Long-term, low temperature simulation of early diagenetic alterations of organic matter from conifers: Aliphatic hydrocarbons

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Early diagenetic alterations of organic matter from conifers were simulated at low temperature of 80°C for five and a half years. Their diagenetic products of aliphatic hydrocarbons were identified and quantified. The results reveal that in the early sedimentation of organic matter from conifers, *n*-alkanes have developed; natural terpenoids have transformed into diagenetic terpenoids, in which some seldom reported evolutionary intermediates—two cadinatetraenes, three norabietatetraenes and one abietatetraene—were detected and identified. The diagenetic pathways for cadalane-type sesquiterpenoids and abietane-type diterpenoids are proposed. The experimental data also show that the diagenesis of aliphatic hydrocarbons is influenced by different inorganic environments. The influences can be concluded as follows: high salinity is propitious to generate and expulse aliphatic hydrocarbons; iron may promote the increase of alkanes, but interrupt the alterations of terpenoids; gypsum, when not coexisting with metal, may slow the degradation from long chain alkanes to short ones; and heavy metal probably delays the early diagenesis of both alkanes and terpenoids. These influences observed under low temperature may enrich studies on early diagenesis of organic matter from higher plants.

Keywords: coniferous organic matter, early diagenetic alternations, aliphatic hydrocarbons, inorganic environment, low temperature simulation

INTRODUCTION

Early diagenesis of terrestrial organic matter from higher plants, in modern sediments, peat, lignite, etc., is one of focuses in geological research (Cranwell, 1985; Del Rio *et al.*, 1992; Dehmer, 1995; Pancost *et al.*, 2002; Hajje and Jaffe, 2006), in which some important discoveries about aliphatic hydrocarbons from higher plants have been reported: *n*-alkanes mainly originate from cuticle waxes and alcohols in higher plants and the dominance of long chain *n*-alkanes with odd carbon number predominance can indicate higher plant origin (Dehmer, 1995). Terpenoids are common constituents of the resins of higher plants, and they are important biomarkers for higher plants, especially for conifers. For example, the diterpenoids with skeletal structure of abietanes, pimaranes and kauranes are nonspecific markers of gymnosperm plants (Otto *et al.*, 1997); the sesquiterpenoids with skeletal structure of the cedranes and cuparanes are specific markers of the species of the *Cupressaceae* (Grantham and Douglas, 1980; Otto and Wilde, 2001); and triterpenoids of oleanane, ursane and lupane classes are biomarkers for angiosperms (Otto *et al.*, 2005). Such

terpenoids, after undergoing complex geochemical and geological transformations, widely exist in the geosphere and their diagenetic pathways are also the interest of some researchers. Simoneit *et al.* (1986) reported that retene may originate from abietic acid and abietadienes; Otto *et al.* (1997) suggested retene may originate from other functionalized abietane-type diterpenoids such as taxodone, dehydrosugiol and ferruginol.

The varied diagenetic pathways of terpenoids are reported to be related to diverse sedimentary environments. For instance, anoxic environments appear to be associated with formation and preservation of aromatic terpenoids (Simoneit and Mazurek, 1982). Oxygenated environments appear to be associated with hydrogenated analogues (Simoneit *et al.*, 1986). Later investigations (Martin *et al.*, 1999) also demonstrated that, through the anaerobic degradation by undefined microorganisms, aromatized and decarboxylated diterpenoids (such as retene) could be generated from resin acids (tricyclic diterpenoids).

However, because of the complexity of biogeochemical processes in the geosphere, investigating the origin and diagenetic pathways of organic matter from higher plants and assessing the influences of sedimentary environments on their diagenesis are difficult. Therefore, on the hypothesis that short-term heating at high temperature can result in similar changes of organic

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Table 1. Sample composition

Sample	Description
P	Contemporary remains from <i>Cedrus deodara</i>
C	Contemporary remains from <i>Platycladus orientalis</i>
A ₂	Organic matter from conifer remains, 50 g; Kaolin, 400 g; Quartzose sand, 400 g
A ₆	Organic matter from conifer remains, 50 g; Kaolin, 400 g; Quartzose sand, 400 g; Iron powder, 20 g; Copper powder, 20 g; Gypsum, 200 g
A ₇	Organic matter from conifer remains, 50 g; Kaolin, 400 g; Quartzose sand, 400 g; Lead powder, 20 g; Zinc powder, 20 g; Gypsum, 200 g
A ₉	Organic matter from conifer remains, 50 g; Kaolin, 400 g; Quartzose sand, 400 g; Copper powder, 20 g; Iron powder, 20 g; Lead powder, 20 g; Zinc powder, 20 g; Gypsum, 197g
A ₁₁	Organic matter from conifer remains, 50 g; Kaolin, 400 g; Quartzose sand, 400 g; Gypsum, 200 g
A ₁₂	Organic matter from conifer remains, 50 g; Kaolin, 400 g; Quartzose sand, 400 g; Salt, 50 g

matter caused by the time-consuming geochemical evolution, thermal simulation experiments have been carried out in the laboratory to study the early generation of hydrocarbons (Shi *et al.*, 1988, 2001; Zhang, L. Y. *et al.*, 1999; Huo *et al.*, 2002). But most of these experiments were conducted at high temperature (200 to 500°C) and for short duration (several hours to days). These experimental conditions may induce some side reactions because of an excessive supply of energy and deprive the researchers of observing the influences of biochemical and inorganic interactions on the early diagenesis of organic matter usually occurring at low temperature (Harvey *et al.*, 1995; Zhang, Z. L. *et al.*, 1999). So a long-term simulation experiment at low temperature (80°C at this temperature, the diagenesis of organic matter can be accelerated while most bacteria are still active) was designed.

So far, the simulated experiment has run continuously for five and a half years and some achievements have been made: The fact that immature oil can generate within a short time after sedimentation was discovered and the influences of inorganic matter such as metals (Fe, Pb, Zn, Cu), gypsum (CaSO₄) and salt (NaCl) on the generation were discussed (Sun *et al.*, 2006). The early generation of hydrocarbon from algae was investigated and influences of inorganic environments on it were elaborated (Qin *et al.*, 2008). The formation of macerals of organic matter from conifers has been analyzed by microscope and conclusions that cutinite, suberinite and huminite have been formed only after one year's simulation, and that cutinite and suberinite always keep good shape and strong fluorescence in the latter simulation were drawn (Sun *et al.*, 2008).

In the present stage of this simulation experiment, the paper tries to investigate the early diagenetic characteristics of aliphatic hydrocarbons (alkanes and terpenoids) from coniferous organic matter under different inorganic environments, which may provide valuable data and better understanding to early diagenesis of organic matter from higher plants.

MATERIALS AND METHODS

Samples and sample preparation

Leaves, branches and barks from contemporary *Cedrus deodara* (Pinaceae) and *Platycladus orientalis* (Cupressaceae) collected from the campus were grounded up and homogenized. Then these organic materials were divided into several groups and mixed with different inorganic materials respectively to keep each sample in a unique condition as shown in Table 1. After that, all samples were put into wide-mouthed bottles where a quartzose sand sheet was placed in advance and another quartzose sand sheet was added to cover the mixed materials. Then, all the contents in each bottle were submerged in purified water which was kept rising from the contents for no less than 2 cm (this was maintained throughout of the simulation experiment beginning from August 1, 2002). Finally, these bottles were closed and put into an oven whose temperature was controlled at 80°C. In order to eliminate possible organic contamination, experimental materials such as bottles, containers, solid reagents and inorganic components, were pre-extracted using redistilled analytical grade solvent, and then heated at 180°C for 8 hours.

Extraction and separation

After the simulation continued for about five and a half years, a part of the samples were taken out from the bottles for organic geochemical analyses. The samples were Soxhlet-extracted for 36 hours using chloroform as solvent, and removal of elemental sulfur was achieved by adding copper foils to the flask during extraction. The extracts were filtered and concentrated by a rotary evaporator. The yields were determined gravimetrically after removal of the solvent. The total extract was separated into aliphatic, aromatic and polar fractions by chromatography column over pre-washed silica gel. The fractions were eluted with hexane, dichloromethane and methanol, respectively. For comparison, fresh samples collected from *Cedrus deodara* and *Platycladus orientalis*

were washed and dried at 37°C, and then the same extraction and separation procedures were repeated.

Gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) analyses of the aliphatic fractions were performed on an Agilent 6890 GC coupled to an Agilent 5973 quadrupole MSD. Separation was achieved on a fused silica capillary column coated with DB5 (30 m × 0.25 mm i.d., 0.25 μm film thickness). The GC operating conditions were as follows: temperature held at 60°C for 5 min, increased from 60 to 300°C at a rate of 4°C min⁻¹ with final isothermal hold at 300°C for 15 min. Helium was used as carrier gas. The sample was injected at split ratio 30:1 with the injector temperature at 290°C. The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy and scanned from 50 to 650 Da. Data were acquired and processed with the Chemstation software. Individual compounds were identified by comparison of mass spectra with literature and library data, and by interpretation of mass spectrometric fragmentation patterns.

RESULTS AND DISCUSSION

Composition of the aliphatic fractions

The low evolutionary degree of organic matter leads to a relative high concentration of polar compounds (34.70–56.58%) but a low concentration of aliphatic (1.47–15.29%) and aromatic (1.58–10.51%) fractions in the total extracts. From Table 2, it can be seen that the composition of extracts is different in different sample conditions. In samples A₆, A₉ and A₁₂, the concentrations of aliphatic hydrocarbons are higher than that in sample A₂ where only organic matter, kaolin and quartzose sand exist. The increased amount of aliphatic hydrocarbons in sample A₆ and A₉ may come from the generation of alkanes from polar compounds under the catalysis of iron or its compounds. For example, iron sulphide in samples can catalyze decarboxylation of fatty acids to produce alkanes (Zhang, Z. L. *et al.*, 1999). The increased amount of aliphatic hydrocarbons (alkanes and terpenoids) in sample A₁₂ may result from the high briny environment, in which generation and expulsion of aliphatic hydrocarbons can be accelerated. This is consistent with the fact that salt lake facies and brackish water facies are favorable to form immature oils (Huang, 1999). In samples A₇ and A₁₁, the concentrations of aliphatic hydrocarbons are lower than that in sample A₂. This may be brought about by prohibitive effect of the heavy metal (lead) and the gypsum, which will be supported in the latter part of this paper.

The total ion chromatograms (TIC) show that the composition of aliphatics is also different in different sample

Table 2. Composition (in rel.%) of extracts, composition (in rel.%) of compound classes in the aliphatic fractions and parameters of *n*-alkanes

Sample	Extracts (%)					Compounds classes (%) in the aliphatic fractions					Parameters of <i>n</i> -alkanes				
	Total* (mg)	Aliphatics	Aromatics	Polars	<i>n</i> -Alkanes	Sesquiterpenoids	Diterpenoids	Total	C number	Max peak	CPI	OEP	ΣC ₂₂ ⁻ ΣC ₃₃ ⁺		
P	70.12	1.47 (1.03)	8.21 (5.76)	39.43 (27.65)	22.23	46.19	7.41	75.94	nC ₁₂ -nC ₃₁	nC ₂₇		13.67	0.07		
C	68.51	4.12 (2.82)	10.51 (7.20)	40.30 (27.61)	2.80	66.30	16.58	85.68	nC ₁₂ -nC ₃₅	nC ₃₃	8.85	13.76	0.05		
A ₂	35.19	2.93 (1.03)	3.98 (1.40)	44.20 (5.21)	16.44	21.13	57.08	96.64	nC ₁₂ -nC ₃₅	nC ₃₃	10.04	11.60	0.36		
A ₆	31.53	10.53 (3.32)	5.71 (1.80)	41.29 (13.02)	58.01		2.12	60.96	nC ₁₂ -nC ₃₅	nC ₃₃	11.62	18.20	0.48		
A ₇	36.00	1.69 (0.61)	2.08 (0.75)	42.28 (15.22)	24.67	13.62	57.52	95.81	nC ₁₂ -nC ₃₅	nC ₃₃	5.77	10.83	0.15		
A ₉	30.01	15.29 (4.59)	6.00 (1.80)	37.15 (11.15)	72.53		1.84	74.37	nC ₁₂ -nC ₃₅	nC ₃₃	8.04	14.00	0.34		
A ₁₁	40.63	2.12 (0.86)	1.58 (0.64)	34.70 (14.10)	77.21	1.33	18.59	97.13	nC ₁₂ -nC ₃₅	nC ₃₃	14.06	16.45	0.04		
A ₁₂	39.96	11.14 (4.45)	5.61 (2.24)	56.58 (22.61)	47.54	9.69	23.88	81.11	nC ₁₅ -nC ₃₅	nC ₁₇	3.53	1.10	3.30		

rel.%: Relative concentrations of extracts are calculated according to the gravimetric results (gravimetric data (mg) are given in parenthesis; * the quantity of extract for separation by chromatography column), and those of compound classes are calculated according to the instrumental integral results.

$$CPI = \frac{1}{2} \left[\frac{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{26} + C_{28} + C_{30} + C_{32} + C_{34}} + \frac{C_{25} + C_{27} + C_{29} + C_{31} + C_{33}}{C_{24} + C_{26} + C_{28} + C_{30} + C_{32}} \right]; \quad OEP = \left[\frac{C_{i-2} + 6C_i + C_{i+2}}{4C_{i-1} + 4C_{i+1}} \right]^{(-)^{i+1}}; \quad (C_i \text{ is the maximum peak}).$$

Fig. 1. GC-MS (TIC) traces of the aliphatic fractions of samples.

conditions (Fig. 1 and Table 2). In contemporary samples P, C and simulated samples A₂, A₇, the amount of sesqui- and diterpenoids dominates the aliphatic fractions; alkanes are minor components. In samples A₁₁ and A₁₂, the amount of alkanes ranks first among aliphatic fractions; the amount of terpenoids comes afterward. However, in samples A₆ and A₉ into which iron powder was added, terpenoids seem to be “used up”, so the amount of alkanes occupies a leading position. The causes of these distinct compositional characteristics will be discussed later.

N-alkane distribution

According to the mass chromatograms (m/z 85 key ion for alkanes) of *n*-alkanes (Fig. 2), long chain *n*-alkanes are dominant components in the contemporary samples P and C, which displays the characteristic of higher plant sources (Dehmer, 1995). In sample P, C₂₇ is the main peak of mass chromatogram; in sample C, C₃₃ is the main peak. The difference of main peak between two samples is mainly caused by their different biological composition of cuticle waxes and alcohols.

Fig. 2. Mass chromatograms (m/z 85) of n -alkanes of samples. Pr = pristane, Ph = phytane, IS = the internal standard (squalane).

Table 3. Sesquiterpenoids and their relative abundance

Name	Composition	MW	Base peak	No.	Relative abundance							ID	References
					P	C	A ₂	A ₆	A ₇	A ₉	A ₁₁	A ₁₂	
Longipinene	C ₁₅ H ₂₄	204	119	I	7.3				4.8			L	
Copaene	C ₁₅ H ₂₄	204	161	II	13.5	3.8			16.2			L	
Bourbonene	C ₁₅ H ₂₄	204	81	III	5.9	3.4			11.2			24.5	
Longifolene	C ₁₅ H ₂₄	204	161	IV	18.3				60.2			L, P	Philp, 1985
Caryophyllene	C ₁₅ H ₂₄	204	93	V	100.0	5.9						L	
α -Cedrene	C ₁₅ H ₂₄	204	119	VI					18.4			47.9	Huang <i>et al.</i> , 1991
γ -Cadinene	C ₁₅ H ₂₄	204	161	VII	22.6	25.1			17.8			L, P	Cong and Li, 2003
Cadina-4,9-diene	C ₁₅ H ₂₄	204	105	VIII	8.7	13.8	30.2		37.8		29.2	47	Cong and Li, 2003
Unknown cadalene isomer	C ₁₅ H ₂₄	204	161	UI	6.2	51.7			37.9			L, P	Cong and Li, 2003
Calamenene	C ₁₅ H ₂₂	202	159	IX			18		30		16.4	21.6	Simoneit and Mazurek, 1982
δ -Cadinene	C ₁₅ H ₂₄	204	161	X	24	100.0	3.5		100.0			16.8	
Cadina-1,3,5,7-tetraene	C ₁₅ H ₂₀	200	157	XI			0.7		17.5			7.2	
Cadina-1(10),6,8-triene	C ₁₅ H ₂₂	202	187	XII			1.8					L, P	Simoneit and Mazurek, 1982
Cadina-1(10),7,6,8-terae	C ₁₅ H ₂₀	200	185	XIII			0.9					I	
Cadalene	C ₁₅ H ₁₈	198	183	XIV			100.0		20	100.0	100.0	L, P	Simoneit and Mazurek, 1982

Relative abundance normalized to major peak = 100, ID = identification: L = NIST98 MS library, P = published mass spectrum, I = interpretation of MS fragmentation pattern. The abundance of calamenene and δ -cadinene is calculated from a multi-peak fitting method which divides their overlapping peaks into individual one.

In all simulated samples excluding A₁₂, C₃₃ is the main peak of mass chromatogram and long chain *n*-alkanes still dominate components. In samples A₂, A₆ and A₉, the percentage of long chain *n*-alkanes is far beyond that of short chain *n*-alkanes, and the values of $\Sigma C_{22}^-/\Sigma C_{23}^+$ are 0.36, 0.48 and 0.34 respectively (Table 3). However, compared with contemporary samples, the short chain *n*-alkanes in these samples have increased. In sample A₁₂, where C₁₇ is the main peak of mass chromatogram, increase of short chain *n*-alkanes is significant and it even surpasses long chain *n*-alkanes ($\Sigma C_{22}^-/\Sigma C_{23}^+ = 3.30$). The obvious increase of short chain *n*-alkanes shows that during the long-term simulation, thermo-chemical processes promote the maturity of short chain *n*-alkanes, in which bacteria may play an important role. According to Otto *et al.* (1994) and Dehmer (1995), microbial activity could contribute to the increase of *n*-alkanes shorter than *n*-C23 from higher plants. The role of bacteria is also suggested by the hump in the TIC curve of every above-mentioned sample (Fig. 1, Peters and Moldowan, 1993). In sample A₁₂, the plot distribution of TIC curve with an obvious hump tends to become smooth, and the value of odd over even carbon preference (OEP) and carbon preference index (CPI) is close to 1. This shows that bacteria remarkably reduced odd carbon preference of *n*-alkanes from organic matter through their degradation during early diagenesis at 80°C (Zhang, 1979). Since sample A₁₂ is kept in a hypersaline condition, it should be halophilic bacteria that assisted the generation and maturity of alkanes in hypersaline environment.

In sample A₇, however, the increase of short chain *n*-alkanes is not obvious. The percentage of its long chain *n*-alkanes in the aliphatic surpasses that in sample A₁₂, A₂, A₆, and A₉. This percentage reaches the highest point in sample A₁₁, whose $\Sigma C_{22}^-/\Sigma C_{23}^+$ is 0.04. This may be caused by the interference of gypsum with biochemical degradation of bacteria. According to Peters and Moldowan (1993), biochemical degradation occurs preferentially from alkanes under the temperature from 65 to 80°C. This process must be isolated from hydrogen sulphide (H₂S) because of its toxicity to associated bacteria. However, in early sedimentation of organic matter, when the temperature is below from 80 to 100°C, this biochemical degradation may be hampered by commonly-happened bacterial sulphate reduction (BSR), in which sulphate is the source of H₂S generation (Disnar and Sureau, 1990; Machel *et al.*, 2001). Therefore, in sample A₁₁, where gypsum (CaSO₄) was added and metals did not exist, gypsum may be reduced to H₂S. It may poison the associated bacteria and hamper the development of short chain alkanes from long chain alkanes. But in other samples (A₆, A₇, A₉) into which gypsum was also added but metals existed, the degradation of the bacteria was less interfered with. It is probably because the hydrogen sulphide

has firstly combined with free metals to form metallic sulphides.

Sesquiterpenoids

In this simulation experiment, the sesquiterpenoids and diterpenoids in the aliphatic fractions, observed from GC-MS data, are mainly unsaturated and aromatic. According to related research, aromatic hydrocarbons of low molecular weight and aromatic terpenoids can appear in aliphatic (saturated) fractions (Stefanova *et al.*, 2005).

In contemporary samples, natural sesquiterpenoids dominate the aliphatic fractions (Fig. 1 and Table 4) where caryophyllene (V) is the maximum component of sample P and δ -cadinene (X) of sample C. However, in simulated samples, most natural sesquiterpenoids evolved into diagenetic sesquiterpenoids through thermal-chemical and biochemical processes. In samples A₂, A₁₁ and A₁₂, some natural sesquiterpenoids disappeared, but some diagenetic cadalane-type sesquiterpenoids often reported in the geosphere were found. For example, evolutionary intermediates calamenene (IX) and cadina-1(10),6,8-triene (5,6,7,8-tetrahydrocadalene, XII), and evolutionary final product cadalene (XIV) (Simoneit and Mazurek, 1982; Otto *et al.*, 1997), whose relative concentration was the highest of above samples, had been detected from the samples. It shows that the cadalane-type biogenic precursors can evolve rapidly during early diagenesis.

In addition, through the study of diagenesis of sesquiterpenoids, two kinds of seldom reported intermediates from cadinatriene to cadalene in sample A₂ were inferred according to their mass fragments (Fig. 3). They were tentatively identified as cadina-1,3,5,7-tetraene (XI) and cadina-1(10),7,6,8-tetraene (XIII). The former type was once reported as analogs in the deep oil from Bella Coola (Simoneit *et al.*, 1986), but their mass spectra were not provided. Based on this, a complementary scheme for early diagenesis of cadalane-type biogenic precursors (cadinenes and cadinols) is proposed (Fig. 4A).

In sample A₇, most of the natural sesquiterpenoids exist and δ -cadinene is still the main component. The relative concentration of cadalene in detected diagenetic sesquiterpenoids is only 20.0%. This indicates that the diagenesis of sesquiterpenoids in this sample is relatively slow. The reason may be that the heavy metal (lead) hindered the biochemical conversion of sesquiterpenoids through poisoning the involved bacteria (the bacterial role in the alterations of terpenoids can refer to the next section of diterpenoids).

In samples A₆ and A₉, no obvious natural and diagenetic sesquiterpenoids were found. This may be caused by the chemical reactions of the iron added in the samples. Theoretically, iron or its uncertain complexes under the simulated conditions may coordinate with the polar precursors or catalyze the polymerization or oxy-

Table 4. Diterpenoids and their relative abundance

Name	Composition	Base peak	No.	Relative abundance							ID	References	
				P	C	A ₂	A ₆	A ₇	A ₉	A ₁₁			A ₁₂
Sandaracopimaradiene	C ₂₀ H ₃₂	257	XV			67.7		2.7		18.0	41.8	L, P	Philp, 1985
Unknown pimaradiene isomer	C ₂₀ H ₃₂	257	U2			55.0		5.6		16.5	25.8		
Isopimaradiene	C ₂₀ H ₃₂	137	XVI			37.0		2.2		10.8	18.8	P	Philp, 1985; Duan and Luo, 1990
Unknown isopimaradiene isomer	C ₂₀ H ₃₂	137	U3		2.0	66.9		6		23.1	60.1		
19-Norabietatriene	C ₁₉ H ₂₈	159	XVII			22.7		7.9		5.8		L, P	Simoneit, 1977
Isopimara-7,15-diene	C ₂₀ H ₃₂	109	XVIII	100.0		27.6		11.9		10.0	23.5	P	Simoneit, 1977; Duan and Luo, 1990
Norabieta-4,8,11,13-tetraene	C ₁₉ H ₂₆	239	XIX			52.9		8.5		9.0	16.9	P	Simoneit and Mazurek, 1982
Norabieta-4(19),8,11,13-tetraene	C ₁₉ H ₂₆	197	XX			25.0		7.1		5.1	6.7	P	Philp, 1985
18-Norabietatriene	C ₁₉ H ₂₈	159	XXI			27.9	32.2	16.2		12.2	13.4	L, P	Simoneit, 1977
Norabieta-3,8,11,13-tetraene	C ₁₉ H ₂₆	239	XXII			24.2		7.7		5.3	5.8	P	Philp, 1985
Abieta-7,8,11,13-tetraene	C ₂₀ H ₃₂	183	XXIII			33.7		15.7		14.1	12.9	I	
Dehydroabietane	C ₂₀ H ₃₀	255	XXIV	17.5	0.4	100.0	100.0	59.2	100.0	100.0	28.9	L, P	Simoneit and Mazurek, 1982
Simonellite	C ₁₉ H ₂₄	237	XXV			15.7		8.6		trace	5.6	P	Simoneit and Mazurek, 1982
Kaur-16-ene	C ₂₀ H ₃₂	272	XXVI	56.2	100.0			100.0			100.0	L, P	Cong and Li, 2003
Retene	C ₁₈ H ₁₈	219	XXVII			91.2		2.3		7.0		L, P	Simoneit and Mazurek, 1982

Relative abundance normalized to major peak = 100. ID = identification: L = NIST98 MS library. P = published mass spectrum, I = interpretation of MS fragmentation pattern. The abundance of dehydroabietane and simonellite is calculated from a multi-peak fitting method which divides their overlapping peaks into individual one.

Fig. 3. Mass spectra of cadinatrienes, cadinatetraenes, norabietatetraenes and abietatetraene. IX calamenene, XI cadina-1,3,5,7-tetraene, XII cadina-1(10),6,8-triene, XIII cadina-1(10),7,6,8-tetraene, XIX norabieta-4,8,11,13-tetraene, XX norabieta-4(19),8,11,13-tetraene, XXII norabieta-3,8,11,13-tetraene, XXIII abieta-7,8,11,13-tetraene.

Fig. 4. Proposed early diagenetic pathways for cadalane-type biogenic precursors (A) and abietane-type biogenic precursors (B). Compounds in boxes are biogenic precursors. (after Simoneit and Mazurek, 1982; Simoneit et al., 1986; Otto and Simoneit, 2001).

generation of cadinenes (Britovsek *et al.*, 2002; Bruijninx *et al.*, 2008), which may distort the diagenetic pathways of sesquiterpenoids.

In brief, all the results indicate that during early diagenesis, natural sesquiterpenoids can evolve into geological ones rapidly, and this process seems to be influenced by inorganic environments.

Diterpenoids

From Fig. 1 and Table 4, it can be seen that in contemporary samples P and C, only a few biogenic diterpenoids in the aliphatic fractions were detected. However, in simulated samples A₂, A₇ and A₁₂, besides biogenic diterpenoids, many diagenetic tricyclic diterpenoid derivatives such as evolutionary intermediates norabietatrienes (XVII and XXI) and simonellite (XXV), and evolutionary final product retene (XXVII) frequently reported in the geosphere (Simoneit, 1977; Otto and Simoneit, 2001) were also found. In addition, two kinds of less frequently reported diterpenoid intermediates were identified through their mass spectra (Fig. 3). They are norabietatetraenes (XIX, Simoneit and Mazurek, 1982; XX and XXII, Philp, 1985) and abietatetraene (XXIII).

Based on data of these detected diterpenoids (Table 4) and relevant reports (Simoneit and Mazurek, 1982; Simoneit *et al.*, 1986; Otto and Simoneit, 2001), an early evolutionary pathway for abietane-type biogenic precursors (dehydroabietane, abietic acid and abietol) is proposed (Fig. 4B), and some new conversions such as the generation of two norabietatetraenes through elimination reaction from dehydroabietol are suggested.

All of these findings show that diterpenoids in simulated samples have evolved in their early diagenesis. The evolutionary speed of diterpenoids in sample A₂ is the fastest of all and its relative abundance of evolutionary final product retene reaches 91.2% (Table 4). The maximum peak of diterpenoids in sample A₁₁ is the same as sample A₂, but its relative abundance of evolutionary products is much lower than that in sample A₂ and simonellite in sample A₁₁ is only detected in trace, which may result from the negative role that gypsum played in the evolution of diterpenoids as it did in that of *n*-alkanes. In samples A₆ and A₉, only a few tricyclic diterpenoids were detected. The lack of diterpenoids, like the case for sesquiterpenoids, may also come from the reactions of iron with the polar precursors such as abietic acids and pimarenoic acids.

Although evolution of diterpenoids is different in different samples, their biogenic precursors, observed from the scheme in Fig. 4B, mainly converted into aromatic derivatives. According to a simulation at 550°C (Dai and Mei, 1988), aromatic diterpenoids, together with minor saturated diterpenoids, could be transformed from biogenic precursors abietic acid by thermal process. Aro-

matic derivatives could also form through biomass burning (Simoneit *et al.*, 2000). This shows that alterations of terpenoids from conifers can be accelerated by thermal-chemical processes, in which biogenetic neutral terpenoids (e.g., dehydroabietane) and functional terpenoids (e.g., abietic acid, phenol and pimarenoic acid), may undergo complicated reactions such as isomerization, dehydrogenation, aromatization, dealkylation and defunctionalization in the long-term simulation experiment.

Whereas, it should be pointed out that some saturated cyclic sesqui- and diterpenoids frequently reported in the geosphere, such as cadinane, abietane, fichtelite, pimarane, and norpimarane, were not encountered in all samples. These saturated terpenoids are regarded as products of reductive diagenesis or disproportionation from their precursors, which are mainly bonded to macromolecular organic fraction (i.e., kerogen), so they are often observed in more mature samples (e.g., petroleum and coal) and more oxygenated environments. However, dehydrogenated and aromatic terpenoids are mainly presented and preserved in recent sediments and anoxic conditions (Simoneit *et al.*, 1986; Dehmer, 1995). Therefore, in the simulated samples, the absence of saturated cyclic terpenoids may likely result from the low maturity of organic matter and the dominance of an anoxic environment, where dehydrogenated and aromatic terpenoids can be easily transformed and preserved through microbial activities (Martin *et al.*, 1999).

In a summary, like sesquiterpenoids, natural terpenoids can transform into diagenetic terpenoids in their early diagenesis rapidly, and this process can be affected by thermal-chemical processes and microbial activities.

SUMMARY AND CONCLUSIONS

The results of the simulation show that the aliphatic hydrocarbons of coniferous organic matter can undergo rapid diagenetic alterations after early sedimentation. After five and a half years of long-term, low-temperature simulation, *n*-alkanes developed and some terpenoid biomarkers, including frequently encountered ones in the geosphere (e.g., cadalene, retene and simonellite) and seldom-reported ones (two cadinatetraenes, three norabietatetraenes and one abietatetraene) in the literature, were detected in most simulated samples. Based on these findings, two complementary pathways of early diagenesis for cadalane-type sesquiterpenoids and for abietane-type diterpenoids are proposed.

Studies on early diagenesis of organic matter from conifers also reveal that the alterations of aliphatic hydrocarbons could be accelerated by thermal-chemical processes, in which bacteria play an important role. Bacteria degraded long chain *n*-alkanes into short chain *n*-

alkanes, which reduces the odd over even carbon number preference of *n*-alkanes, and promoted the formation of aromatic sesquiterpenoids and diterpenoids. The alterations could also be influenced by inorganic sedimentary environments. According to the experimental results, the following conclusions can be drawn: briny environment is propitious to the early generation, maturation and expulsion of aliphatic hydrocarbons; iron, through its inorganic interactions, may promote the development of alkanes, but interrupt the alternations of terpenoids; heavy metal and gypsum, through the toxicity or hindrance to associated bacteria, may hamper the early diagenesis of aliphatic hydrocarbons.

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APPENDIX

(see p. 259)

Appendix. Sesquiterpenoids and diterpenoids.