LIPID GEOCHEMISTRY OF RECENT SEDIMENTS FROM SUN-MOON LAKE, CENTRAL TAIWAN

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ABSTRACT

Recent sediments collected from the Sun-Moon Lake have been analyzed by gas chromatography-mass spectrometer (GC-MS) for lipids. Normal alkanes, triterpenoids, methylketones, methylesters, n-fatty acids, and n-alkanols are investigated and discussed in terms of organic source inputs and the paleoenvironments. The n-C_{16}-n-C_{37} alkane series detected shows predominance at C_{25}-C_{31} and high CPI value. Several C_{24} and C_{25} tetracyclic triterpenoids, including des-A-lupane, are detected. Many of these compounds are supposedly derived from terrestrial triterpenoids and can be used as biomarkers for organic input, more specifically the angiosperms. Pentacyclic triterpenoids detected include hopanes and their corresponding methylhopanes. Both these two homologous series of triterpenoids show similar distributions, indicating common organisms, very likely bacteria, responsible for their precursors. Long chain methylketons (C_{23}-C_{35}), methylesters (C_{17}-C_{35}), aldehydes (C_{24}-C_{34}), n-fatty acids (C_{10}-C_{28}) and n-alkanols (C_{20}-C_{32}) are present and show dominance of compounds with high molecular weight. Most of these compounds are early diagenetic products of plant wax esters.

The organic inputs of the sediments in the Sun-Moon Lake are supposed mainly from the terrestrial higher plants judged from the n-alkane, methylketones, methylesters, n-alkanols and tetracyclic triterpenoids composition. Some proportion of organic input from the bacteria and aquatic organisms to the sediments is inferred by the presence of methylhopanes and short chain n-fatty acids. Vegetational change through time is indicated by comparison the n-alkane distribution of samples from upper and lower sections.

The low CPI value of samples in the upper 35 cm may infer the recent pollution of the petroleum-derived hydrocarbons or the cultivation of the outcropped ancient sedimentary rocks in the surrounding area of the Sun-Moon Lake in last two centuries.

Key words: Organic Geochemistry, Lipid, Biomarker, Sun-Moon Lake

INTRODUCTION

Recent sediments contain organic matter and compounds including lipids derived from various plants and aquatic organisms. These lipids provide particularly useful information about the sources, diagenetic alteration and depositional environments of the organic matter and therefore are especially important to geochemical studies (Peters and Moldowan, 1993, and references therein). The structural and stereochemical specificity of lipids biosynthesized by organisms enables such compounds to serve as indicators of the sources of organic matter in sedimentary deposits (Cranwell, 1986).

Lipids have been extensively analyzed in recent marine and lacustrine sediments. This paper reports the lipid composition and distributions in the late Quaternary sediments deposited at the Sun-Moon Lake. Organic compounds including n-alkanes, triterpenoids, methylketones, methylesters, n-fatty acids, and n-alkanols are investigated and discussed in terms of organic source inputs and the paleoenvironments.

The Sun-Moon Lake is the largest lake in Taiwan covering 20 square kilometers owing to the hydro-electric dam construction in 1950. The lake is situated in Nantou County, Central Taiwan, at longitude 120°54' East and latitude 23°51' North, and is surrounded by mountains higher than 1,000 m. This lake, about 726 m above sea level, developed during Late Pleistocene to Holocene, where lacustrine deposits of more than 30 meters in thickness were accumulated. The regional geology of the Sun-Moon Lake can be referred to Lou (1996).

SAMPLES AND EXPERIMENTAL METHODS

Samples

A piston core (3.69 m in length) covering the sediments of last 20,000 years was taken from the Sun-Moon Lake. The coring site and core description can be referred to Lee *et al.* (2002). The sediments recovered are mainly constituted of peat layers, mud layers and organic debris. Pollen analyses and paleomagnetic study have been carried out on this core for the purpose of paleo-climate changes in central Taiwan (Lou, 1996; Lee *et al.*, 2002). Four levels of the core, at depths of 48 cm, 201 cm, 309 cm and 367 cm, have been dated by C-14 as 540 ± 50, 5150 ± 50, 10,960 ± 60 and 19,460 ± 230 yr B.P., respectively (Lou, 1996). In this study, a total of 12 samples collected from two sections of this core, 10-70 cm and 170-230 cm, were analyzed for the lipids following the procedures shown in Figure 1.

Experimental Methods
Extraction and fractionation

The samples were dried by using the EYELA freeze dryer prior to analysis. The dried samples weighed 10 grams each were Soxhlet-extracted with 300 ml solvent mixture of methanol and dichloromethane (v:v=1:4) for 24 hours. The extracts were condensed to 1-2 ml and then subjected to chromatographic analysis in columns (15 cm x 2.4 cm I.D.) of fully activated alumina oxide (Al_2O_3). Normal hexane, a mixture of n-hexane and diethyl ether, and methanol were used as eluents, sequentially.
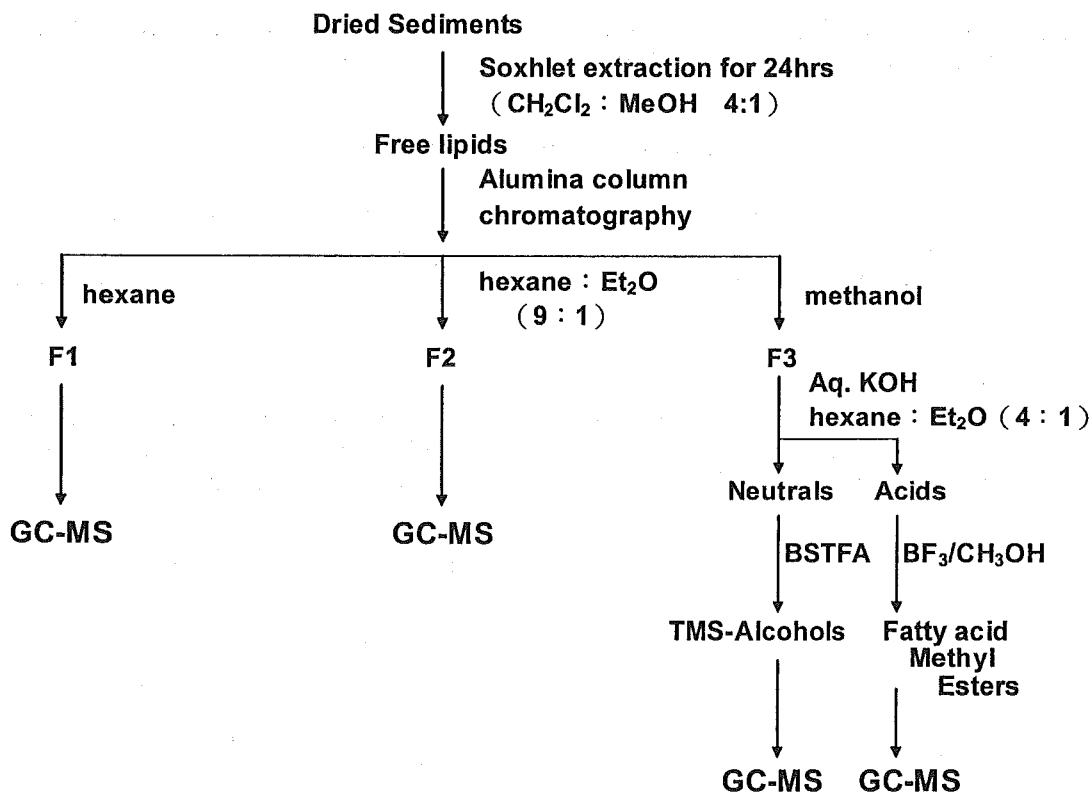
Dried Sediments

Soxhlet extraction for 24hrs
$(CH_2Cl_2 : MeOH$ **4:1** $)$

Free lipids

Alumina column
chromatography

hexane   **hexane : Et$_2$O**   **methanol**
                    (9 : 1)

F1   **F2**   **F3**

                                                  Aq. KOH
                                                  hexane : Et$_2$O (4 : 1)

                                                  Neutrals   Acids

GC-MS   **GC-MS**

                                          BSTFA **BF$_3$/CH$_3$OH**

                                    TMS-Alcohols   Fatty acid
                                                      Methyl
                                                        Esters

                                          GC-MS   GC-MS

Figure 1. Flow chart showing the analytical procedures for lipid analysis.

     The fraction (F1 fraction) eluted through the column with n-hexane contains non-polar hydrocarbons. The F2 fraction eluted with the mixture of n-hexane and diethyl ether contains slightly polar lipids. The F3 fraction eluted with methanol contains polar compounds, including fatty acids, alkanols and pigments. The F1 and F2 fractions were then analyzed by using gas chromatography-mass spectrometry (GC-MS) to determine the lipid distributions. The F3 fraction, which contains polar compounds, was separated into two subfractions, one contains fatty acids and the other contains alkanols. The fatty acids were derivatised into fatty acid methyl esters (FAME) by borontrifluoride/methanol and the alkanols were derivatised into their trimethylsilyl (TMS) ethers by N,O-bis(trimethyl silyl)-trifluoroacetamide (BSTFA) prior to GC-MS analyses.

Gas chromatography-mass spectrometry (GC-MS)

     GC-MS analysis was performed on an HP 5890 gas chromatograph interfaced directly into an HP 5971 mass spectrometer. A DB-5 fused silica capillary column (0.25 µm film, 30 m x 0.25 mm I.D.) was used for gas chromatography. The injector temperature was held at 300 °C isothermally. The column temperature was initially 50 °C for 2 min., then increased at a rate of 8 °C/min to 315 °C, and held isothermally for 5 min. Helium, at a flow rate of 1 ml/min, was used as a carrier gas.

     For the mass spectrometry, EI mode at 70 eV, 200 °C and data acquisition of full scan mode at a scan rate of 2 scan/sec was performed. Identification of individual compounds was

made by comparison of retention sequences and mass spectra with standards and those published (Philp, 1985; Summons and Jahnke, 1992).

RESULTS AND DISCUSSION

Lipid compounds including n-alkanes, triterpenoids and their unsaturated counterparts in the non-polar F1 fraction, methylketones, methylesters and aldehydes in the F2 fraction, n-fatty acids and n-alkanols in the polar F3 fraction were detected in the sediment samples of the Sun-Moon Lake.

n-Alkanes

The n-alkanes with carbon numbers ranging from 16 to 37 were detected in the sediments of the Sun-Moon Lake. The n-alkane distribution generally shows predominance at $C_{25}\sim C_{31}$, inferring input from terrestrial higher plants (Fig. 2). The $n-C_{16}\sim n-C_{19}$ alkane series, which is
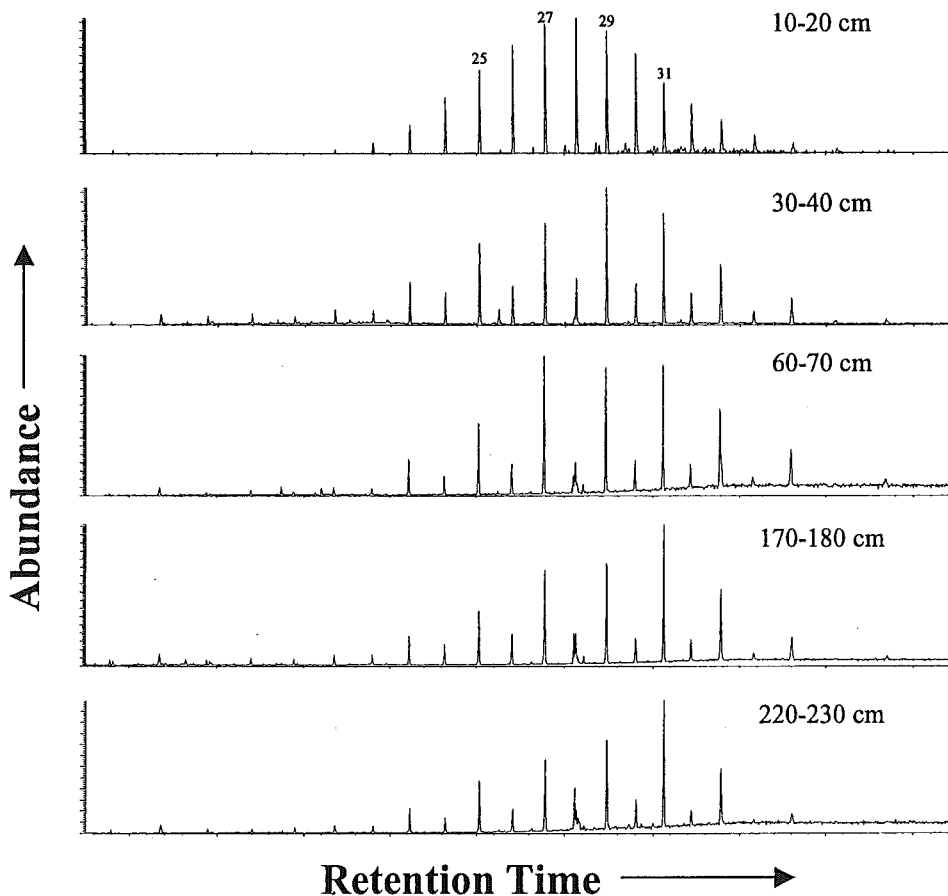
Figure 2. Partial m/z 85 fragmentograms showing the n-alkane distribution of samples from various depths. Carbon numbers of n-alkanes are indicated.

believed to be sourced from microbial and/or algae to the samples, is in much smaller amounts compared to the n-C_{20}~n-C_{35} homologous. The samples in the lower section (170-230 cm) generally showed a maximum at n-C_{31}, probably inferring terrestrial plants input different from those in the upper section.

Although the n-alkane distribution of the recent sediments shows a distinct odd carbon number preference. The carbon preference index (CPI), i.e., the ratio by weight of odd to even carbon molecules (Bray and Evans, 1961) for the samples is measured and given in Fig. 3. The CPI value is generally around 4, consistent with that of 4-6 in the peat of the Toushe Basin, Central Taiwan (Oung *et al.*, 1998), reflecting the low maturity level of the sediments.

Of more interest is the fact that the CPI decreases dramatically with the burial depth decreasing for the samples in the upper 35cm section (Fig. 3). This may be the result from
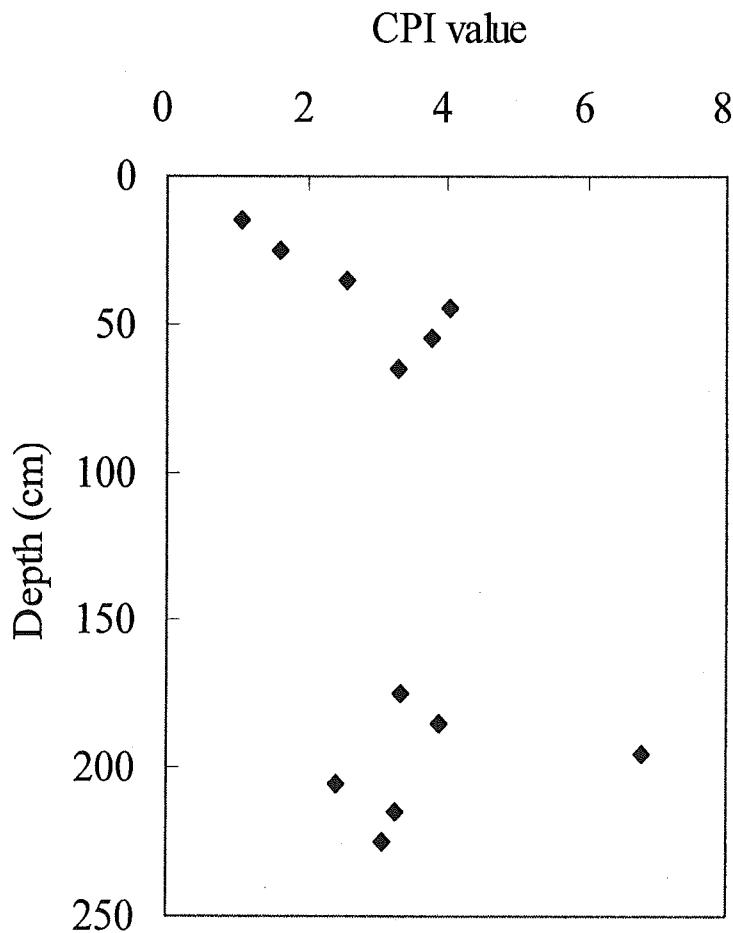
$$CPI=0.5*[(C_{25}+C_{27}+C_{29}+C_{31})/(C_{24}+C_{26}+C_{28}+C_{30})+(C_{25}+C_{27}+C_{29}+C_{31})/(C_{26}+C_{28}+C_{30}+C_{32})]$$

Figure 3. CPI depth profile showing the variation of CPI through depth.

recent pollution of petroleum-derived hydrocarbons, which always show features of fossil hydrocarbons. The cultivation of human activity around the Sun-Moon Lake area since last two centuries could be another reason. It is supposed that the ancient and mature sedimentary rock formations outcropping in the surrounding area contain the n-alkane distribution with a lower CPI value. Erosion as a consequence of human activities and redeposition of this clastic material is responsible for the presence of these hydrocarbons in lake sediments. The construction of the hydro-electric dam in 1950 may result partly the input of ancient sediments with a low CPI value. The inference is supported by the pollen and paleomagnetic studies in this core. Lou (1996) studied the pollen assemblages and pointed out that the sediments had been disturbed due to the construction of the dam. High magnetic susceptibility measured by Lee *et al.* (2002) in the upper 32 cm section is also probably attributed to the event.

Triterpenoids

Triterpenoids including both tetracyclic and pentacyclic compounds are detected in the sediments of the Sun-Moon Lake. Samples in the lower section contain triterpenoids in higher amounts than those in the upper section.

Tetracyclic triterpenoids

At least 10 tetracyclic triterpenoids, including six C_{24} tetracyclenes (molecular weight 328), three C_{24} tetracyclanes (mw. 330) and one C_{25} tetracyclane (mw. 344) are detected and partial fragmentograms given in Figure 4 to show their occurrences. Little about the chemical structure of these compounds is reported except for the peak 5 and peak 8. Peak 5 is identified as des-A-lupane based on its spectrum and retention time. Peak 8 gives a spectrum similar to Wehner *et al.* (1992) and is tentatively assigned as a C_{24} tetracyclene related to an arborane or filicane skeleton from which the A-ring has been totally removed and replaced by an unsaturation between the previous bridging atoms. It is believed that most of these tetracyclic compounds present in the samples are derived from triterpenoid precursors having appropriate functional groups that would promote the opening of the terminal rings in the early diagenetic stage. Such triterpenoid precursors occur in numerous genera of angiosperms (Pant and Rastogi, 1979; Chandel and Rastogi, 1980). For example, des-A-lupane is supposedly derived from lupenols, which are pentacyclic triterpenoids present exclusively in angiosperms, as shown by A-ring cleavage in the early diagenetic stage (Woolhouse *et al.*, 1992).

All the 10 compounds have been detected in the peat deposit in the Toushe Basin (Oung *et al.*, 1998) and are considered as useful biomarkers of higher terrestrial plants. What should be mentioned is that many C_{24} tetracyclic triterpenoids, e.g., des-A-lupane, des-A-oleanane, and des-A-ursane are ubiquitous in Tertiary terrigenously derived oils and sediments in Taiwan (Oung and Philp, 1994). These compounds are also regarded as biomarkers for higher plant inputs, more specifically, the angiosperms (Philp and Gilbert, 1986; Czochanska *et al.*, 1988; Oung and Philp, 1994). A useful comparison of the tetracyclic compounds present in the recent sediments and in the ancient sediments shows that des-A-lupane is the only one compound present in both recent and Tertiary sediments. This may infer that precursor of this compound has been ubiquitous in plants since Tertiary in Taiwan. In addition, the occurrence of des-A-lupane in these very recent sediments infers that the conversion of its precursor to form this geological product should be very feasible.

Pentacyclic triterpenoids

Pentacyclic triterpenoids with carbon numbers ranging from 27 to 32 are detected. Partial fragmetograms showing the typical pentacyclic triterpenoid distribution are given in Figure 5.
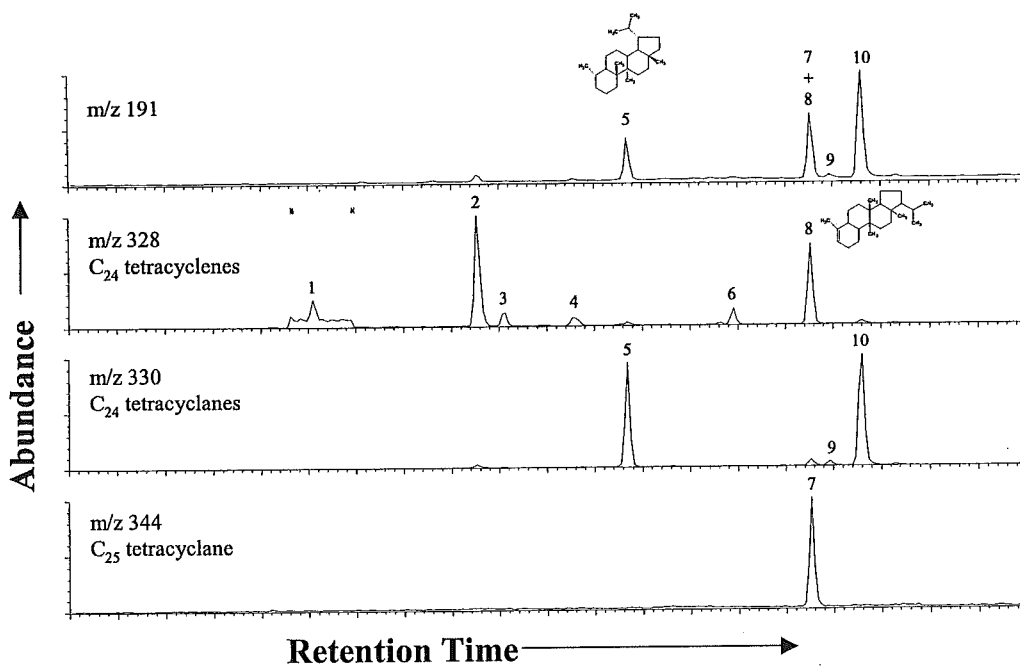
Figure 4. Partial fragmentograms showing the tetracyclic triterpenoid distribution. Structures of peak 5 (des-A-lupane) and peak 8 are included.
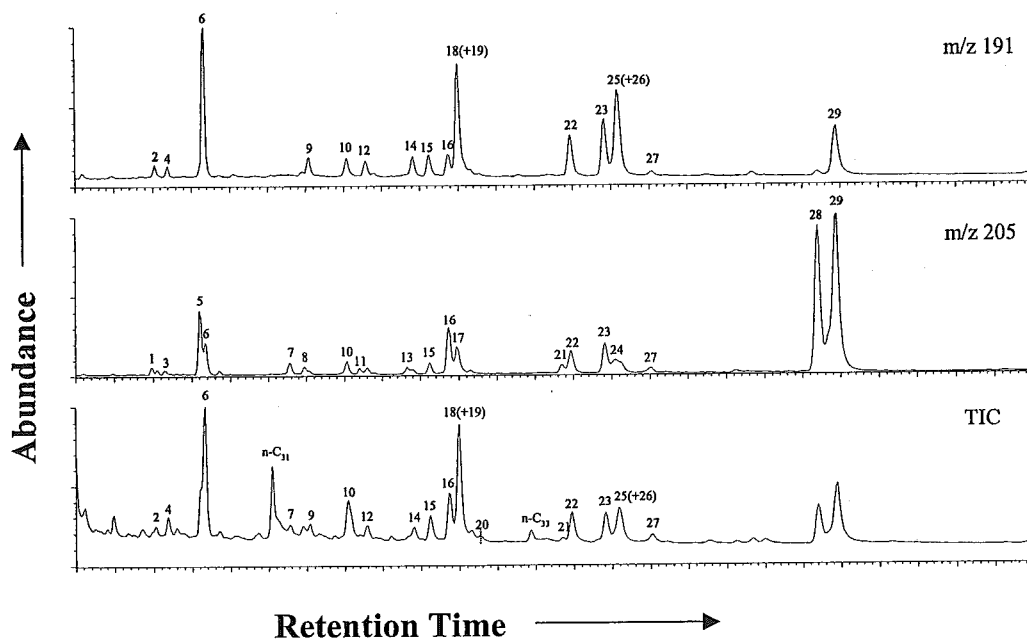

Figure 5. Partial m/z 191 and m/z 205 fragmentograms showing the pentacyclic triterpenoid distribution. Assignments of peaks are shown in Table 1.

Peak assignments are shown in Table 1. These compounds can be classified into unsaturated hopenes including hop-17(21)-enes and hop-13(18)-ene, saturated hopane series and methylhopane series.

Table 1. Assignments of the pentacyclic triterpenoids present in the sediments of the Sun-Moon Lake.

Peak No.*	Assignments	Formula	Molecular weight
1	Methylhopene (Methyl-22,29,30-Trisnorhop-17(21)-ene?)	$C_{28}H_{46}$	382
2	22,29,30-Trisnorhop-17(21)-ene	$C_{27}H_{44}$	368
3	Methylhopane (Methyl-17α(H)-22,29,30-Trisnorhopane?)	$C_{28}H_{48}$	384
4	17 α (H)-22,29,30-Trisnorhopane (Tm)	$C_{27}H_{46}$	370
5	Methylhopane (Methyl-17β(H)-22,29,30-Trisnorhopane?)	$C_{28}H_{48}$	384
6	17 β (H)-22,29,30-Trisnorhopane	$C_{27}H_{46}$	370
7	Methylhopene (stereoisomer of peak 1 ?)	$C_{28}H_{46}$	382
8	Methyl-17 α (H),21 β (H)-30-norhopane	$C_{30}H_{52}$	412
9	17 α (H),21 β (H)-30-Norhopane	$C_{29}H_{50}$	398
10	Hop-17(21)-ene	$C_{30}H_{50}$	410
11	Methyl-17 β (H),21 α (H)-30-norhopane	$C_{30}H_{52}$	412
12	17 β (H),21 α (H)-30-Norhopane (Normoretane)	$C_{29}H_{50}$	398
13	Methyl-17 α (H),21 β (H)-hopane	$C_{31}H_{54}$	426
14	17 α (H),21 β (H)-Hopane	$C_{30}H_{52}$	412
15	Hop-13(18)-ene	$C_{30}H_{50}$	410
16	Methyl-17 β (H),21 β (H)-30-norhopane	$C_{30}H_{52}$	412
17	Methyl-17 β (H),21 α (H)-hopane	$C_{31}H_{54}$	426
18	17 β (H),21 β (H)-30-Norhopane	$C_{29}H_{50}$	398
19	17 β (H),21 α (H)-Hopane (Moretane)	$C_{30}H_{52}$	412
20	Homohop-17(21)-ene	$C_{31}H_{52}$	424
21	Methyl-17 α (H),21 β (H)-homohopane (22R)	$C_{32}H_{56}$	440
22	17 α (H),21 β (H)-Homohopane (22R)	$C_{31}H_{54}$	426
23	Methyl-17 β (H),21 β (H)-hopane	$C_{31}H_{54}$	426
24	Methyl-17 β (H),21 α (H)-homohopane (22R)	$C_{32}H_{56}$	440
25	17 β (H),21 β (H)-Hopane	$C_{30}H_{52}$	412
26	17 β (H),21 α (H)-Homohopane (Homomoretane)	$C_{31}H_{54}$	426
27	Pentacyclic triterpene (unknown structure)	$C_{31}H_{52}$	424
28	Methyl-17 β (H),21 β (H)-homohopane (22R)	$C_{32}H_{56}$	440
29	17 β (H),21 β (H)-Homohopane	$C_{31}H_{54}$	426

* Peak No. refers to the fragmentograms in Fig. 5.

The hopane distribution is dominated by a homologous series of C_{27}-C_{32} hopanes with biological 17β(H),21β(H) configuration. Hopanes with 17α(H),21β(H) and 17β(H),21α(H) configurations (i.e., moretanes) are also detected but in much smaller amount than their 17β(H),21β(H) analogues. The C_{31} 17α(H),21β(H)-homohopane is in pure biological 22R form and the 22S geological homologue is not detected. Predomonance of 17β(H),21β(H) hopanes and C_{31} 22R homohopane, as well as the unsaturated tetracyclic and pentacyclic triterpenes, indicates that the samples are still in an early diagenetic stage.

Hopanes in geological samples are believed to be derived from diploptene and C_{35} tetrahydroxylhopane, which are widely distributed among bacteria and cyanobacteria (blue-green algae), tropical trees, some grasses and lichens and several ferns (Ourisson *et al.*, 1979). C_{35} triterpene alcohol, a bactereiohopane-tetrol with 17β(H),21β(H) configuration is a constituent of the membranes of bacteria other than Archaebacteria (Orrisson *et al.*, 1979).

Besides the hopanoid series, a homologous series of A/B-ring methylated hopanes, which gives a diagnostic fragment of m/z 205, are detected in the sediments from the Sun-Moon Lake as shown in Fig. 5. Based on the comparison of mass spectra and retention time sequence with those published (McEvoy and Giger, 1985; Summons and Jahnke, 1992) C_{28}, C_{30}, C_{31}, and C_{32} 2β-methylhopanes with 17β(H),21β(H), 17α(H),21β(H) and 17β(H),21α(H) configurations, respectively, are identified (Table 1). The C_{29} methylhopanes, which are analogous to C_{28} hopanes, are absent. The methylhopanes are eluted immediately prior to their corresponding hopanes. It is noted that the distribution of methylhopane series is almost analogous to that of hopane series and dominated by the 17β(H),21β(H)-methylhopanes.

Methylhopanes have been reported to occur in oils and ancient sediments (Seifert and Moldowan, 1979; Summons and Jahnke, 1992). It is proposed that they are derived from A-ring methylated tetriterpenoids present in prokaryotes such as cyanobacteria and methylotrophs (Bisseret *et al.*, 1985; Zundel and Rohmer, 1985), which flourish in the upper oxic waters as well as at the oxic/anoxic interface of the water column. The similarity in the distributions of methylhopanes and their hopane counterparts infers common biosynthetic pathways of their precursor components. The prokaryotic species responsible for the precursors of the hopanoids may also be responsible for the precursors of the methylated components.

Methylketones, Methylesters, and Aldehydes

Partial fragmentograms of m/z 58, m/z 74 and m/z 82 of the F2 fraction are given in Figure 6 to show the distributions of methyketones, methylesters and aldehydes present in the samples, respectively. The methylketones with carbon number ranging from 23 to 35 are detected. Its distribution shows a distinct odd-carbon number dominance with maximum at C_{29}. The C_{17}-C_{35} methylesters are detected and show bimodal distribution with maximum at C_{17} and C_{29}, respectively. Odd-carbon number is highly dominant over the even-carbon number components. Long chain aldehydes with carbon number ranging from 24 to 34 are present and show distinct even carbon dominance with the maximum at C_{26} and C_{28}.

· Little variation in the methylketone and methylester distributions of samples of different depth is observed. The similarity in the distributions of methylketones and methylesters implies that they have a common origin. It is supposed that these lipids are early diagenetic products derived mainly from the wax components present in the higher plants.
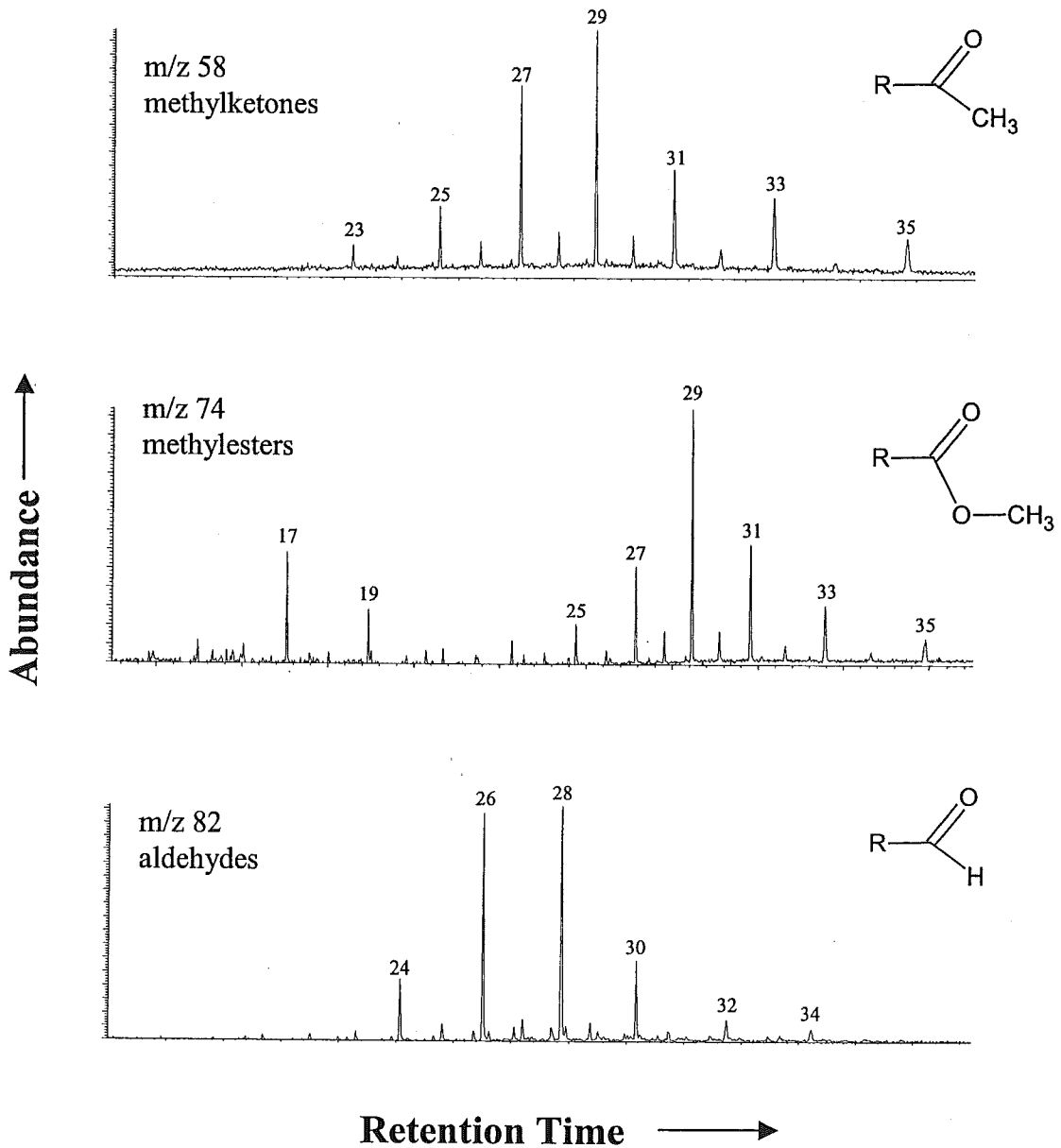
Figure 6. Partial fragmentograms of m/z 58, m/z 74 and m/z 82 of the F2 fraction showing the typical distributions of methylketone, methylesters, and aldehydes, respectively, in the Sun-Moon Lake sediments. The carbon length is indicated.
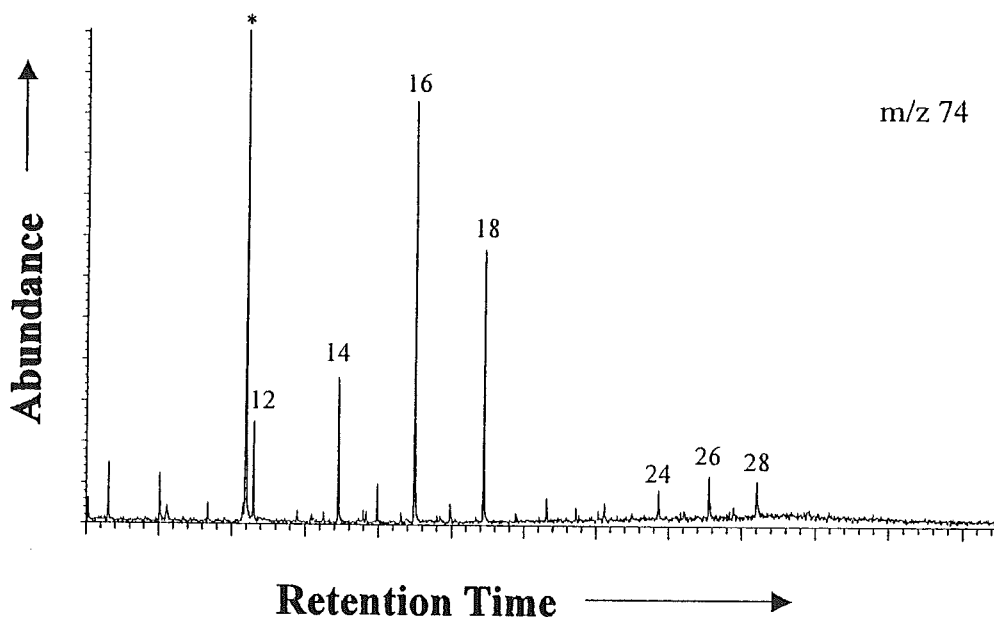
Figure 7. Partial m/z 74 fragmentogram of the methylated acid fraction showing the typical n-fatty acid distribution in the Sun-Moon Lake sediments. The carbon length is indicated. The peak labeled with * is an unknown compound.

n-Fatty acids

n-Fatty acids with carbon number ranging from 10 to 28 are present in the sediments. The n-fatty acid distribution, measured as methyl esters, is shown in Fig. 7. All samples have n-fatty acid distribution dominated by even carbon-numbered components and show a dominant maximum at C_{16}. Little variation in the n-fatty acid distribution of samples of different depth is observed.

The short-chain n-fatty acids (C_{12} -C_{18}) are dominant and in much higher amount than the long-chain n-fatty acids (>C_{20}), reflecting planktonic and bacterial contributions to the sediments. This implication is not consistent with the major contribution from higher terrestrial plants to the sediments inferred by the n-alkane, methylketone and methylester distributions. A possible reason for this discrepancy is that a proportion of the long-chain n-fatty acids derived from higher terrestrial plants have been degraded during the organic matter transportation.

n-Alkanols

n-Alkanols with carbon number ranging from 20 to 32 are detected in the sediments. A representative n-alkanol distribution, measured as TMS ethers, is shown in Fig. 8. The n-alkanols show an even over odd predominance and a major peak at C_{28}. Long-chain n-alkanols (C_{22}-C_{28}) are usually thought to originate from terrigenous plants. The short chain n-alkanols, which are considered to be a source indicator for aquatic organisms, such as plankton or macrophytes, are absent or in trace in the sediments.
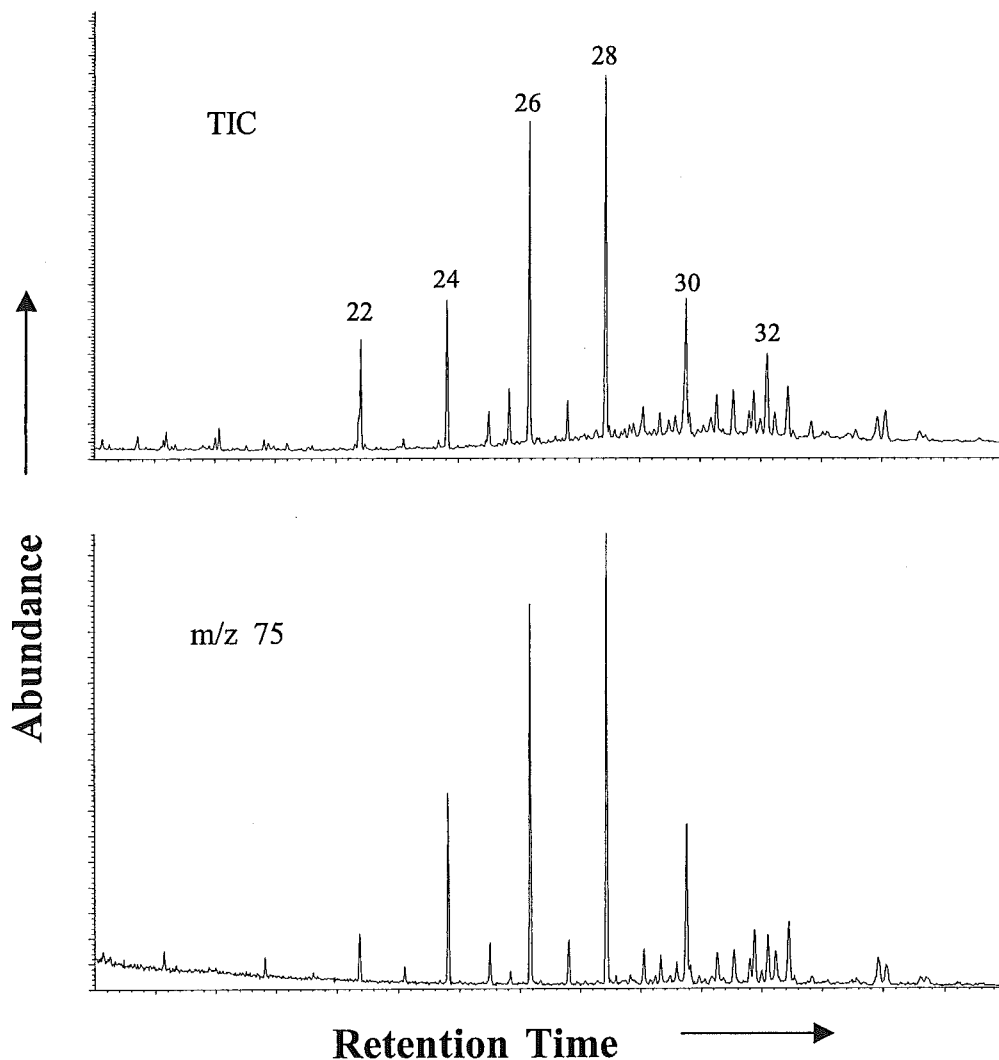
Figure 8. Partial total ion chromatogram and m/z 75 fragmentogram of the derivatised alkanol fraction showing the typical n-alkanol distribution in the Sun-Moon Lake sediments. The carbon length is indicated.

CONCLUSIONS

Lipid compounds including n-alkanes, triterpenoids, methylketones, methylesters, n-fatty acids and n-alkanols are presented in the sediments of the Sun-Moon Lake.

The distributions of these lipid compounds reveal that the organic input of the sediments involves both higher terrestrial plants and aquatic organisms/bacteria. The dominance of high

molecular n-alkanes, methylketones, methylesters, aldehydes and n-alkanols indicates that higher terrestrial plants are the major organic matter source for the recent sediments in the Sun-Moon Lake. Occurrence of tetracyclic triterpenoids, including des-A-lupane, indicates a contribution of higher terrestrial plants of angiosperms. Pentacyclic triterpenoids, including hopanes and methylhopanes with various stereochemical configurations and carbon number of 27-32 may reflect the contribution of organic matter from bacteria. Vegetational change through time is indicated by comparison the n-alkane distribution of samples from the upper and lower sections.

The predominance of 17β(H),21β(H) hopanes and methylhopanes, C_{31} 22R homohopane, unsaturated tetracyclic and pentacyclic triterpenes, as well as the high CPI value of the n-alkane distribution reflect the characteristics of sediments of an early diagenetic stage. The decreasing of CPI value of samples in the upper 35 cm section may infer the recent pollution of the petroleum-derived hydrocarbons or the cultivation of the outcropped ancient sedimentary rocks in the surrounding area of the Sun-Moon Lake in last two centuries.

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