursor molecules may also occur at this carbon. Therefore, carbon in the C-22 position in the side chain of steroids is readily attacked via reservoir biodegradation. As a consequence, short chain sterane analogues might later be released through cleavage of weak C–S bonds. Typically, the presence of sulfur containing steroids (20-thienylpregnanes and thienylandrostanes) provides strong support for this enzymatic cleavage mechanism of androstanes and pregnanes in our oils.

Likewise, this mechanism can be used to explain the formation of short chain lanostanes in this study, which might represent cleavage of either side of the C-22 carbon, a position shown to contain a sulfur linkage in lanostane sulfides reported by Peng et al. (1998). Finally, in subsurface oil pools with high levels of sulfur (as high as 14.7%) and H_2S associated gas (as high as 92%), biodegradation of sulfurized lanostanoids and steroids within the reservoir can be used to explain the formation of the short chain C_{24}–C_{25} lanostanes and the distinctive occurrence of short chain steranes in this study.

6. Conclusions

Unusual short chain lanostanes (C_{24} and C_{25}) and C_{30} lanostane were identified in sulfur rich crude oils from the Jinxian Sag, Bohai Bay Basin, northern China. Besides the regular steranes (C_{27}–C_{30}), a series of 4-methyl steranes (C_{22-23}, C_{27-30}), 4,4-dimethyl steranes (C_{22-24}, C_{28-30}) and short chain steranes (C_{23-26}) and abundant pregnanes (C_{21-22}) and androstanes (C_{19-20}), together with sulfur containing steroids (20-thienylpregnanes and thienylandrostanes), were detected in the aliphatic and branched/cyclic hydrocarbon fraction of these crude oils. The saturated and aromatic hydrocarbon fractions of 17 drill core samples in the vicinity of these oil pools were analysed by GC–MS in order to investigate the possible source, origin and formation mechanism of these short chain lanostanes.

The literature survey of some long chain steranes (e.g., A-norsteranes, norcholestanes, C_{30} steranes, lanostanes) and pregnanes seems to point to a sponge and/or dinoflagellate source. 4-Methyl (Bird et al., 1971; Bouvier et al., 1976; Summons et al., 1994), 4,4-dimethyl steroids (Bouvier et al., 1976; Patt and Hanson, 1978) and lanosterols (Patt and Hanson, 1978; Schouten et al., 2000) can be derived from methanotrophic bacteria. Furthermore, 4,4,14-trimethyl steroids share the basic skeleton of lanostanes. Thus, from the viewpoint of methyl moieties, a biological origin from a prokaryotic methylotroph can be used to explain the common source of abundant short chain steranes (C_{23-26}), 4-methyl (C_{22-23}) and 4,4-dimethyl steranes (C_{22-24}), as well as lanostanes (C_{24-25} and C_{30} analogues) in our samples. However, further interpretation from the viewpoint of carbon number ranges of these compounds was apparently needed, as short chain analogues dominated the steroids and lanostanes in these oils.

During early diagenesis, the steroids seem to have been extensively sulfurized and the C-22 site was the location of diagenetic sulfur substitution and abiotic oxidation or enzymatic cleavage of the precursor molecule. Thus the C-22 position in the side chain was easily attacked during biodegradation. As a consequence, short chain sterane analogues might have been released later through the cleavage of the weak C–S bonds. In this study, an unusual abundance of pregnanes and androstanes emerged from sulfur containing steroids-20-thienylpregnanes and thienylandrostanes in our samples. Likewise, short chain lanostanes (C_{24}–C_{25}) could have originated from the cleavage of either side of the C-22 carbon in lanostane sulfides. In the Jinxian Sag, biodegradation (e.g., H_2S directly generated from biological sulfate reduction) might be possible in subsurface oil pools with levels of sulfur as high as 14.7% and H_2S associated gas as high as 92%. Finally, from the viewpoint of carbon numbers, biodegradation of sulfurized lanostanoids and steroids in the reservoir can be used to explain the formation of the short chain C_{24}–C_{25} lanostanes and the distinctive occurrence of short chain steranes in this study.

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