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Volatiles from Two Gymnosperm Fossils: Miocene Leaves of *Metasequoia glyptostroboides* and Early Cretaceous Seed Cone of *Pityostrobus* spp. *

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Abstract Fifty-one and nineteen compounds were detected respectively from the volatile extracts from the fossils of Miocene leaves of *Metasequoia glyptostroboides* and early Cretaceous seed cone of *Pityostrobus* spp. by gas chromatography - mass spectrometry (GC - MS) analysis. These compounds were found belonging to *n*-alkanes, *n*-alkenes, *n*-alkanols, long chain fatty acids and their esters, phthalates, terpenoids and aromatic compounds, and three compounds of them, abietane diterpenoid, heptadecanoic acid and retene were considered as biomarkers for gymnosperm plants. Fig 1, Tab 2, Ref 22

Keywords fossil; *Metasequoia glyptostroboides*; *Pityostrobus* spp.; biomarker
CLC Q946.8

中新世水杉叶片和白垩纪松型球果化石中的挥发性成分*

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摘　要　采用气相色谱-质谱联用分析技术从中新世水杉叶片和白垩纪松型球果两种裸子植物化石中分别鉴定了51个和19个挥发性成分,类型涉及烷烃、烷烯、烷醇、长链脂肪酸及其酯、邻苯二甲酸酯、萜类和芳香族化合物.其中3种成分——松香烷型二萜、饱和十七碳脂肪酸和惹烯是裸子植物的生物标志分子. 图1 表2 参22

关键词　化石; 水杉; 松型球果属; 生物标志分子
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Chemical studies of ancient plants provided crucial information on chemical preservation of fossils and sources of organic matters in sediments. Characteristic natural products detected from fossils could be used as chemosystematic marker or biomarkers for their biological origin[1]. Previous chemical investigation of plant fossils led to the identification of diterpenoids[2], urea derivatives[3] and steroids[4], and indicated a high level of phytochemical fidelity between respective fossils and extant genera[5-7]. Pinaceous fossil cones are divided into three genera: *Pityostrobus*, *Pseudoaraucaria* and *Pinus*[8]. *Pityostrobus* is an artificial genus including the cones of indefinite affinity. *Metasequoia*, a 'living fossil'[9], has existed since the Cretaceous period about 120 million years before. To reveal the organic constituents of the Miocene leaves of *Metasequoia glyptostroboides* and early Cretaceous seed cone of *Pityostrobus* spp., a phytochemical investigation was undertaken on the two fossils retaining their whole configurations. Fifty-one and nineteen compounds were detected respectively from the volatile extracts from the fossils of the Miocene leaves of *M. glyptostroboides* and early Cretaceous seed cone of *Pityostrobus* spp. by gas chromatography - mass spectrometry (GC - MS). The gymnosperm biomarkers were discussed.

1　Experiment

1.1　Plant fossil material

The Miocene leaves of *M. glyptostroboides* were collected in Svalband, Norway and identified by Professor WU Sugong at Kunming Institute of Botany, Chinese Academy of Sciences (CAS). The specimen (NWM-1) is deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS. The early Cretaceous seed cone of *Pityostrobus* spp. was collected at Yixian in Liaoning, China and identified by Professor ZHANG He. The specimen is deposited in Fairy Lake Botanical Garden, Shenzhen Palaeontology Museum.

1.2　Extraction

The early Cretaceous seed cone (38.350 g) of *Pityostrobus* spp. and the whole Miocene leaves of *M. glyptostroboides* were

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** Corresponding author (E-mail: jzhou@mail.kib.ac.cn) both extracted for 7 days with methanol at room temperature, respectively. After removing methanol under reduced pressure, two residues (45 mg and 5 mg) were obtained. Each residue was partitioned between *n*-hexane and methanol to give the *n*-hexane layer and methanol layer, respectively. Two n-hexane extracts (15 mg and 2 mg) were subjected to gas chromatography-mass spectrometry (GC-MS) analysis.

1.3 GC - MS analysis

The GC - MS experiment was carried out with FISONS MD800 GC/MS/DS instruments, at the following conditions: held from 80 ℃ and ramped at 5 ℃ min^{-1} to 280 ℃. The MS was operated in full scan mode (35 ~ 450, 1 scan s^{-1} 70 eV ionization energy). Peaks were identified by comparison of their relative retention times and mass spectral characteristics, with NBS mass spectral library software.

2 Results and discussion

Nineteen compounds (Table 1) from the early Cretaceous seed cone of *Pityostrobus* spp. and fifty-one (Table 2) from the Miocene leaves of *M. glyptostroboides* were detected by GC - MS analysis. These volatiles included *n*-alkanes (C_{15} ~ C_{25}), *n*-alkenes, *n*-alkanols, long chain fatty acids and their esters, phthalates, terpenoids and aromatic compounds, indicating that the plant fossils showed excellent preservation of lipids and other organic compounds, though some natural products altered and transferred during the period of burial in sediments.

Table 1 Volatiles from the early Cretaceous seed cone of *Pityostrobus* spp.

No.	Compounds	Retention time	Molecular formula	Relative content (%)
1	Acetic acid, 2-methylpropyl ester	3.57	$C_6H_{12}O_2$	10.92
2	3,7-dimethyl-1,3,6-octatriene	5.45	$C_{10}H_{16}$	0.11
3	Pyridine, 3-1-methyl-2-pyrrolidinyl	16.07	$C_{10}H_{14}N$	0.12
4	10 (14)-aromadendrene	17.87	$C_{15}H_{24}$	0.31
5	Pentanedioic acid, bis (1-methylpropyl) ester	21.82	$C_{13}H_{24}O_4$	0.39
6	Hexadecane	22.37	$C_{16}H_{34}$	0.79
7	Heptadecane	24.84	$C_{17}H_{36}$	0.81
8	Octadecane	27.17	$C_{18}H_{38}$	1.49
9	Diisobutyl phthalate	28.98	$C_{16}H_{22}O_4$	0.47
10	Nonadecane	29.42	$C_{19}H_{40}$	1.04
11	Hexadecanoic acid, methyl ester	30.12	$C_{17}H_{34}O_2$	15.63
12	Dibutyl phthalate	31.12	$C_{16}H_{22}O_4$	14.98
13	Heptadecanoic acid, methyl ester	31.59	$C_{18}H_{36}O_2$	7.81
14	6,9-octadecadienoic acid, methyl ester	33.59	$C_{19}H_{34}O_2$	6.24
15	9,12,15-octadecatrienoic acid, methyl ester	33.59	$C_{19}H_{32}O_2$	4.68
16	Octadecanoic acid, methyl ester	34.14	$C_{19}H_{38}O_2$	4.27
17	8,11,13-abietatrien-18-oic acid, methyl ester	38.64	$C_{21}H_{30}O_2$	0.94
18	Bis (2-ethylhexyl) phthalate	41.95	$C_{24}H_{38}O_4$	3.12
19	Squalene	48.57	$C_{30}H_{50}$	2.34

Table 2 Volatiles from the Miocene leaves of *M. glyptostroboides*

No.	Compounds	Retention time	Molecular formula	Relative content (%)
1	3-(*E*)-penten-2-one	0.92	$C_5H_{10}O$	0.97
2	2,3-dimethyl-3-buten-2-ol	1.23	$C_6H_{12}O$	1.02
3	2-(*E*)-hexenal	1.63	$C_6H_{10}O$	0.56
4	6,8-dioxabicyclo 3. 2. 1 octane	1.80	$C_8H_{12}O$	0.26
5	2-hexanol	1.92	$C_6H_{14}O$	0.41
6	4-hydroxy-4-methyl-2-pentanone	2.52	$C_6H_{12}O$	1.07
7	2-methyl-cyclopentanol	2.57	$C_6H_{12}O$	0.62
8	Tetrahydro-3,6-dimethyl-2H-pyran-2-one	2.88	$C_7H_{12}O_2$	0.47
9	2-heptanol	3.45	$C_7H_{16}O$	0.46
10	1,2-dimethylcyclopentane	4.95	C_7H_{14}	0.71
11	4-hydroxybenzenesulfonic acid	5.47	$C_6H_6O_4S$	0.31
12	Isocyanato-cyclohexane	5.77	$C_7H_{11}NO$	0.26
13	Formic acid, octyl ester	7.63	$C_9H_{18}O_2$	0.51
14	(*E*)-2-undecen-1-ol	8.57	$C_{11}H_{22}O$	1.53
15	1,7,7-trimethyl-bicyclo 2. 2. 1 heptan-2-one	10.03	$C_{10}H_{16}O$	1.07
16	1-(methylphenyl)-ethanone	10.80	$C_9H_{10}O$	0.92
17	*L*-(-)-menthol	10.85	$C_{10}H_{20}O$	0.97
18	Naphthalene	11.30	$C_{10}H_8$	1.37
19	Decanal	11.55	$C_{10}H_{20}O$	0.72
20	1,2-benzisothiazole	12.50	C_7H_5NS	1.17
21	2-methyl-3-(1-methylethenyl)-cyclohexanol	13.27	$C_{10}H_{20}O$	0.56
22	1-methyl-naphthalene	14.55	$C_{11}H_{10}$	1.28
23	2-methyl-naphthalene	15.05	$C_{11}H_{10}$	0.69
24	2,3-dimethyl-N-phenylpyrrolidine	17.84	$C_{12}H_{17}N$	1.41

25	2-hexyl-1-decanol	19.32	$C_{15}H_{32}O$	1.34
26	Pentadecane	19.85	$C_{15}H_{32}$	1.63
27	Dodecanoic acid, methyl ester	20.54	$C_{13}H_{26}O_2$	1.52
28	Dibenzofuran	20.90	$C_{12}H_8O$	0.87
29	Hexadecane	22.52	$C_{16}H_{34}$	3.46
30	1-(1-naphthalenyl)-ethanone	23.52	$C_{12}H_{10}O$	1.58
31	Benzophenone	23.75	$C_{13}H_{10}O$	1.76
32	Heptadecane	25.00	$C_{17}H_{36}$	1.70
33	2,6,10-trimethyl-hexadecane	25.17	$C_{19}H_{40}$	2.85
34	Tetradecanoic acid, methyl ester	25.59	$C_{15}H_{30}O_2$	0.49
35	Octadecane	27.40	$C_{18}H_{38}$	2.65
36	2,6,10,14-tetramethyl-hexadecane	27.64	$C_{21}H_{44}$	2.34
37	Anthracene	27.74	$C_{14}H_{10}$	1.43
38	Diisobutyl phthalate	29.20	$C_{10}H_{22}O_4$	1.39
39	Nonadecane	29.69	$C_{19}H_{40}$	1.79
40	Hexadecanoic acid, methyl ester	30.27	$C_{17}H_{34}O$	3.06
41	Dibutyl phthalate	31.32	$C_{10}H_{22}O_4$	3.68
42	Eicosane	31.82	$C_{20}H_{42}$	1.23
43	Heneicosane	33.94	$C_{21}H_{44}$	2.53
44	Octadecanoic acid, methyl ester	34.44	$C_{19}H_{38}O$	0.77
45	Docosane	35.84	$C_{22}H_{46}$	1.33
46	Retene	37.07	$C_{18}H_{28}$	1.17
47	Tricosane	37.67	$C_{23}H_{48}$	1.49
48	Tetracosane	39.40	$C_{24}H_{50}$	1.18
49	Pentacosane	41.07	$C_{25}H_{52}$	0.94
50	Bis (2-ethylhexyl) phthalate	42.24	$C_{24}H_{38}O_4$	1.94
51	Squalene	48.90	$C_{30}H_{50}$	0.92

Plant fossils contain organic compounds that are derived from natural product precursors biosynthesized by living organisms. These biomolecules are degraded before and after burial in sediment to their diagenetic products. Despite various chemical transformations[2, 10, 11], the diagenetic products retain their characteristic basic structural skeletons and can thus be used as biomarkers for their biological origin. Lipid biomarkers have been widely used to assess depositional environments and types of organic input[12]. Three types of constituents, retene, abieta and heptadecanoic acid (Fig. 1) detected from these two gymnosperm fossils are preserved as the biomarkers for gymnosperm input of organic constituents in sediments.

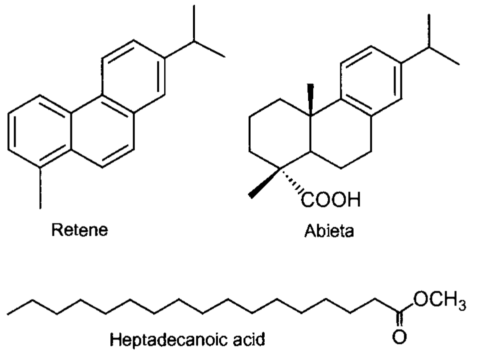

Fig. 1 Structures of biomarkers from two gymnosperm fossils

Retenes were widely distributed in coals, sediments and fossil resins[13, 14], and were used as biomarkers derived from higher plants (such as conifers) resins[15~17]. The formation of the retenes may result from chemical transformations of abietane skeleton by processes, such as aromatisation, alkylation, dealkylation and isomerisation[13, 18]. Abietane diterpenes are well-known natural products found in extant gymnosperms such as Pinaceous plants. The fatty acid composition could be used for the classification of gymnosperms[19]. The heptadecanoic acid also called branched-17 : 0 fatty acid has been identified in the wood of several pines[20] and needles[21]. The role and the pathway of its biosynthesis remain totally unknown[22]. From the volatiles of the Miocene leaves of *M. glyptostroboides*, the unsaturated fatty acids[19] found in photosynthetic tissues of extant genera were not detected for probable hydrogenation process.

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