Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Organic Geochemistry 34 (2003) 635–641
Organic Geochemistry
www.elsevier.com/locate/orggeochem

Note

# Gel permeation chromatography for fractionation and isotope ratio analysis of steranes and triterpanes in oils

Toshinori Inaba[1,*], Noriyuki Suzuki

*Division of Earth and Planetary Sciences, Graduate School of Science, Hokkaido University, N10, W8, Sapporo 060-0810, Japan*

Received 1 October 2002; accepted 3 January 2003
(returned to author for revision 14 October 2002)

**Abstract**

Gel permeation chromatography (GPC) was applied to concentrate steranes and triterpanes in oils and measure their compound-specific carbon isotope ratios ($\delta^{13}C$). Trace steranes and triterpanes were concentrated from *n*-alkane-rich Miocene Yabase marine oils by GPC. $\delta^{13}C$ was determined by gas chromatography–combustion/isotope ratio–mass spectrometry (GC–C/IR–MS). The $\delta^{13}C$ of cholestane from the Yabase oil ranges from −24.4 to −26.3‰. This is nearly consistent with that of the *n*-$C_{18}$ to *n*-$C_{20}$ alkanes, suggesting significant algal and zooplankton contribution. The $\delta^{13}C$ of ethylcholestane from the Yabase oil is comparatively light, ranging from −26.7 to −30.8‰. It is similar to the $\delta^{13}C$ of *n*-$C_{27}$, *n*-$C_{29}$, *n*-$C_{31}$ alkanes and oleanane, suggesting a significant contribution of terrestrial higher plant material.


## 1. Introduction

In the late 1970s, the basic concept of a device combining an isotope ratio mass spectrometer with a gas chromatograph was developed, to measure compound-specific carbon isotope ratios ($\delta^{13}C$) of trace organic compounds (Sano et al., 1976; Matthews and Hayes, 1978). The gas chromatography–combustion/isotope ratio–mass spectrometer (GC–C/IR–MS) came into practical use in the 1990s. The compound-specific $\delta^{13}C$ measurement of organic matter in sedimentary rock, oil, and gas has been accepted in petroleum geochemistry. Determination of source organic matter (e.g. Kennicutt and Brooks, 1990) and petroleum generation mechanism (e.g. Bjorøy et al., 1992) are typical applications. Compound-specific $\delta^{13}C$ analysis has also been applied to some trace biomarkers of source rocks (e.g. Bjorøy et al., 1992) and heated samples (e.g. Ishiwatari et al., 1997). However, the measurement of compound specific $\delta^{13}C$ by GC–C/IR–MS requires sufficient concentration of the individual target compounds. $\delta^{13}C$ analyses of steranes and triterpanes, especially in oils, are limited (e.g. Schoell et al., 1992). Low relative abundance of steranes and triterpanes and the significant presence of co-eluents in gas chromatography present difficulties for the isotope measurement of these useful biomarkers in oils.

Gel permeation chromatography (GPC) was widely adopted for molecular separation after the development of dextran gel (Porath and Flodin, 1959) and polystyrene gel (Moore, 1964). Gel has a three-dimensional cross-linked network of long polymer chains. The network defines an exclusion limit, which is the maximum size of molecules that can pass through the gel network. If the molecule's size is larger than the maximum diameter of gel pores, it cannot penetrate any gel pores and is completely excluded from the pores. Such molecules appear first on a GPC chromatogram. If the molecule's size is smaller than the gel pore diameters,

* Corresponding author. Tel.: +81-3-5440-8611; fax: +81-3-5440-8585.
*E-mail address:* t_inaba@epedeco.co.jp (T. Inaba).
[1] Present address: The Egyptian Petroleum Development Co., Ltd., 11-10, Minamiazabu 2-Chome, Minato-ku, Tokyo 106-0047, Japan.

0146-6380/03/$ - see front matter 
doi:10.1016/S0146-6380(03)00017-2

the molecule enters the gel. Such molecules are strongly retained in the gel, and appear only in the later part of a GPC chromatogram.

In the present paper, GPC is applied to concentrate steranes and triterpanes in Miocene Yabase oil to determine their carbon isotope ratios. Our results show that GPC has significant potential as a preparation method for the compound-specific $\delta^{13}C$ analyses of these biomarkers. Implications of the $\delta^{13}C$ values of oil steranes and triterpanes are also discussed.

## 2. Samples and experimental

The Yabase oil samples are from the Kitaakita R-1a, Sotoasahikawa R-22, R-37, R-100, R-114, Koya R-76, R-83, and R-135 wells (see Fig. 1 of Inaba et al., 2001; both Sotoasahikawa R-22 and R-37 are 0.5 km north of Koya R-76, Koya R-83 is close to Koya R-76, and Koya R-135 is 1.2 km south of Koya R-76). Yabase oil was derived from a source rock that is rich in marine organic matter (plankton) based on cholestane and methylcholestane predominance, $n$-$C_{15}$ and $n$-$C_{17}$ significance, and low hopanes/steranes ratio (e.g., Hirai et al., 1990). A small amount of oleanane, a contribution of terrestrial higher plants to the source rock, was also reported (e.g. Suzuki et al., 1995). Organic-rich siliceous mudstones of the Middle-Late Miocene Onnagawa Formation are considered to be the major source rock for the Yabase oils (Hirai et al., 1990).

Experimental procedures for bitumen extraction and column chromatography are given in Inaba et al. (2001). In the GC–C/IR–MS system, a Hewlett-Packard HP6890 GC was coupled with a Finnigan MAT 252 isotope-ratio mass spectrometer using a Finnigan MAT Interface GC-Combustion III. A GC equipped with a DB-5 fused silica capillary column (30 m, 0.25 mm i.d., J&W) was programmed isothermally at 40 °C for 2 min, from 40 to 120 °C at 20 °C/min, from 120 to 300 °C at 4 °C/min, and then held at 300 °C for 25 min.

GPC conditions were set using the sterane-rich bitumen sample extracted from an Onnagawa mudstone sample. GPC for the saturate hydrocarbon fraction was carried out as follows. Column: TSKgel G2000$H_{HR}$; mobile phase: dichloromethane; flow rate: 0.79 ml/min; injected sample volume: 50 μl and approximately 32 μg/μl; detector: refractive index detector (RI); and column temperature: 40 °C. The GPC solute was separated into several fractions, which were analyzed by GC and GC–MS.

## 3. Results and discussion

A GPC chromatogram for saturate hydrocarbons from the Sotoasahikawa R-114 oil is shown in Fig. 1(A). GCs of the bulk sample and six fractions obtained by GPC are also shown in Fig. 1(B; F1–F6). Retention time intervals of these fractions are: F1 9.26–9.68 min; F2 9.68–10.39 min; F3 10.39–10.81 min; F4 10.81–11.11 min; F5 11.11–11.86; and F6 11.86–12.41 min. Major peaks in F2–F5 are $n$-alkanes. The longer chain $n$-alkanes elute earlier in GPC as shown in Fig. 1. Higher molecular weight cyclic alkanes were recovered in F4 and F5 fractions.

The $m/z$ 217 and $m/z$ 191 mass chromatograms of the F4 and F5 fractions show that the major cyclic alkanes in these fractions are steranes and triterpanes, respectively (Fig. 2). The F4 fraction is mainly composed of cholestanes, methylcholestanes and ethylcholestanes, and does not contain any $n$-alkanes that co-elute with these steranes. The major cyclic alkanes in the F5 fraction are norhopane, hopane and homohopanes, and no $n$-alkanes are detected as their co-eluents. Steranes and triterpanes elute much slower than the $n$-alkanes with carbon numbers similar to steranes and triterpanes in GPC. This probably results from differences in their apparent molecular size (head to tail length). Earlier elution of steranes and homohopanes compared with norhopanes and hopanes is consistent with their different apparent molecular sizes. GPC also reduces the amount of unresolved complex mixture. Compound-specific $\delta^{13}C$ values of n-alkanes from the bulk and the F2–F4 fractions of the Sotoasahikawa R-114 oil indicate little isotopic fractionation (generally less than 0.5‰) in the GPC column (Table 1). The $\delta^{13}C$ values of 17α,21β(H)-hopane also show little isotopic fractionation between fraction F5 and F4 (Table 2).

Compound-specific $\delta^{13}C$ values of trace biomarkers were successfully measured for steranes and triterpanes concentrated from Yabase oils by GPC, and the $\delta^{13}C$ values are shown in Tables 1 and 2, and Fig. 3. The maturity level of the Yabase oil is low, as indicated by ethylcholestane $20S/(20S+20R)$ ratios ranging from 0.34 to 0.41 (except for the Kitaakita R-1a oil). The $\delta^{13}C$ values are, therefore, controlled principally by source organic matter type and not by maturation. The $\delta^{13}C$ values of $n$-$C_{18}$ to $n$-$C_{20}$ alkanes from the Yabase oil are comparatively heavy, ranging from −23.7 to −26.0‰, suggesting that these alkanes are derived mainly from algae and associated zooplankton. The $\delta^{13}C$ of $n$-$C_{21}$ to $n$-$C_{24}$ alkanes is characterized by a comparatively wide range (−24.4 to −29.2‰), with a general decrease with increasing carbon number. The $\delta^{13}C$ values of $n$-$C_{25}$ to $n$-$C_{33}$ alkanes are comparatively light, ranging from −25.8 to −29.8‰. The $\delta^{13}C$ values of $n$-$C_{31}$ and $n$-$C_{33}$ are lighter than those of $n$-$C_{30}$ and $n$-$C_{32}$, strongly suggesting a higher plant input to the source organic matter of the Yabase oil.

The $\delta^{13}C$ values for cholestanes in the Yabase oils range from −24.4 to −26.3‰. This range is nearly consistent with the $\delta^{13}C$ range for $n$-$C_{18}$ to $n$-$C_{20}$ alkanes, suggesting

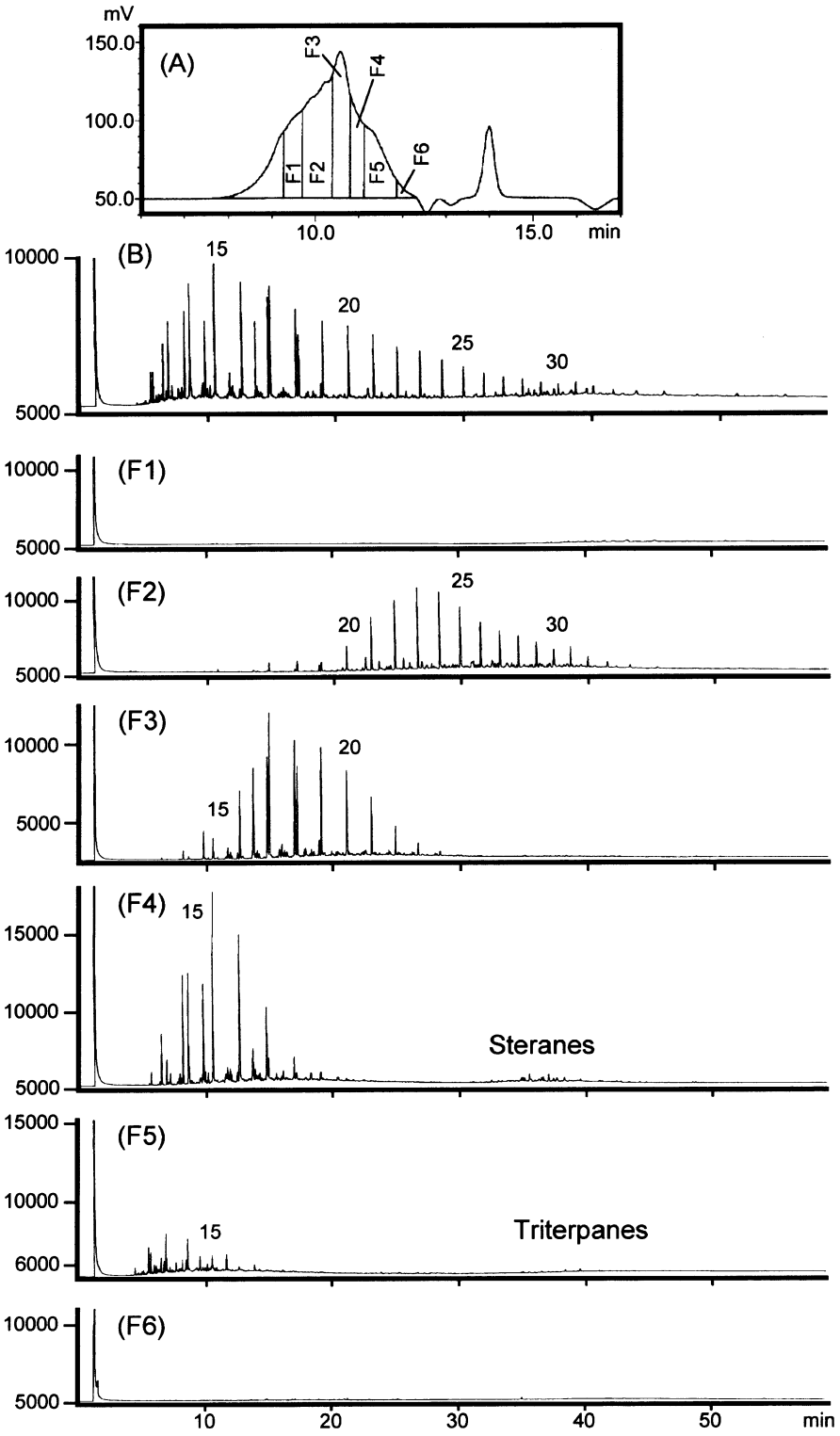


Fig. 1. (A) Representative gel permeation chromatogram of saturated hydrocarbons from the Sotoasahikawa R-114 oil, gas chromatograms of (B) bulk saturated hydrocarbon fraction and six fractions (F1–F6) separated by GPC as shown in (A). Numbers on chromatograms correspond to carbon number of *n*-alkanes.

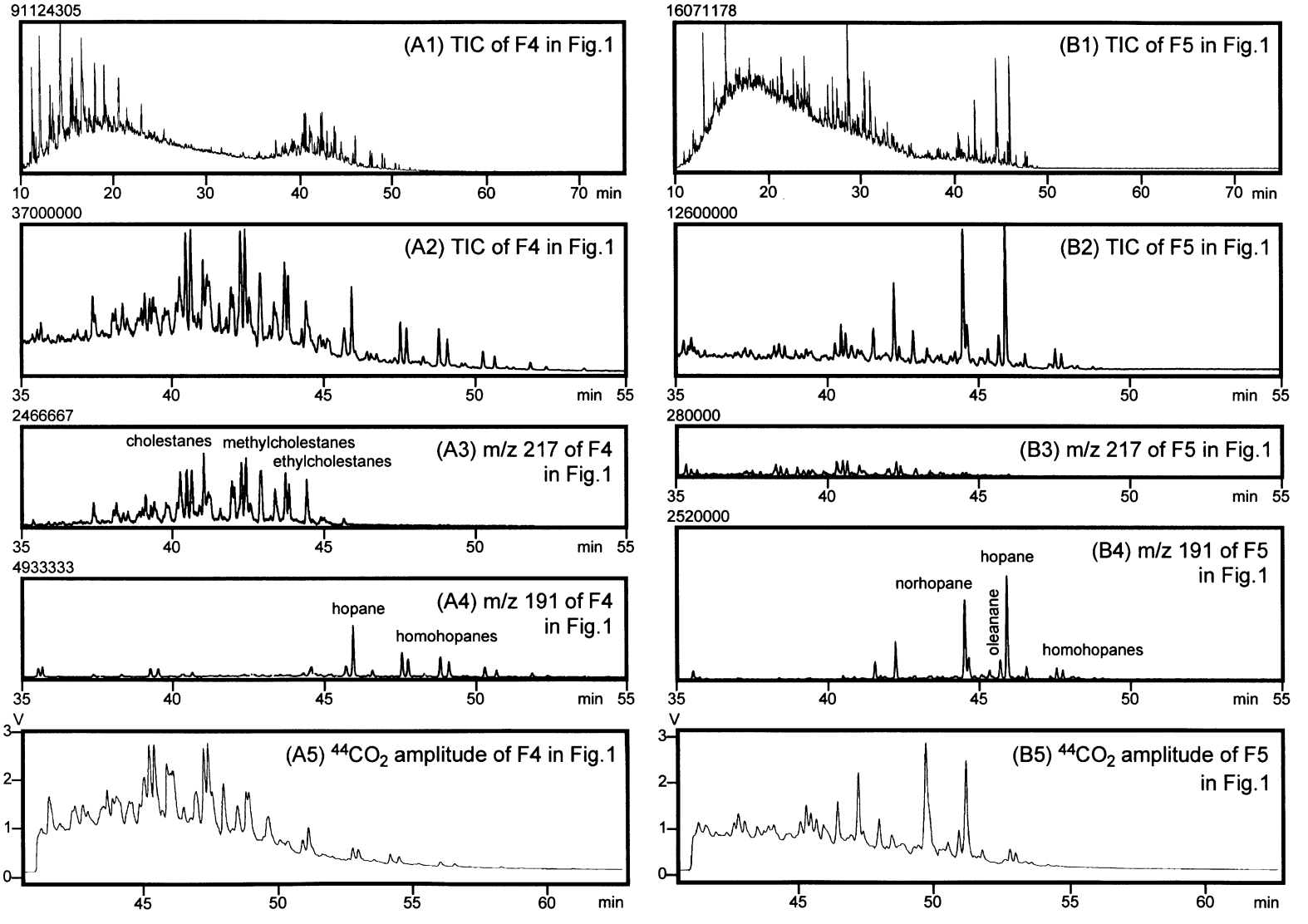


Fig. 2. Total ion chromatograms, *m*/*z* 217 and *m*/*z* 191 mass chromatograms, and $^{44}CO_2$ amplitude chromatograms of F4 and F5 fractionated by GPC (Fig. 1) for saturated hydrocarbons from the Kitaakita R-1a oil. (A1), (A2), (B1) and (B2) show representative total ion chromatograms; (A3) and (B3) show *m*/*z* 217 mass chromatograms, corresponding to (A2) and (B2); (A4) and (B4) show *m*/*z* 191 mass chromatograms, corresponding to (A2) and (B2); and (A5) and (B5) are $^{44}CO_2$ amplitude chromatograms by GC–C/IR–MS.

Table 1
Compound-specific carbon isotope ratios of *n*-alkanes of produced oils in the Yabase oil field, Northeast Japan

| Well | 18* | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kitaakita R-1a | −24.3 | −25.1 | −25.4 | −27.0 | −28.9 | −29.2 | −28.6 | −28.4 | −27.4 ±1.1 [3] | −27.4 ±0.8 [3] | −27.5 ±0.2 [3] | −26.8 | | −28.1 | | | |
| Sotoasahikawa R-22 | −23.7 | −24.2 | −24.5 | −25.2 | −26.9 | −28.3 | −28.8 | −28.6 | −28.1 | −28.5 | −27.2 | −27.4 | −27.2 | −28.7 | −27.6 | −28.2 | −27.0 |
| Sotoasahikawa R-37 | | −25.0 ±0.2 [2] | −25.0 ±0.1 [2] | −25.0 ±0.0 [2] | −25.2 ±0.1 [2] | −25.5 ±0.3 [2] | −25.8 ±0.3 [2] | −26.9 ±0.4 [2] | −27.7 ±0.2 [2] | −28.1 ±0.3 [2] | −27.9 | −29.8 | −28.5 | −29.6 | | | |
| Sotoasahikawa R-100 | | | −24.9 | −25.1 | −25.2 | −25.1 | −25.6 | −26.6 | | | | | | | | | |
| Sotoasahikawa R-114 | | | | | | | | | | | | | | | | | |
| Bulk *n*-alkanes | −26.0 | −26.0 | −25.8 | −25.8 | −26.0 | −26.6 | −25.9 | −26.3 | −26.7 | −26.7 | −25.8 | −26.2 | −26.6 | −29.4 | −28.2 | −28.9 | |
| Sotoasahikawa R-114 | | | | | | | | | | | | | | | | | |
| GPC Fraction F2 | | | −25.0 | −26.1 | −26.0 | −26.2 | −25.6 | −26.6 | −26.8 | −28.2 | −27.0 | −28.0 | −26.6 | −29.2 | −28.1 | | |
| Sotoasahikawa R-114 | | | | | | | | | | | | | | | | | |
| GPC Fraction F3 | −25.4 | −25.6 | −26.2 | −26.5 | −26.3 | | | | | | | | | | | | |
| Sotoasahikawa R-114 | | | | | | | | | | | | | | | | | |
| GPC Fraction F4 | −24.8 | −26.0 | | | | | | | | | | | | | | | |
| Koya R-76 | | −25.6 | −25.6 | −25.5 | −25.6 | −25.7 | −26.2 | −27.8 | −28.9 | −28.3 | | | | | | | |
| Koya R-83 | −25.2 ±0.0 [2] | −25.3 ±0.0 [2] | −25.7 ±0.6 [2] | −25.8 ±0.2 [2] | −26.2 ±0.2 [2] | −27.2 ±0.2 [2] | −27.7 ±0.1 [2] | −28.1 ±0.7 [2] | −27.8 ±0.5 [2] | −29.0 ±1.1 [2] | −26.0 | −27.3 | | −27.4 ±0.0 [2] | −26.2 | | |
| Koya R-135 | −24.4 | −24.4 | −24.1 | −24.4 | −25.1 | −26.6 | −28.2 | −28.8 | −27.6 | −28.6 | −27.4 | −27.1 | −27.0 | −29.3 | | | |

Unit is permil (‰). Some isotope ratios are shown as average±σ [number of data].
* Carbon number.

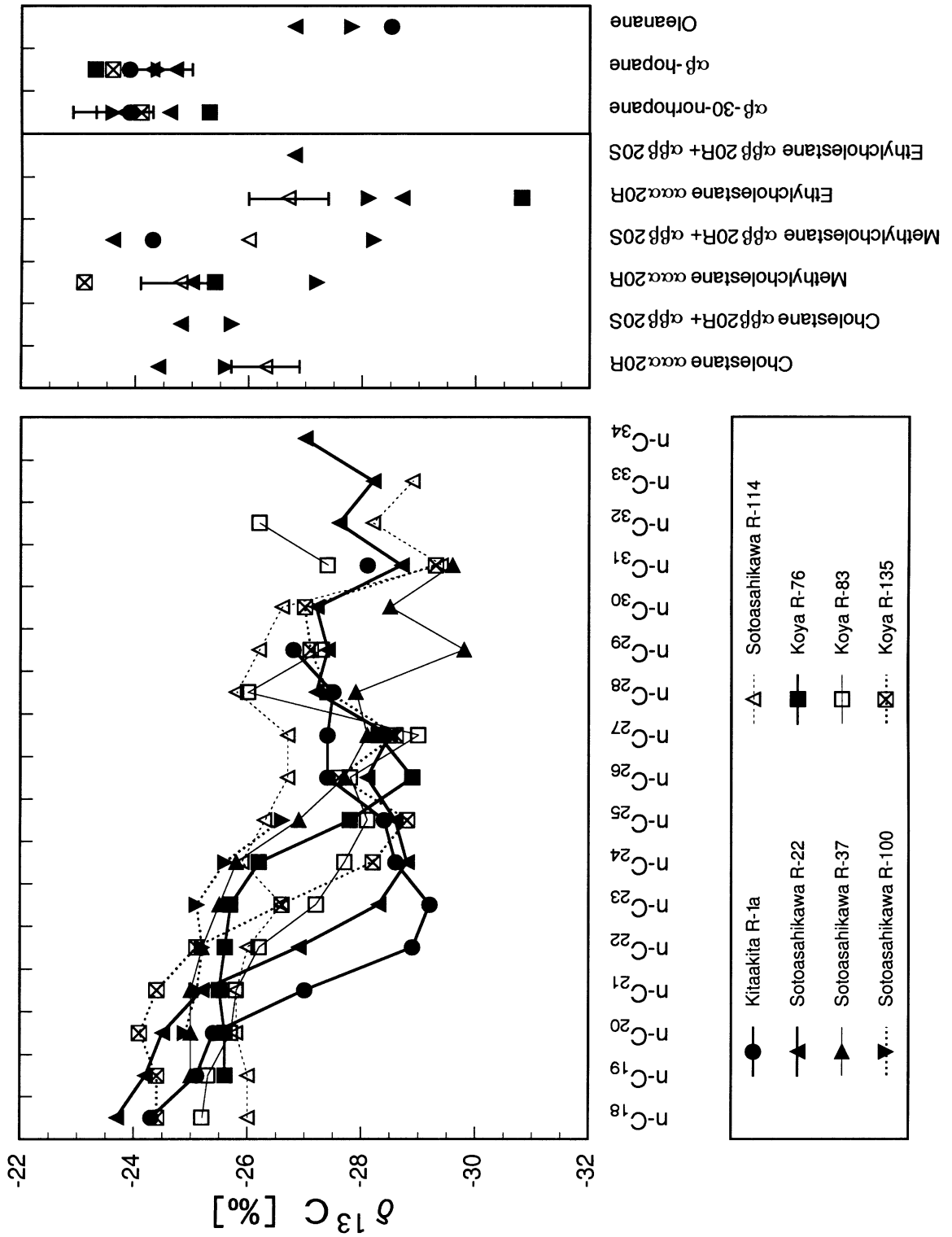


Fig. 3. Carbon isotope ratios of *n*-alkanes, steranes and triterpanes from Yabase oils.

Table 2
Compound-specific carbon isotope ratios of steranes and triterpanes of produced oils in the Yabase oil field, Northeast Japan

| Well | Cholestanes | | Methylcholestanes | | Ethylcholestanes | | Triterpanes | | |
|---|---|---|---|---|---|---|---|---|---|
| | αββ20R* | ααα20R | αββ20R* | ααα20R | αββ20R* | ααα20R | Norhopane | Oleanane | Hopane |
| Kitaakita R-1a | | | −24.3 | | | | −23.9 | −28.5 | −23.9 |
| Sotoasahikawa R-22 | −24.8 | −24.4 | −23.6 | −25.0 | −26.8 | −28.7 | −24.6 | −26.8 | −24.7 |
| Sotoasahikawa R-100 | −25.7 | −25.6 | −28.2 | −27.2 | | −28.1 | −23.6 ±0.7 [3] | −27.8 | −24.4 ±0.6 [3] |
| Sotoasahikawa R-114 GPC Fraction F4 | | −26.3 ±0.6 [4] | −26.0 | −24.8 ±0.7 [3] | | −26.7 ±0.7 [3] | | | −23.8 |
| Sotoasahikawa R-114 GPC Fraction F5 | | | | | | | −23.8 ±0.5 [3] | | −24.3 ±0.5 [3] |
| Koya R-76 | | | | −25.4 | | −30.8 | −25.3 | | −23.3 |
| Koya R-135 | | | | −23.1 | | | −24.1 | | −23.6 |

Unit is permil (‰). Some isotope ratios are shown as average ± σ [number of data].
* Mixture of 5α(H),14β(H),17β(H)-(20R + 20S) isomers.

significant algal and zooplankton contributions to oil. The $\delta^{13}C$ of methylcholestanes from Yabase oils ranges from −23.1 to −28.2‰, and is close to that of cholestane. The $\delta^{13}C$ of ethylcholestanes is, however, light, ranging from −26.7 to −30.8‰. It is similar to $\delta^{13}C$ of *n*-$C_{27}$, *n*-$C_{29}$, *n*-$C_{31}$ alkanes and oleanane, suggesting significant contribution of higher plants to these oil ethylcholestanes. The $\delta^{13}C$ values of 17α,21β(H)-30-norhopane and 17α,21β(H)-hopane are comparatively heavy, ranging from −23.6 to −25.3‰ and from −23.3 to −24.7‰, respectively. Heterotrophic bacteria can be a major source of these hopanoid hydrocarbons in the Yabase oil.

## Acknowledgements

Teikoku Oil gave us the opportunity to conduct this research in the Graduate School of Science of Hokkaido University. The authors thank Professor Koichi Saitoh of Tohoku University, Research Center for Higher Education, and Dr. Hidenori Kaneta of Teikoku Oil, for their beneficial advice on GPC. Tosoh Corporation technically assisted the GPC preparation. This paper is a part of the PhD dissertation of Inaba (2000). Dr. Joseph A. Curiale and an anonymous reviewer provided helpful comments that significantly improved the manuscript.

*Associate Editor*—**J. Curiale**

## References

Bjorøy, M., Hall, K., Hustad, E., Williams, J.A., 1992. Variation in stable carbon isotope ratios of individual hydrocarbons as a function of artificial maturity. Organic Geochemistry 19, 89–105.

Hirai, A., Sato, T., Takashima, T., 1990. Geochemical study on the Yabase oil field, Akita. Journal of the Japanese Association for Petroleum Technology 55, 37–47. (Abstract in English).

Inaba, T., 2000. Source rock prediction based on petroleum biomarkers and their compound-specific carbon isotope ratios. Dissertation of Hokkaido University, Graduate School of Science, Department of Earth and Planetary Sciences, p.138.

Inaba, T., Suzuki, N., Hirai, A., Sekiguchi, K., Watanabe, T., 2001. Source rock lithology prediction based on oil diasterane abundance in the siliceous-clastic Akita sedimentary basin, Japan. Organic Geochemistry 32, 877–890.

Ishiwatari, M., Ishiwatari, R., Yamada, K., 1997. Two possible pathways of formation of pristane and phytane from chlorophylls on diagenesis: Carbon isotopic results from laboratory heating of chlorophyll-a. Chemistry Letters 1997, 355–356.

Kennicutt II, M.C., Brooks, J.M., 1990. Unusual normal alkane distributions in offshore New Zealand sediments. Organic Geochemistry 15, 193–197.

Matthews, D.E., Hayes, J.M., 1978. Isotope-ratio-monitoring gas chromatography-mass spectrometry. Analytical Chemistry 50, 1465–1473.

Moore, J.C., 1964. Gel permeation chromatography. I. A new method for molecular weight distribution of high polymers. Journal of Polymer Science: A 2, 835–843.

Porath, J., Flodin, P., 1959. Gel filtration: a method for desalting and group separation. Nature 183, 1657–1659.

Sano, M., Yotsui, Y., Abe, H., Sasaki, S., 1976. A new technique for the detection of metabolites labeled by the isotope $^{13}C$ using mass fragmentography. Biomedical Mass Spectrometry 3, 1–3.

Schoell, M., McCaffrey, M.A., Fago, F.J., Moldowan, J.M., 1992. Carbon isotope compositions of 28,30-bisnorhopanes and other biological markers in a Monterey crude oil. Geochimica et Cosmochimica Acta 56, 1391–1399.

Suzuki, N., Sampei, Y., Matsubayashi, H., 1995. Organic geochemical difference between source rocks from Akita and Niigata oil fields, Neogene Tertiary, Japan. Journal of the Japanese Association for Petroleum Technology 60, 62–75. (Abstract in English).