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IP_{25} AND BRASSICASTEROL: THE BIOMARKER AS ICE PROXY FOR THE SEA ICE COVERING IN THE ARCTIC OCEAN

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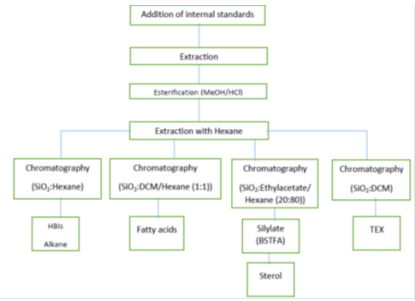
Graphical abstract



Abstract

Stable biomolecules play an important role in the oceans because they can be used to determine the origin and the translocation of the organic material such as sterols which are synthesized by plants. In recent times, a major focus has been on a new biomarker in the high latitudes, a highly branched Isoprenoid (HBI) as the new sea ice proxy 2,6,10,14-tetramethyl-7-(3-methylpent-4-enyl) Pentadecane (IP25, Ice Proxy with 25 carbon atoms). The stability of the proxies such as sterols and HBI is important because only an almost inert character of the molecules ensures its reproducible use as biomarker proxy.In this research, different analytical methods were used to check whether these components actually fulfill the requirements of duration and storage to be stable that possible degradation processes such as microbial degradation and autoxidation. However, duration and storage are proven negligible or can be treated as the secondary essential factors. For this purpose, samples of a sediment core from the continental shelf of the East Siberian Sea were analyzed. Samples from this core were stored (1) deep frozen directly after sampling (-30°C) and (2) at 5°C for an extended time period.The results show that the degradation processes are negligible or least only of secondary importance. The concentrations of the biomarker show good correlations. The ratio between Brassicasterol and IP_{25} (PIP_{25}-Index) is not affected by the different storages. In addition, all measurements using gas chromatography and mass spectrometry result in nearly the same concentrations of the compounds.

Keywords: IP_{25}, biomarker, 24 methylcholesta-5, 22e-diene 3β-ol, brassicasterol

1.0 INTRODUCTION

Polar sea ice has a complex relationship with the global climate and this relationship is shown in the interplay of ice, scope, duration and the exchange of heat, moisture and gas between ocean and atmosphere. Moreover, it significantly affects the oceanic heat transport and salinity between south and the north hemisphere. It has a regulating effect on the thermohaline circulation in the northern North Atlantic. Sea ice plays a role in heat exchange to keep cool the atmosphere by reflecting solar radiation. In addition, sea ice plays a role for the carbon cycle in high latitudes. Organic materials exist on the carbon cycle and they are synthesized under the prevailing conditions on earth through the fixation of carbon dioxide in autotrophic organisms. The organic substances may be respired to CO_2 by heterotrophic organisms again and these organic substances are circulated and stored in cycle under chemical. Among the heterotrophic organisms, sea ice algae absorbs carbon, which exist in the melting ice, faster than the pelagic phytoplankton and the largest amount of carbon is found in the sedimentation.

Certain biogenic organic substances have the important roles in specific organisms or organism's classes. These substances include various classes of substances such as sterols, alkanes, alkenes, fatty acids, and ketones. They allow the biological activities from the past biological activity by analysis of organic trace substances. The distribution of organisms in the sediment must be reconstructed in order to find out the stability of the biomarker.

Moreover, the maturation of the sediment material determines the geological phase through conversion of the sterols. The proportion of long chain, variety of unsaturated ketones (alkenones) showed the result on the sediments in the oceans surface's temperature during sedimentation. The study about time trace period can be carried out by analysis of sterols.

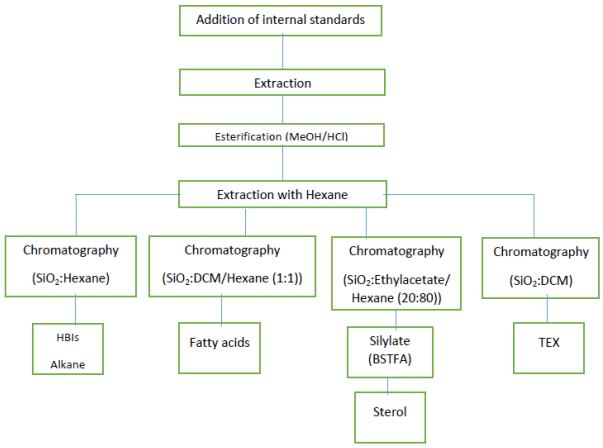


Figure 1 Flow Schema of Performing Work

2.0 EXPERIMENTAL

A. Material

Sediment core from PS72/350-2 (79°40.19'N, 169°50.30'E) was taken during the Polar stern Expedition ARK-XXIII/3. Then, the samples were grinded in a mortar and they are respectively freeze-dried at 5^0C and -30^0C over several years. The other materials used were IP_{25} and sterol where both were as biomarker.

B. Method

Figure 1 show the flow schema of performing work. After grinded, the samples were frozen in a freezer at about -25°C for 24 hours. Thereafter, they were dried in a freeze dryer for 3 days. Then, they were homogenized using a mortar and pestle. The samples homogenized were stored in brown glass bottles and extracted in ASE 200 and at this stage some copper were added. This aimed to remove the sulfur from the sample. Approximately 4-5 g sample were weighed, added100 µl Squalene (0.024 µg/µl), 20 ml cholesterol d6 (1,01µg/µl), 20 µl 7-Hexylnonadecane (0,0038µg/µl), and GDGT C26 (10 µg/ml) into the mentioned sample as the internal standard. Finally, the extractions cells were added with sea sand (at 500°C). Copper, sample, and sea sand were separated by the heated glass fiber filters. The extraction was processed using dichloromethane (DCM): methanol (MeOH) (2: 1) at a temperature of 100 ° C and a pressure of 1000psi.

The extract was transferred into conical flask and at 40°C / 600> 400> 250 mbar at rotary evaporator to a residue of about 1 ml. The extract was transferred to a test tube then 1 ml of 3N MeOH-HCl was added. The sample was esterified at 50°C for 12 hours and 3 ml of hexane was added to the extract in the test tube using a Vortex shaker. The upper phase was removed and dried by Na_2SO_4. This step was repeated 2 times with 2 ml hexanes. Subsequently, about 1 ml was concentrated on a rotary evaporator at 300 mBar.

For chromatography sample, a Pasteur pipette was filled with 5 cm purified cotton and silica (SiO_2). Afterwards 1 pipette ethyl acetate: hexane (20:80), 1 pipette dichloromethane, and 5 pipettes hexane were filled in a Pasteur pipette and finally filled by the extract in the previous stage. Fractions were separated chromatographically using 5 ml of hexane for the alkane fraction, 7 ml DCM: hexane (1: 1) for the fatty acid fraction, 6 ml of ethyl acetate: hexane (20:80) for the sterol fraction and 6 ml MeOH for Southwestern fraction. All fractions were concentrated on a rotary evaporator (40°C / 300 mbar) to approximately 0.5 ml. Afterwards, under a gentle stream of nitrogen in the sample vial is further obtained in form of concentration.

The sterol fraction was purified from the residual solvent using nitrogen and then added by 500 µl BSTFA. Then the sample was silylated on heat block for 2 hours at 60°C. After cooling process, the extract was concentrated under nitrogen in order to obtain the complete dryness of the sample and added another 100 µl of hexane and moved to auto sampler vials. The alkane fraction and the sterol fraction were measured by GC-MS (Gas Chromatography- Mass Spectrometry). The Measurements were performed with an Agilent 6850 GC (30 m DB-1 MS Column, Diameter 0.25 mm, film thickness 0.25 microns) with an Agilent 5975 MSD VL C (Mass Selective Detector). The IP_{25} measurement was measured under the following conditions:

Init.temp : 60°C
Init.time : 3 min
Flow : 1 mL / min

Rate	Final Temp.	Final Time
Level 1 15°C/min	150°C	--------
Level 2 10°C/min	320°C	15 min

The IP_{25} measurement was measured as a SIM measurement and Full Scan measurement. The SIM parameters were detected from minute 6 to Minute16 with mass 99 m/z. From minute 16 to minute 19, the Hbis with mass 346, 348 and 350 m/z were detected where the IP_{25} had the mass of 350 m/z. After 19 minutes, the internal standards were measured with mass of 99 m/z and 266 m/z. In the Full Scan measurement, the samples were detected with 50 to 550 m/z.

The sterol measurements were measured under the conditions:

Init.temp : 60°C
Init.time : 2 min.
Flow : 1 mL / min

Rate	Final Temp.	Final Time
Level 1 15°C/min	150°C	-------
Level 2 3°C/min	320°C	20 min

The sterol fraction was also measured at the GC. The measurement was performed by Agilent 6850 GC (60m db, 1MS Column Diameter 00:32 mm and film thickness 0.25µm). They were measured under the following conditions.

Init.Temp : 60 ° C
Init.time : 3 min
Flow : 1.1 mL/min

Rate	Final Temp.	Final Time
Level 1 15°C/min	150°C	-----
Level 2 3°C/min	320°C	30 min

The GC had a Cooled Injection System (CIS) and the injection temperature was 60°C. It was heated up to 340°C at a heating rate of 12°C/sec and the final temperature is maintained for 2 minutes. Both GC and GC-MS used helium as the carrier gas and the injection volume was 1 µl.

Beside the measurement with GC-MS, Total organic Carbon (TOC) measurement was carried out. In this stage, each 50 mg sample was weighed into a ceramic crucible then the sample was wetted 3 drops of ethanol, and then 500 µl 37% concentrated hydrochloric acid. Afterwards, the sample was heated on the heating plate for 2 hours at 250°C. Iron and tungsten were added into the sample to determine the TOC composition.

3.0 RESULTS AND DISCUSSION

Generally, many studies have discussed the organic geochemical analysis in which the method used was the storage of sediment samples at -30 °C without a solvent. This method did not affect the chemical compositions of the sample (e.g. Tupas et al., 1994). In this study, the biomarker samples using gas chromatography and massenspectroscopy were compared and were observed at the different storage temperatures (5°C and -30°C). This was carried out because many cases were not directly used after the sediments had been collected but they were stored with temperature of 5°C. Generally, the unsaturated components were sensitive for autoxidation (Thiele, 1986). Autoxidation constitutes the oxidation that needs atmospheric oxygen. The organic molecules need light, particularly in short light wavelength, and oxygen from the air to form Hydroperoxides as is shown in Figure 2. It produces a variety of products which are undesirable in identifying the components and analyzing the results. In addition, degradation role by special microbial plays an important role in auto-oxidation

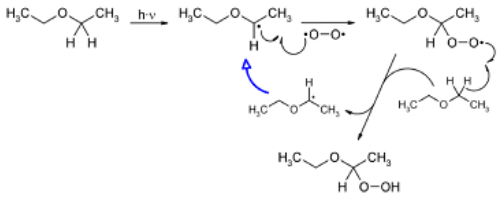


Figure 2 Autoxidative Formation of Hydroperoxides

Figure 3 shows the difference between the degradation by microbial degradation photo and hydrogenation process. The degradation mechanisms are shown with Δ^5-sterole (double bond between C5 and C6), in which the sterols observed in this study belong to this group. Referring the comparison results of the storage difference of sterols (Brassicasterol, Campesterol and β-Sitosterol) and ice proxy IP_{25}, it can be concluded that the autoxidation and the microbial degradation are not significantly relevant. Thus, it is possible to use the old sample materials even though they are stored under optimum temperature conditions. However, the other researchers should pay attention to the interpretation and the comparison of different methods used.

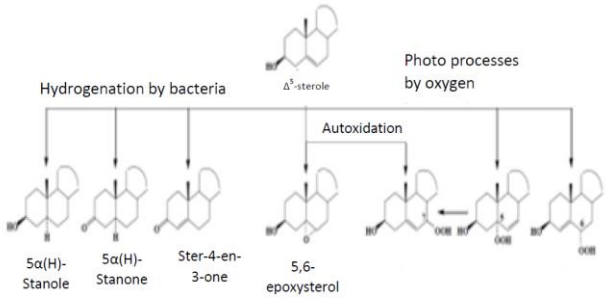


Figure 3 Δ5-sterole Degradation's Schema

The absolute concentration is not the only thing important in the application of biomarkers for paleontological issues, so is the ratio of molecules concentrations. The latest studies have shown that the combination of the different markers enhance the findings on ice reconstruction (Müller et al, 2011; Fahl and Stein, 2012; Stein and Fahl, 2013; Stein et al, 2012). This new approach has been originally resulted from the fact that the researchers are unable to distinguish whether an IP_{25} is from the place with a fully covered ice or free of water. Müller et al. (2011) conducted the study that results a marker for water free area and the formula for PIP_{25} Index (Phytoplanktonmarker-IP_{25}-Index).

Table 1 Calculated PIP_{25} index values for the investigated the core PS72 / 350-2

$$PIP_{25} = \text{Concentration } IP_{25} / (\text{Concentration } IP_{25} + (\text{concentration Brassicasterol} * F))$$

The factor F is only used for different concentration ranges between IP_{25} and Brassicasterol while the value of F = 0.0695 is for the core PS72 / 350-2.

Table 1 shows the calculated PIP_{25} index values for the investigated the core PS72 / 350-2. In addition, the PIP_{25} index values are nearly identical, so it can be summarized that the degradation in both storage temperatures work in a parallel way as stated in Fahl and Stein (2012). Therefore, the PIP_{25} can be used as an indicator to interpret paleontological issues related to degradation processes mentioned before. Another result of the analysis using the internal standard sample shows the similar response to the previous conclusion. The limitations of this study are the extraction may lose during all processes needed or unreliability of the tools used. Figure 4 shows a PIP_{25} comparison between the storaged samples in 5°C and -30°C.

Depth	PIP_{25} 5°C	PIP_{25} -30°C
0	0,6	0,5
31	0,6	0,5
51	0,6	0,6
70,5	0,5	0,5
91	0,5	0,6
110	0,3	0,6
130	0,4	0,5
150,5	0,5	0,4
170,5	0,5	0,5
189,5	0,4	0,4
210	0,4	0,4

Figure 4 PIP_{25} Comparison between the Storaged Samples in 5°C and -30°C

Because the sea ice plays a major role to determine the seasonality in the marginal seas of the Arctic Ocean, the focus of this study is on the distribution of ice proxies IP_{25} in the sediment core PS72 / 350-2. Sea ice is the most sensitive component of the total system since its change and the changes in the atmospheric circulation can influence the radiation balance (ocean-atmosphere). Thus, it is important in the climate development in this area. In addition, the extent of the sea ice impacts the biological productivity because a covering ice impedes the primary production during a covered ice situation or an ice-free ocean increases the production as is illustrated in Figure 5 (Wassmann, 2011).

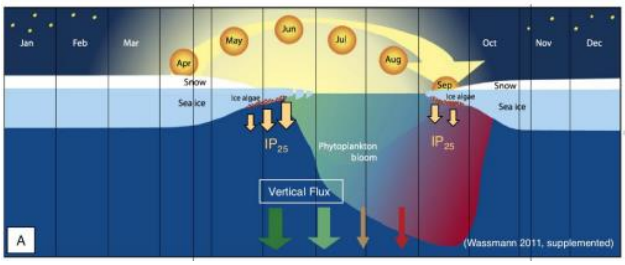


Figure 5 Schematic Representation of the Basic Processes, which Productivity and Flux from Phytoplankton, Zooplankton and Ice Algae mit Ice Algae Proxy IP_{25} Influenced by Light and Sea Ice

The interpretation of the IP_{25} indicated that the IP_{25} from ice-free or ice-covered areas are not differentiated because the values are zero. Müller et al. (2011) figured the way out of this problem using the second marker: "Phytoplanktonmarker Brassicasterol". The Brassicasterol serves as an indicator of 100% ice-free situation and is resulted from the new index PIP_{25}.

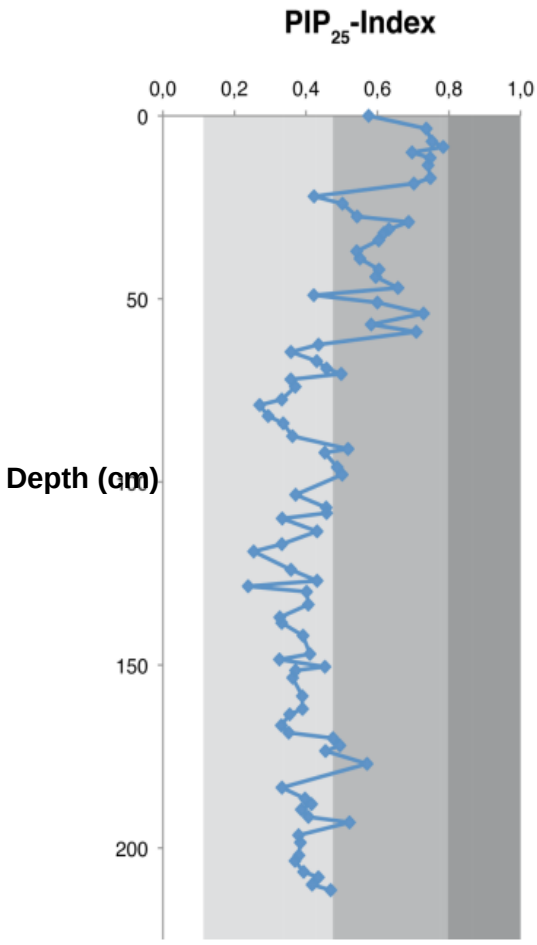


Figure 6 PIP_{25} Index from PS72/350-2

Figure 6 indicates the index calculation of PS72 / 350-2. It can be seen that the PIP_{25} indexes are in the bottom core section that is lower in the upper horizon (0-100 cm). The different sizes are shown in Figure 5 in different shades of gray as highlighted. Therefore, the values suggest that the core position is over the entire period represented as the core of nearly-covered ice situation. This condition enhances the primary production through melting process and an ice-associated alga as found in some studies: Fahl and Stein, 1997; Sakshaug, 2004; Müller et al., 2011; Fahl and Stein, 2012

4.0 CONCLUSION

Stable biomolecules play an important role in the oceans because they can be used to determine the origin and the translocation of the organic material such as sterols which are synthesized by plants (e.g. Campesterol and β-Sitosterol) or the aquatic sterolscholesta-5-en-3β-ol (cholesterol) and 24 Methylcholesta-5, 22E-diene 3β-ol (Brassicasterol) (Boon et al., 1979; Volkman, 1989). The latter is synthesized by diatoms, while cholesterol is associated with secondary producers (zooplankton).

In recent times, a major focus has been on a new biomarker in the high latitudes, a highly branched Isoprenoid (HBI), (Belt et al., 2007). It is the new sea ice proxy 2,6,10,14-tetramethyl-7-(3-methylpent-4-enyl) Pentadecane (IP25, Ice Proxy with 25 carbon atoms).

The studies of sediment traps and sediments from the Arctic Ocean have demonstrated a definitely association of this component to sea ice (Belt et al., 2007; Mueller et al., 2009; Mueller et al., 2011; Fahl and Stein, 2012; Stein et al., 2013; Xiao et al., 2012). It is assumed that the molecule is synthesized by ice algae Haslea spp. (Belt et al., 2007). Therefore, these components can be used to reconstruct the sea ice conditions in the Arctic regions qualitatively and even semi-quantitatively. The undoubtful importance of sea ice distribution in relation to the current climate emphasizes the importance of this proxy. Sea ice is ultimately a most sensitive component in the change of climate which attracts the changes of other variables such as atmospheric heat budget and gas exchange. The stability of the proxies such as sterols and HBI is important because only an almost inert character of the molecules ensures its reproducible use as biomarker proxy. In this research, different analytical methods were used to check whether these components actually fulfill the requirements of duration and storage to be stable that possible degradation processes such as microbial degradation and autoxidation. However, duration and storage are proven negligible or can be treated as the secondary essential factors. For this purpose, samples of a sediment core from the continental shelf of the East Siberian Sea were analyzed. Samples from this core were stored (1) deep frozen directly after sampling (-30°C) and (2) at 5°C for an extended time period.

The results show that the degradation processes are negligible or least only of secondary importance. The concentrations of the biomarker show good correlations. The ratio between Brassicasterol and IP_{25} (PIP_{25}-Index) is not affected by the different storages. In addition, all measurements using gas chromatography and mass spectrometry result in nearly the same concentrations of the compounds.

Acknowledgement

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